Characterization of *Neorautanenia brachypus* (Harms) C.A.Sm tubers for anthelmintic properties in ruminant livestock

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A dissertation submitted in fulfillment of the requirements for an award of the degree of Master of Philosophy in Animal Production and Technology

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Dedication

This dissertation is dedicated to God, my sister Vivian Masimba, and my mother Concilia Kachepa for their unfailing support and faith in me throughout my studies. This is for you, mother, and I miss you a lot.

Statement of Declaration

I Mellisa C. Mpofu (C14123645Q) declare that the work contained in this thesis entitled- Characterization of phytocompounds and pharmacological study of *Neorautanenia brachypus* (Harms) C.A.Sm tubers for anthelmintic effects in ruminant the purpose of developing dosing drugs for livestock has not been previously submitted for a degree or as part of requirements for a degree to any university or institution other than Chinhoyi University of Technology. I further certify that the thesis is an original piece of work written by me. I have appropriately acknowledged all help that I received in my research work and in the preparation of the thesis itself. I also certify that all information sources and literature are indicated in the thesis.

Signature………M.C.M……………………. Date… 15-8-2022

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Abstract

Infections from helminths are common in livestock, resulting in economic losses. Anthelmintic developed from either killed or attenuated bacteria have been used over many centuries but their effectiveness has been lowered by anthelmintic resistance exhibited by helminths. Use of plants with anthelmintic properties has been found to offer a significant alternative pathway that is climate smart. In this research tuberous plants traditionally used in the control of helminths were explored and then focus was on *Neorautanenia brachypus*, a recently discovered plant to have anthelmintic properties worth exploring. In this research a systematic literature review on tuberous plants with anthelmintic properties was done and then through isolation and characterisation of phytochemicals in one such important tuber (*Neorauternenia brachypus*) found in Zimbabwe. *Neorautanenia brachypus* was futher subjected to in vitro and in vivo anthelmintic pharmacological studies. Forty-eight ethnobotanical investigations recorded 43 plants with tuber portions that were utilized to combat helminths. The phytochemical analysis involved extraction, phytochemical screening, quantitative analysis using a spectrophotometer, and Gas Chromatography-Mass Spectrometry analysis. *Neorautanenia brachypus* extracts contained essential oils, terpenoids, quinones, saponins, coumarins, phenols, flavonoids, alkaloids, and tannins, according to phytochemical screening tests. In terms of GAE, the total phenolic content of methanol and distilled water extracts was determined to be 365.18mg GAE/g and 89.43mg GAE/g, respectively. The Total Tannin Content of methanol and distilled water extracts was determined to be 2.33 mg TAE/g and 1.42 mg TAE/g, respectively.he pharmacological tests included a toxicity test, the Egg Hatch Inhibition Test, Larval Mortality Test, Adult Worm Mortality Test, and Faecal Egg Count Reduction Test. *Neorautanenia brachypus* extracts had a significant effect $\boldsymbol{P}<0.05$, on the inhibition of egg hatching, mortality of larvae, and death of adult *Eisenia foetida* worms. The undiluted fresh blended tuber extract and undiluted methanol tuber extracts had a statistically significant anthelminthic activity comparable to Albendazole conventional drug diluted to 75% on egg hatch inhibition. The highest IC_{50} dilution 78.88% was recorded for larval mortality compared to egg hatch inhibition 71.01%. The highest mortality of *Eisenia foetida* worms was recorded when *N. brachypus* extracts were undiluted and after 24 hours of exposure. Treatments Albe100, Albe75, Blend100, Soak100, Meth100, Meth75, and DW100 showed some anthelmintic activity after 1 hour of exposure. The treatment Albe100 showed 100% worm mortality after 2 hours of exposure. Meth100, Soak100, and Blend100 showed 100% worm mortality after 6 hours of exposure. Albe75, Albe50, Albe25, Blend75, Blend50, Soak75, Soak50, Meth75, Meth50, and Meth25 exhibited 100% worm mortality after 24 hours of exposure. All treatments except for the negative control showed anthelmintic activity ±80 % after 24 hours of exposure. *In vivo* study showed that the ranks for reduction in eggs per gram for both coccidia and Strongyloides across species increased from week 1 to week 5 for the untreated group. Significant differences in eggs per gram reduction of Strongyloides ($P<0.05$) were noticed from week 4 to week 5. However, the ranks for reduction in eggs per gram for both coccidia and Strongyloides in goats and cattle decreased from week 1 to week 5. There was no significant change in the weight of goats and cattle between the start and end of the experimental period ($\boldsymbol{P}>0.05$). *Dioscorea deltoidea, Dioscorea bulbifera, Dioscorea alata, Gloriosa superba, Curcuma longa, Dioscorea pentaphylla,* and *Cyperus rotundus* were shown to be the most culturally important plants for the control of helminths. As a result, conservation measures for these culturally significant plants are needed. There is also a need to investigate other tuberous plants, especially those found in Africa to identify unique compounds that are active against helminths to develop more robust anthelminthic drugs and reduce the rate at which anthelmintic resistance occurs. It was concluded that white *N. brachypus* were the most significant to use for extraction of phytochemicals giving a significant extract yield when air-dried and Soxhlet extracted using chloroform 50%: ethyl acetate 50% as a solvent. While Methanol was the most acceptable solvent when extracting the greatest number of different classes of phytochemicals and also gave a significant extract yield. The chemical constituencies of *N. brachypus* confirmed by GCMS analysis affirm the therapeutic applications of the tubers. White *N. brachypus* tubers air-dried and Soxhlet extracted using methanol and distilled water can be used as alternatives in drug discovery because of their low toxicity to erythrocytes. The in vitro anthelmintic activity of tested plant preparations was characterized by a decrease in egg hatching, larvae, and adult worm mortality. *Neorautanenia brachypus* treatments may reduce the hatchability of the eggs excreted in the feces, resulting in both a reduced risk of reinfection and lightened worm loads by decreasing pasture contamination. Accordingly, *N. brachypus* extracts have the potential to contribute to controlling gastrointestinal parasites of ruminants. Fresh blended samples are recommended for use to control the eggs and larvae of helminths as they had acceptable IC_{50} concentrations in both assays. The results of this study are suggestive of promising anthelmintic activity of the herbal-based drug for both Strongyloides and coccidia in cattle and goats.

Keywords: Anthelmintic, tubers, extraction, phytochemicals, *Neorautanenia brachypus, in vitro, in vivo*, livestock, anthelmintic resistance, Strongyloides, coccidia

Table of Contents

List of Tables

List of Figures

Abbreviations

FC: Frequency of citation

RFC: Relative frequency of citation

FIV: Family importance value

N: number of citations

JI: Jaccard index

Chl: chloroform

EA: ethyl acetate

Meth: methanol

DW: distilled water

Chl-Meth: chloroform and methanol

Chl-EA: chloroform and ethyl acetate

Meth-EA: methanol and ethyl acetate

GC-MS: Gas Chromatography-Mass Spectrometry

GAE: Gallic Acid Equivalent

TAE: Tannic Acid Equivalent

Blen: blended juice extract

Albe: Albendazole

Soak: soaked extract

EPG: Eggs per gram

List of Appendices

CHAPTER 1 Introduction

1.1 Background

People's livelihoods are heavily reliant on livestock, particularly in drought-prone areas. Approximately more than 150 million poor people in sub-Saharan Africa survive on livestock-based products (Berihulay, Abied, He, Jiang, & Ma, 2019). Cattle serve many functions in community spaces, including producing milk, dung, meat, hides, draught power, and serving as a measure of one's wealth status (Gusha, Palmer, & Villano, 2018; Maburutse, Mutibvu, Mbiriri, & Kashangura, 2012; Masikati, 2011). These advantages also apply to other ruminant species. It is consequently critical that animals be protected from a nutritional and health standpoint as millions of people depend on them for their livelihood.

There are various restrictions to ruminant production in Zimbabwe, including a high prevalence of illnesses and parasites, a low level of management, inadequate pasture supply, and poor marketing management (Tavirimirwa *et al*., 2013). Helminthiases affect almost every region of the planet, including Zimbabwe, causing significant losses in ruminant productivity and jeopardizing animal welfare (Alawa *et al*., 2010). Helminthiases, often known as worm infection, is a macro-parasitic disease of people and animals in which parasitic worms known as helminths invade a component of the body (Deepak, 2019). Helminths are multicellular worms that can be classified into three types: nematodes, cestodes, and trematodes (Duguma *et al*., 2011; Nandhini & Sumathi, 2014).

Helminths impair weight increase, cause anemia, diarrhea, decreased reproductive performance, low live mass, dull rough coat, organ condemnation, and mortality (Johansson, 2017; León, Delgado, & Florez, 2019; Morgan *et al*., 2013). As a result, a considerable percentage of the population, particularly HIV-positive people, are at risk of malnutrition. Conventional anthelmintics are mostly used to control the parasites. Benzimidazole (BZ) (broad-spectrum, effective against nematodes and trematodes), levamisole (effective against nematodes and trematodes in higher doses), salicylanilides (effective against trematodes), praziquantel (effective against cestodes), and ivermectin (effective against nematodes) are the major anthelmintic classes (Vercruysse & Claerebout, 2014).

However, due to their high cost, general toxicity, drug residue problems in milk and meat, and the development of anthelmintic resistance in helminths, the use of contemporary medications for animal health and productivity faces obstacles (Matekaire & Bwakura, 2004; Mwale, Bhebhe, Chimonyo, & Halimani, 2005; Oliveira *et al*., 2009; Zaman, Zafar, Sindhu, Abbas, & Qamar, 2017). Dealing with anthelmintic resistance has also been problematic due to the high expenses of new chemical research (Besier, 2007; Schlander, Hernandez-Villafuerte, Cheng, Mestre-Ferrandiz, & Baumann, 2021). The control of parasitic helminths has been hampered by climate change.

Animals are affected directly by climate change (e.g., heat stress), indirectly via changes in their surroundings and available resources (e.g., changes in the availability of grass and water), and indirectly through changes in host-pathogen interactions. Worm parasites, in particular, that have a free-living stage or rely on the availability of an intermediate host to complete their life cycle, will be affected by climate change (Mas-Coma, Valero, & Bargues, 2008). The geographical distribution of helminths, as well as the seasonal pattern of occurrence, will be altered (Van Dijk, David, Baird, & Morgan, 2008). There are currently reports available on such developments (Jenkins, Schurer, & Gesy, 2011; Kenyon, Sargison, Skuce, & Jackson, 2009). Furthermore, as global warming continues, helminths prevalence is expected to increase (Morgan & Van Dijk, 2012).

Because of their accessibility, affordability, and availability, ethnoveterinary plants have become more relevant in managing helminths in the face of the aforementioned obstacles. Herbal medications have certain unique qualities when compared to conventional drugs, such as low toxicity, less residual effects, and very slow evolution of resistance, as well as being environmentally benign (Waller *et al*., 2001). The availability of numerous active phytocompounds in one plant, which might act in various ways, may be the fundamental explanation for the slow development of anthelmintic resistance in isolated rural locations (Athanasiadou, Githiori, & Kyriazakis, 2007). This was later backed up by Nandhini *et al.* (2014), who stated that the advantage of natural products is that they are a combination of components acting synergistically to produce an anthelmintic effect, as opposed to synthetic drugs, which only have one molecule acting on the parasite when not in a combination formulation. A medical plant, according to the World Health Organization, is any plant that contains compounds that can be utilized for therapeutic purposes or are precursors of chemo pharmaceutical semi production in one or more of its organs (Singh, 2011).

Several ethnobotanical surveys, *in vivo*, and *in vitro* research studies have indicated hundreds of plants that have anthelmintic effects. *Artemisia afra*, *Aloe ferox*, *Leonotis leonurus*, and *Elephartorrhiza elephantine* are anthelmintic plants used in South Africa (Mazhangara, Sanhokwe, *et al*., 2020). *H. contortus* larvae were negatively affected by extracts of *Senecio congestus*, *A. ferox*, *Senecio barbertonicus*, and *Gardenia* sp. (Chitura *et al*., 2019). *Bridelia ferruginea*, *Combretum glutinosum*, and *Mitr-agyna inermis* were found to have anthelmintic effects on *H. contortus* eggs and larvae in vitro (Alowanou *et al*., 2019). In a concentration-dependent way, *Ozoroa pulcherrima* Schweinf extracts and fractions increased cercariae and worm mortality (Feussom *et al*., 2020). *Artemisia campestris* at a dose of 5000 mg/kg demonstrated substantial nematicidal action (Abidi *et al*., 2018). In vitro anthelmintic activity was found in *Camellia sinensis* L. and *Albizia lebbeck* L. against *H. contortus* (Zaheer, Hussain, Khalil, Mansha, & Lateef, 2019). *Origanum majorana* essential oil at 5000 mg/kg reduced egg and adult worm counts by 76.3% and 74%, respectively (Abidi, Sebai, Dhibi, Darghouth, & Akkari, 2020).

Traditional herbal therapy has been developed and used in Africa since the Stone Age, and it is more common than conventional treatment. African traditional medicine is defined as a comprehensive healthcare system divided into divination, spiritualism, and herbalism, with significant overlap in some cases (Chavunduka, 1999; Mahomoodally, 2013). Ethnoveterinary medicine is a body of knowledge based on local people's beliefs, skills, methodologies, and practices related to animal health and production. "The holistic, interdisciplinary study of local knowledge and its associated skill, practices, beliefs, practitioners, and social structures on the health care and healthful husbandry of food, work, and other in-coming producing animals, always with an eye to practical development application within livestock production and livelihood," wrote McCorkle (1995). Ethnoveterinary medicine has been documented in various books (Mathias-Mundy & McCorkle, 1989; McCorkle, Mathias-Mundy, & Schillhorn-van-Veen, 1996), and databases and websites such as NUFFIC, Ethnovet online, PRELUDE, and SPIRAL exist.

Medicinal plants used to treat animals and those used to treat people frequently cross paths (McCorkle & Mathias-Mundy, 1992). It's thought that livestock caretakers have adapted human treatments for use in animals, or vice versa, over the centuries. Similar therapies are used to treat similar ailments in humans and their livestock, and the same is true for anthelmintics. Gakuya (2001), previously stated that human beings and livestock herbalists are often interchangeable and that there is a need for a collaborative approach when dealing with medicinal plants because most herbs are used to treat both human and animal ailments.

Plants are one of the most important natural producers of different compounds, ranging from simple skeletal structures to complex ones. Several well-known components, such as quinine (chloroquine and mefloquine), artemisinin, taxol (paclitaxel), camptothecin, khellin, sodium chromoglycate, galegine, metformin, papaverine, and verapamil, are based on plant based medicines (Cragg & Newman, 2013; Khan & Ahmad, 2019; Koparde, Doijad, & Magdum, 2019).

Neorautanenia brachypus (Zhombwe), which is widely distributed in semi-arid south-eastern Zimbabwe, has only recently gained scientific attention. Zhombwe (Shona) or Mapombwe are the local names for the plant. The plant was discovered in Zimbabwe by a local farmer in the south-east Lowveld during the severe two-year drought of 1991–1992 (Murungweni, Andersson, Van Wijk, Gwitira, & Giller, 2012). *Neorautanenia brachypus* belongs to the Leguminosae-Papilionaceae family. The plant produces purple flowers, which develop into dehiscent pods densely covered in hairs (Murungweni et al., 2012). The plant produces a tuberous underground root that looks like yams (*Colocasia esculentum*) and cassava (*Manihot esculentum*).

Tubers of *N. brachypus* tubers are used for cow feeding, dosing animals, curing severe wounds, and catching fish, according to an ethnobotanical study (Murungweni et al., 2012). In feeding studies with goats, the tuber's anthelmintic value was assessed. Infected animals fed *N. brachypus* demonstrated lower rates of Strongyloides worm infection in small ruminants ($\boldsymbol{P}<0.05$) and large ruminants ($\boldsymbol{P}<0.01$), equivalent to animals given conventionally indicated medicines(Murungweni et al., 2012) .

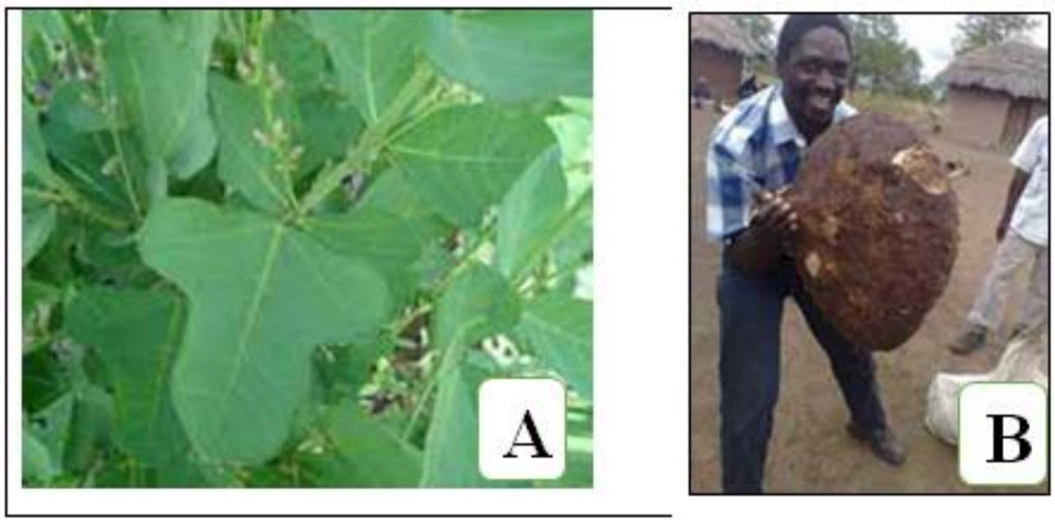

Figure 1.1: A is a picture of *N. brachypus* leaves and B is a picture a man holding the tuber in Chikombedzi. Retrieved from (Murungweni, Andersson, Van Wijk, Gwitira, & Giller, 2012)

Tuber plants are one of the plant classes being researched for their anthelmintic properties. Tubers are large fleshy underground structures that form from stems or roots, such as potatoes and yams, and they are classified into a variety of botanical families. *Orchidaceae* is one of the largest botanical families that produce tubers, with between 20000 and 35000 species divided into 600-850 genera (Gutiérrez, 2010; Hossain, 2011). *Fabaceae*, *Liliaceae*, *Alismataceae*, *Cyperaceae*, *Vitaceae*, *Asphodelaceae*, *Zingiberaceae*, *Ranunculaceae*, *Dioscoreaceae*, *Primulaceae*, *Arecaceae*, and *Capparidaceae*, among others, are some of the tuber-producing families.

The major goals of this research were to identify phytocompounds found in N. *brachypus* and ascertain their anthelmintic value. Ethnobotanical surveys, phytochemical analysis and isolation, and pharmacological studies are all part of medicinal plant research (*in vitro* and *in vivo*). The literature review explains these processes in detail.

1.1.2 Effects of helminths on meat production

The economic impact of helminthiases on the condemnation of carcasses and offals after slaughter is significant and should not be overlooked, as it deprives livestock farmers of much-needed cash. Furthermore, most of the condemned meat that could have been helpful is thrown away instead of being converted into processed meat, bone meal, or pet food. The magnitude of such losses can only be understood in the context of developing countries' high poverty rates, hunger, and food insecurity. According to Phiri (2006), *Fasciola gigantica* infections made livers and lungs the most condemned offals in terms of number and weight (20.1% and 0.7% respectively). Hydatidosis was also responsible for 0.9% lung and 0.1% liver losses. Only 0.05% of all tongues, hearts, and skulls examined contained Cysticercus bovis.

In a study by Nur *et al*. (2016), 97 (32.3%) and 146 (48.7%) of 300 calves slaughtered in an abattoir were found to be infected with Fasciola spp. and hydatid cysts, respectively. 190 visceral organs were revealed to be afflicted with hydatid cysts. The average prevalence of *Taenia saginata* cysticercosis was reported to be 1.6% in Zimbabwe's Matabeleland Province and was greater during the wet season. The majority of the infected cattle (1.4%) had live cysts, while a few had dead cysts (0.2%), and the majority of the cattle condemned were under the age of two years (Sungirai, Masaka, & Mbiba, 2014). *T. saginata* cysticercosis is usually classified as high when it occurs at least 10% of the time, moderate when it occurs between 1 and 10% of the time, and low when it occurs less than 1% of the time (Zdolec *et al*., 2012). Cysticerci can easily be missed during meat inspection since they are not present on routine cuts or as a result of light infections in the carcasses (Dorny, Phiri, Gabriël, Speybroeck, & Vercruysse, 2002).

With an infection rate of 7%, Abuseir, Epe, Schnieder, Klein, and Kühne (2006) estimated $USD 1.8 billion in annual economic losses in Africa due to *T. saginata* cysticercosis. In cases where meat is held for further processing, which involves holding the carcass at temperatures below -7oC for up to 3 weeks to inactivate the parasite, there are also additional expenditures linked to refrigeration and handling. Losses of 85,051.70 USD, 2,567,586 USD, and 5,110,499 USD were reported in Nigeria, Kenya, and Iran, respectively (Cadmus & Adesokan, 2009; Khaniki, Kia, & Raei, 2013; Kithuka, Maingi, Njeruh, & Ombui, 2002). A study in Turkey recorded a loss of 760 USD (Yibar, Selcuk, & Senlik, 2015).

Fascioliasis and hydatidosis control strategies, which may involve the use of anthelmintics, grazing management, and intermediate intervention, can significantly minimize all of these losses (Nur *et al*., 2016). Plant extracts can also be utilized instead of current anthelmintics with success.

1.1.3 Zoonotic helminths

According to Mas-Coma *et al*. (2008), helminths diseases may be classified as infectious disorders, and extra attention should be devoted to them in the future due to climate change. Helminths of the genus *Fasciola* are parasitic flatworms belonging to the Trematoda class. Many herbivorous mammals, such as ruminants, equines, and camelids, as well as omnivores like pigs and humans, are the definitive hosts. Specific freshwater lymnaeid snail species serve as intermediary hosts. *Fasciola hepatica* and *Fasciola gigantica* worms cause fascioliasis. Only *F. hepatica* is found in Europe, America, and Oceania, but both species are found in Africa and Asia (Lu *et al*., 2018; Mas-Coma, Valero, & Bargues, 2009). Reduced fertility, abortions in the late stages of pregnancy, anemia, reduced milk output, and mortality are all production consequences of *Fasciola* infection. *Fasciola* has been reported in humans on all five continents, with an estimated 2.4 million people infected in 61 countries and many more at risk (Molina-Hernández *et al*., 2015; Torgerson & Macpherson, 2011).

The beef tapeworm, *Taenia saginata*, is a member of the order *Cyclophyllidea* and the genus *Taenia*. In humans, it causes taeniasis (a form of helminthiases), and in cattle, it causes cysticercosis (Zdolec *et al*., 2012). It's known as beef measles because the larvae mature into cysts that look like measles and are discovered on the animal's muscles. The adult stage of the cestode causes diarrhea, depression, and weakness in humans (Kumar & Tadesse, 2011), and taeniasis infections in humans have been estimated to affect roughly 60 million individuals (Raether & Hänel, 2003). According to the Organization (1996), 50 million cases of such infestation occur worldwide each year, resulting in 50,000 deaths. In humans, appendicitis, intestinal blockage, and gall bladder perforation are more significant side effects (Hendrickx *et al*., 2019).

Praziquantel, Niclosamide, and albendazoles are effective treatments for these helminths. However, helminths have acquired resistance to these medications, and they are also expensive. Natural plants, on the other hand, can effectively inhibit zoonotic helminths. At 50% concentration, the plant *O. gratissimum* was found to kill 100% of *T. saginata* ova, whereas G.

latifolium and *O. gratissimum* extracts killed 70% of *F. gigantica* and *Schistosoma spp*. (Daniel, Ohalete, Ibiam, & Okechukwu, 2015). An anthelmintic effect of 1% citronella oil (*Cymbopogan nardus*) on live *Fasciola gigantica* was discovered in another investigation. *F. gigantica* was reported to be inhibited by alcoholic extracts of *Allium sativum* and *Piper longum*, according to Singh *et al*. (2014). *Areca catechu* plant extracts were found to be more effective than conventional medications in controlling helminths (Jeyathilakan, Murali, Anandaraj, & Abdul Basith, 2010).

1.1.4 Prevalence of helminths in Zimbabwe

Nineteen gastrointestinal nematode species from seven families have been found to infect cattle in Zimbabwe, according to research (Pfukenyi & Mukaratirwa, 2013). The Trichostrongylidae Family includes the genera *Cooperia*, *Haemonchus*, *Trichostrongylus*, *Ostertagia*, and Chabertiidae including the genus *Oesophagostomum* which has the highest incidence (Zvinorova *et al*., 2016). *Trichuris* and *Strongyloides,* in addition to *Haemonchus*, *Trichostrongylus*, and *Oesophagostomum*, have been found in additional research (Tsotetsi & Mbati, 2003). The highest incidence of these nematodes may be due to adult females' ability to produce thousands of eggs every day, resulting in rapid larval pasture contamination and hemonchosis outbreaks (de Matos *et al*., 2017; Kotze & Prichard, 2016). In their study, Zvinorova *et al*. (2016) found a low prevalence of trematodes (amphistomes), with *Moniezia spp*. being the only cestode found in Zimbabwe. *Fasciola gigantica* and *Fasciola hepatica* are the two primary species of *Fasciola* parasitic on animals and humans in the tropics. In Zimbabwe, just one genus of liver fluke, *F. gigantica*, has been found to infect cattle (Condy, 1962; Vassilev, 1994). The *Schistosoma mattheei* is the only Schistosoma species found in cattle.

However, because the medications are only available through importation, controlling and managing helminths is exceedingly expensive in Zimbabwe. In addition, the country, like any other country in the world, is dealing with anthelmintic resistance (AR). Farmers in South Africa have been reported to spend on average ZAR 15-30 per animal for *Fasciola* infection treatment alone (Jaja, Mushonga, Green, & Muchenje, 2017).

1.1.5 Anthelmintic resistance (AR)

Anthelmintic resistance develops when parasites that are normally treated by a single dose become resistant to the treatment. Because resistance is passed down through the generations, the surviving worms will pass on their resistance alleles to their offspring (Coles, 2005). The frequency and dosage of treatment are the most critical elements in the development of resistance. Under-dosing is the major cause of parasite resistance in resource-constrained farms (Verma, Lata, & Das, 2018). As demonstrated in Figure 1, there is a wealth of knowledge on AR in ruminants.

Table 1.1: Table showing the introduction of anthelmintic drugs for ruminants and the development of resistance to the drug (De Graef, Claerebout, & Geldhof, 2013).

Anthelmintic drug class	**Mode of action**	**Generic drug name**	**Introduced on the market**	**Resistance reported**	**Reference**
Heterocyclic compounds	Blocking dopaminergic transmission Agonist of the inhibitory GABA-receptor	Phenothiazine	1940	1957	(Leland *et al.*,1957)
Heterocyclic compounds	Blocking dopaminergic transmission Agonist of the inhibitory GABA-receptor	Piperazine	1954	1966	(Leland *et al.*,1957)
Benzimidazoles	Inhibiting polymerization of microtubules	Thiabendazole	1961	1964	(Drudge *et al.*, 1964)
Benzimidazoles	Inhibiting polymerization of microtubules	Cambendazole	1970	1975	(Berger,1975)
Benzimidazoles	Inhibiting polymerization of microtubules	Oxibendazole	1970	1985	(Drudge *et al.*, 1985)
Benzimidazoles	Inhibiting polymerization of microtubules	Mebendazole	1972	1975	(Berger,1975)

Benzimidazoles	Inhibiting polymerization of microtubules	Albendazole	1972	1983	Cawthorne and Whitehead, 1983)
Benzimidazoles	Inhibiting polymerization of microtubules	Fenbendazole	1975	1982	(Boersema and Lewing-van der Wiel, 1982)
Benzimidazoles	Inhibiting polymerization of microtubules	Oxfendazole	1976	1981	(Le Jambre *et al*., 1981)
Benzimidazoles	Inhibiting polymerization of microtubules	Triclabendazole	1983	1998	(Mitchell *et al*., 1998)
Imidazothiazoles and Tetrahydropyrimidines	Agonist of nicotinergic acetylcholine receptors	Levamisole	1979	1979	Sangster *et al*., 1979)
Imidazothiazoles and Tetrahydropyrimidines	Agonist of nicotinergic acetylcholine receptors	Pyrantel	1974	1996	(Chapman *et al*., 1996)
Imidazothiazoles and Tetrahydropyrimidines	Agonist of nicotinergic acetylcholine receptors	Oxantel	1976	-	-
Imidazothiazoles and Tetrahydropyrimidines	Agonist of nicotinergic acetylcholine receptors	Morantel	1970	1979	(Sangster *et al*., 1979)
Macrocyclic lactones	Allosteric modulators of the glutamate-gated chloride channels	Abamectin	Late 1970's	2001	(Wooster *et al*., 2001)
Macrocyclic lactones	Allosteric modulators of the glutamate-gated chloride channels	Ivermectin	1981	1988	(van Wyk and Malan, 1988)
Macrocyclic lactones	Allosteric modulators of the glutamate-gated chloride channels	Moxidectin	1991	1995	(Leathwick, 1995)
Macrocyclic lactones	Allosteric	Doramectin	1993	2007	(Borgsteede

	modulators of the glutamate-gated chloride channels				*et al*., 2007)
Macrocyclic lactones	Allosteric modulators of the glutamate-gated chloride channels	Eprinomectin	1996	2003	(Loveridge *et al*., 2003)
Amino-acetonitrile derivative	Agonist of nicotinergic acetylcholine receptors	Monepantel	2009	-	-
Spiroindole	Agonist of cation channels	Derquantel	2010	-	-

However, there have been initiatives to establish novel helminths control measures in livestock as well as reduce AR, both of which have been partially successful. An approach including the use of various anthelmintic classes was proposed to delay the onset of AR (Dobson *et al.*, 2012; Leathwick & Hosking, 2009). However, according to Charlier *et al*. (2018), the strategy will not be sustainable until the manner the drugs are used changes, and resistance to the new combination products may emerge at the same time (Besier, 2007; Hodgson & Mulvaney, 2017). Another technique is "refugia," which is based on the idea that retaining a section of the parasite population unaffected by anthelmintic medications slows the spread of AR (Muchiut, Fernández, Steffan, Riva, & Fiel, 2018). The difficulty lies in determining the appropriate proportion of "refugia" to reduce AR growth while retaining adequate performance.

Vaccination, genetic selection, biological approaches, and pasture management were also suggested as methods to control helminths infections in addition to these tactics (Vercruysse *et al*., 2018). These strategies were also less effective since they required strong epidemiological information, which is not always available to farmers, particularly in places with few resources.

The most viable and sustainable helminths control strategy was indicated as an integrative approach combining grazing management and application of anthelmintics through focused decision-making (Charlier *et al*., 2018). Furthermore, ethnoveterinary plants have gained popularity as the most successful way of controlling helminths that are also environmentally friendly and have an animal welfare goal (Scholten, De Boer, Gremmen, & Lokhorst, 2013), and no notable resistance has been recorded to date. *Acacia karoo, Cassia singueana*, *Ozoroa insignis, Vernonia amygdalina,* and *Ximenia caffra* have all been shown to exhibit anthelmintic properties against *H. diminuta* (Mølgaard *et al*., 2001). Because phytochemicals are present, these plants have bioactivities.

1.1.6 Phytochemicals that have anthelmintic effects

Non-nutritive plant compounds with disease-preventive or protective qualities are known as phytochemicals. Tannins, alkaloids, flavonoids, terpenoids, phenols, saponins, and essential oils have all been shown to have anthelmintic properties (Ajah & Eteng, 2010; Athanasiadou, Kyriazakis, Jackson, & Coop, 2001; Wang, Zhou, et al., 2010). Tannins impede ATP generation in parasites, affecting linked oxidative phosphorylation (Davuluri, Chennuru, Pathipati, Krovvidi, & Rao, 2020; Martin, 1997). Tannins bind to the cuticle of the helminths' body surface, paralyzing the parasites and causing death, according to other studies (Botura et al., 2013; Thompson & Geary, 1995). Alkaloids act on the central nervous system, causing parasite paralysis and death (Bate-Smith, 1962; Dubois *et al*., 2019). Saponins stop the enzyme acetylcholinesterase from working, influence the permeability of worm cell membranes and irritate the gastrointestinal mucous membrane channel of worms preventing food absorption and resulting in death (Melzig, Bader, & Loose, 2001; Santos *et al*., 2018). Flavonoids work by suppressing arachidonic acid metabolism, which can lead to neuron degeneration and death in the worm's body (Chetia & Das, 2018; Ferrandiz & Alcaraz, 1991). Phenols have an inhibitory/cidal effect on helminths due to their prooxidant activity (Sprenger *et al*., 2015).

1.1.7 Fabaceae plants' phytochemistry and anthelmintic impact

The Fabaceae family of legumes produces more nitrogen-containing secondary metabolites than other plant groups (Wink, 2013). These compounds include the alkaloids and amines (quinolizidine, pyrrolizidine, pyridine, pyrrolidine, etc.), non-protein amino acids (NPPA), cyanogenic glucosides, and peptides (lectins, cyclotides). Phenolics (flavonoids, isoflavones, catechins, tannins, coumarins, and furanocoumarins), polyketides (anthraquinones), and terpenoids (steroidal saponins, tetraterpenes, and triterpenoid) are phytochemicals that do not contain nitrogen (Wink, 2013).

Although the plant kingdom supplies a huge diversity of herbal plants with anthelmintic qualities, these natural resources have not been fully utilized to control helminthiases. The shortcomings in ethnoveterinary plant research and exploitation were highlighted by Vercruysse *et al*. (2018). They stated that there are few systematic, scientific analyses of efficacy, mode of action, and active component identity and that no plant-based anthelmintic is now commercially accessible. Difficulties in registering herbal plants, an unclear method of action, and the existence of opportunistic pathogens are all obstacles to their widespread usage.

Difficulties in registration, unclear method of action, probable presence of other uncharacterized secondary metabolites, residue, quality assurance issues, and manufacturing and distribution obstacles are among the roadblocks to widespread usage of herbal plants. Murthy and Joseph (2011), stated that more than 100 plant items have been proved to have anthelmintic properties but have not been turned into marketable medications for a variety of reasons.

1.1.8 Anthelmintic Drugs Made from Plant Extracts

Schitozim (not registered) is an example of an over-the-counter herbal medicine used to treat schistosomiasis infection in some locations (Ayonga, 2014). It's used to treat infections caused by *S. mansoni* and *S. haematobium*. It is cheaper than Praziquantel and is manufactured from a blend of many plant extracts put into a tablet. Tannins, steroids, flavonoids, glycosides, and saponins were found in the phytochemical analysis of Schitozim (Ayonga, 2014). For the treatment of helminthiases, several medications from the Orchidaceae family have been created, including Agrimophol from *Agrimonia supatoria*, Arecoline from *Areca catechu*, and qulsqualic acid from *Quisqualis indica* (Kong, Goh, Chia, & Chia, 2003).

Niclosamide, Oxyclozanide, and bithionol are examples of synthetic phenolic anthelmintics (Suryavanshi, Rai, & Malviya, 2012). Anthelmintic resistance, on the other hand, will emerge quickly as a result of widespread usage of an active chemical isolated from a particular medicinal plant (Chagas, 2015). Natural chemicals are more stable and structurally diverse than synthetic compounds, making them effective against a wide spectrum of parasites.

1.2 Problem statement

Infection by helminths is a major concern in ruminant livestock production, resulting in considerable financial losses, lack of food security, impeded rural development projects, and slow economic growth (Negasi, Bogale, & Chanie, 2012). Infection with *Fasciola spp*. causes a 3.8% to 15.2% decline in milk supply in the dairy industry, with global production losses exceeding $3 billion per year (Toet, Piedrafita, & Spithill, 2014). Due to helminth's capacity to remove Red Blood Cells, immediate effects include a rough dull coat, weakness, diarrhea, apathy, tail rubbing, submandibular edema (bottle jaw), loss of appetite, weight loss, and anemia (Dogo, Karaye, Patrobas, Galadima, & Gosomji, 2017). Despite the availability of anthelmintic treatments, a considerable portion of the world's population lives in isolated rural areas with little access to contemporary medications. Anthelmintic drug resistance to practically every marketed anthelmintic drug is also a major issue around the world (De Graef *et al*., 2013). Multiple-drug resistance has prompted a few farmers in South Africa, New Zealand, and Australia to abandon sheep and goat production (Geary, 2005; Kaplan, 2004). Such circumstances put the livestock business at risk of imploding and exacerbating the food security deficit.

Furthermore, management of is extremely costly, not just for individual farmers but also for a country's government. Externalities, such as trade consequences and public health effects, must be considered by the government. For example, research in the United Kingdom assessed the cost of treating parasitic nematodes in sheep to be around 120 million US dollars per year, whereas studies in Switzerland estimated the cost of treating liver fluke illness in cattle to be around 63 million US dollars per year (Schweizer, Braun, Deplazes, & Torgerson, 2005). Another disadvantage is that some parasitic helminths can be transmitted between vertebrate animals and humans (i.e., these helminths are zoonotic), with roughly 20 species producing severe or deadly illnesses. Furthermore, some anthelmintics present an environmental concern due to residues from these drugs being released into the environment and in foods of animal origin (Salgado & Santos, 2016). This may have an impact on consumer health and has prompted research into alternate control strategies. In this case, medicinal plants and their metabolites may be a viable parasite control option (Hoste & Torres-Acosta, 2011; Rochfort, Parker, & Dunshea, 2008).

“The smuggling and selling of counterfeit veterinary drugs is on the increase in Zimbabwe, putting livestock at risk”, an official with the Medicines Control Authority of Zimbabwe (MCAZ) said (https://www.newsday.co.zw/2016/11/smuggling-counterfeit-veterinary-drugs-increase-mcaz/). Even though imports account for 95% of the country's medicines, the country is experiencing foreign currency shortages, resulting in the procurement of a few classes of drugs that are then sold to farmers at a premium price. As a result, anthelmintic medication resistance is an inevitable end in the country. As a result of the helminths infestation, livestock productivity in Zimbabwe has decreased. Thus, in light of the current situation, extensive research in the field of medicinal plants is required to investigate their anthelmintic efficacy and, as a result, the isolation and characterization of bioactive compounds from them, which will aid in the development of better, safer, and cost-effective novel drugs in the future.

Conventional anthelmintic drugs’ exhibition of anthelmintic actions after biotransformation induces a high level of reactive nitrogen species (RNS) and reactive oxygen species (ROS) production in biological systems, thereby promoting oxidative damage of tissues (Dimitrijević, Borozan, Katić-Radivojević, & Stojanović, 2012; Velık *et al*., 2004). Numerous toxicological studies have established that Albendazole and other derivatives of Benzimidazoles lead to a state of oxidative stress by overproduction of ROS (Locatelli *et al*., 2004; Pedrosa *et al*., 2001). In addition, administration of repeated doses of Albendazole and Mebendazole was associated with lipid peroxidation and an imbalance of the glutathione homeostasis in rat livers (Locatelli *et al*., 2004).

Furthermore, chemical products also affect the ecosystems through their toxicity to aquatic and terrestrial organisms and plants. This was noticed by the pathway of fecal excretion contaminated with Albendazole and its metabolites (Wagil *et al.*, 2015). Subsequently, due to the obvious risk to the environment, these drugs are classified as emerging environmental contaminants (Raisová, Podlipná, Szotáková, Syslová, & Skálová, 2017; Robles-Molina, Gilbert-López, García-Reyes, & Molina-Díaz, 2014).

Because of their accessibility, affordability, and availability, ethnoveterinary medicines have become more important in the control of helminths. However, its use to combat helminths must be scientifically proven and validated. This initiative aims to document the importance of Zhombwe as an Ethnoveterinary plant in ruminant helminths control. The importance of scientific validation of indigenous medicinal herbs in animal health management is critical.

1.3 Justification

Murungweni *et al*. (2012)'s discovery of *Neorautanenia brachypus* has created the opportunity to investigate the tuber's anthelmintic capabilities. This could potentially alleviate cattle and human survival concerns in resource-limited and drought-prone locations. If animal production in dry places improves, poaching in National Parks for meat for protein supplementation in communities' diets may decrease. Unfortunately, farmers in drier places face resource constraints, making it difficult to purchase feed supplements and commercial defensive and restorative chemicals to assist their animals to fight diseases. They rely on local natural resources, such as ethnoveterinary plants, but knowledge of these alternatives is poorly organized and, in many cases, uncharacterized. As a result, if communities are to fully benefit from these naturally occurring, largely drought-tolerant plants and their many components, a deeper understanding of them is required.

Zimbabwe can create pharmaceuticals using *Neorautanenia brachypus* as a model. *Neorautanenia brachypus* was shown to have anthelmintic characteristics in a study by Murungweni *et al*. (2012). However, it is necessary to discover and define active chemicals with anthelmintic characteristics in the tuber. Characterization is critical since it leads to the identification of active chemicals that can be employed as medications in the future. Furthermore, such information is critical for retaining alternatives for using traditional treatments, as well as for lowering costs and strengthening trust in herbal medications.

In addition, *N. brachypus'* method of action, effective dosage, and efficacy against helminths in vitro are unknown. Do they have control over all helminth's classes, or is it just one class or genus? Do they function alone or in combination to suppress helminths, and if in combination, which combinations are the most effective, and at what concentrations and dosages? Only by conducting this research could these questions be answered. This information will help the community in understanding the active compounds present in the medicinal plant, as well as pave the way for the future development of traditional anthelmintic drugs derived from *Neorautanenia brachypus*, reducing the amount of foreign currency required to procure drugs through imports. Knowing whether active chemicals in the tuber have therapeutic properties increases the plant's value and marketability. New sources of medicinal plants may provide an opportunity for the industry to diversify anthelmintic treatments. *Neorautanenia brachypus* is a plant that has the potential to control helminths, but further research is needed to determine its efficacy.

1.4 Objectives

1.4.1 Main objective

To establish the anthelmintic properties of *Neorautanenia brachypus* for the purpose of developing a herbal-based dewormer for use in ruminant livestock health

1.4.2 Specific Objectives

1. To investigate and establish use of tuberous plants in the management of helminths in livestock by farmers in the whole world.
2. To determine the effect of extraction method on ability to extract different types of phytochemicals and on extracting a high amount of phytochemicals present in *Neorautanenia brachypus* (Zhombwe).
3. To determine the effect of phytochemical extracts from *Neorautanenia brachypus* on helminths under laboratory conditions (*in vitro*).
4. To determine, the efficacy of best-bet phytochemical extract from *Neorautanenia brachypus* on the two important helminths commonly affecting livestock (Strongyloids and Coccidia) in Zimbabwe.

1.5 Hypotheses

1. H_0: Tuberous plants have no use in management of helminth in livestock.
2. H_0: Extraction method has no effect on number and yield of phytochemicals from *Neorautanenia brachypus*.
3. H_0: Phytochemical extracts from *Neorautanenia brachypus* have no effect on helminths associated with ruminant livestock production *in vitro*.
4. H_0: Extracts from *Neorautanenia brachypus* have no effect on Strongyloids and Coccidia in affected ruminant livestock.

1.6 Outline of the dissertation

The thesis is comprised of three complementary research chapters and a systematic review paper. Chapter 2 is a systematic review paper on tuberous plants with active compounds against helminths in livestock. Chapter 3 compares extraction methods, drying methods, and solvents. This was done to establish which methods led to the extraction of a greater number of phytochemicals in *N. brachypus* and their identification through GCMS analysis. The anthelmintic effect of these phytochemicals was determined using *in vitro* and *in vivo* tests in Chapters 4 and 5 respectively. The findings of the research are discussed wider in Chapter 6.

CHAPTER 2 Tuberous Plants with Anthelmintic Value for Livestock Gut Health

Tuberous plants with active compounds against helminths in livestock: A systematic review

This article is under review in the journal of Ethnobotany Research and Applications – No. 3759 as Mpofu, M.C., Gomo, C., Mugumbate, G., Mashingaidze, A.B., Chikwambi, Z., and Murungweni, C. 2022. Tuberous plants with active compounds against helminths in livestock: A systematic review. Journal of Ethnobotany Research and Application.

Abstract

Background: The rise in drug resistance to helminthiases is posing a serious challenge to conventional techniques of controlling parasitic illnesses in livestock. Using less conventional approaches such as plant extracts can improve the situation. This research aims to explore the emerging role of tuberous plants in developing anthelmintics. From October 2020 to July 2022, a comprehensive literature search was conducted using the search engines Google Scholar, NISCAIR Online Periodicals Repository, NCBI, Taylor and Francis Online, Wiley Online Libraries, Science Direct, ResearchGate, and Springer Link. The evaluation included only tuberous plants with anthelmintic properties. Qualitative-quantitative analysis techniques were used to analyse the data collected. Forty-eight ethnobotanical investigations recorded plants with tuber portions that were utilized to combat helminths. There were 43 plants identified, divided into 24 families. Seven plants were found to be the most culturally important in the management of helminths. The common phytochemical classes were phytosterols, tannins, alkaloids, saponins, essential oils, flavonoids, and terpenoids. Twenty-six of these tuberous plants have been tested for their anthelmintic effect against trematodes, cestodes, nematodes, protozoa, coccidian, and eoacanthacephala. Thirty-two plants have been reported to exhibit some toxicity effects. Twelve tuberous plants have been indicated to be endangered. *Dioscorea deltoidea, Dioscorea bulbifera, Dioscorea alata, Gloriosa superba, Curcuma longa, Dioscorea pentaphylla,* and *Cyperus rotundus* were the most culturally important plants for controlling helminths. These plants are mostly found in India and Nepal. The review gave more insights into

the therapeutic safety of the popular folklore drugs and provided grounds for an assessment of possible measures to be introduced before conducting clinical studies. The article provides public and Government recognition of endangered plants species and spurs conservation efforts toward saving both plants and folk medicinal knowledge. There is a need to investigate other tuberous plants to identify unique compounds that are active against helminths to develop more robust anthelmintic drugs and reduce anthelmintic resistance levels.

Keywords: Anthelmintic, ethnobotanical, tubers, active compound, anthelmintic resistance, livestock

Background

Helminths are found throughout the world, generating significant losses in ruminant production by exposing animal welfare to risk (Alawa *et al*., 2010). Low reproductive performance, reduced growth rate, low live mass, dull rough coat, organ condemnation, and mortality are all effects of helminths on cattle productivity (Cannel, 1998). Helminths are multicellular worms that can be classified into three types: nematodes, cestodes, and trematodes (Duguma *et al*., 2011; Nandhini *et al*., 2014). Anthelmintics are the most common treatment for helminths. The most commonly used anthelmintics: benzimidazoles, the nicotinic agonists, praziquantel, triclabendazole, and the macrocyclic lactones act either by interfering with target sites unique to the parasite or differ in their structural features from those of the homologous counterpart present in the vertebrate host (Köhler, 2001).

Benzimidazole is a broad-spectrum anthelmintic effective against nematodes and trematodes. Levamisole is effective against nematodes and trematodes in higher doses. Mixing benzimidazole and levamisole can reduce resistance to nematodes in sheep (McKenna, 1990). The group salicylanilides, contain compounds primarily used against trematodes, praziquantel is effective against cestodes, and ivermectin is primarily used against nematodes (Cezar *et al*., 2010). However, due to their high cost, general toxicity, drug residue concerns in milk and meat, and the development of drug resistance, the utility of current medications for animal health and productivity is being questioned (Mwale *et al*., 2005; Oliveira *et al*., 2009; Zaman *et al*., 2017). Dealing with anthelmintic resistance has also proven problematic due to high costs and long wait times for new compounds to reach the market (Besier, 2007).

Because of their accessibility, affordability, and availability, ethnoveterinary plants have become more relevant in managing helminths in the face of the aforementioned obstacles. Furthermore, medicinal plants, including tuberous plants, have been shown to play an important role in drug discovery and development processes (McCorkle *et al*., 1992; McGaw & Eloff, 2010). It's thought that livestock farmers have adapted human treatments for use in animals or vice versa over the millennia. Similar therapies are used to treat similar ailments in humans and their livestock, and the same is true for anthelmintics. Gakuya (2001), previously stated that human and livestock herbalists are often the same and that there is a need for a collaborative approach when dealing with medicinal plants because most herbs are used to treat both human and animal ailments.

Plants are an important natural supply of a wide range of chemicals, ranging from simple skeleton structures to sophisticated ones. Many well-known components, such as quinine (chloroquine and mefloquine), artemisinin, taxol (paclitaxel), camptothecin, khellin, sodium chromoglycate, galegine, metformin, papaverine, and verapamil, are based medicinal plants (Cragg *et al*., 2013; Khan *et al*., 2019; Koparde *et al*., 2019). Tuberous plants are one of the plant classes being researched for their anthelmintic properties.

Tubers are large fleshy underground structures that form from stems or roots of plants such as potatoes and yams, and they belong to a variety of botanical families. Orchidaceae is one of the largest botanical families that produce tubers, with between 20000 and 35000 species divided into 600-850 genera (Gutiérrez, 2010; Hossain, 2011). However, leaves, barks, stems, seeds, latex, and flowers were found to be the most regularly employed parts in the treatment of helminths by the majority of researchers (Ataba *et al*., 2020; ba Ndob, Mengome, Bourobou, Banfora, & Bivigou, 2016; Kuma, Birhanu, Hirpa, & Nekemte, 2015; Muthee *et al*., 2011). The fact that these parts are easier to collect and process than tubers and roots may explain why they are more commonly used (Ghimire, Gimenez, Pradel, McKey, & Aumeeruddy-Thomas, 2008; Giday, Asfaw, Elmqvist, & Woldu, 2003). However, their collection may harm regeneration and lead to species extinction. To prevent the loss of ethnobotanical knowledge of these plants, this review study documents species with specific tuber portions employed as anthelmintics as stated in ethnobotanical studies.

Anthelmintic action of Phytochemicals

Tannins, alkaloids, flavonoids, terpenoids, phenols, saponins, and essential oils have all been shown to have anthelmintic properties (Ajah *et al.*, 2010; Athanasiadou *et al*., 2001; Wang, Zhou, *et al*., 2010). Phenols affect the decoupling of the oxidative phosphorylation responsible for ATP production interfering with energy production and leading to the death of parasites (Salhan *et al*., 2011). Tannins have ovicidal action related to their interaction with enzymes responsible for the hatching of eggs (Molan & Faraj, 2010). In addition, they can interact with metabolites, increasing cell permeability, which leads to their interaction with free proteins or cuticle glycoproteins of parasites hindering nutrient absorption, mobility, reproduction, and consequently causing their death (Botura *et al*., 2013). Quinones inhibit cell development by different mechanisms, such as apoptosis induction, intercalation and binding with DNA, and inhibition of the enzyme topoisomerase (Pe´rez-Pertejo *et al*., 2019). Terpenes in essential oils exhibit anthelmintic effects by enhancing suppression effects of many biochemical targets such as tyramine receptors, chloride channels, and acetylcholinesterase (Lynagh, Cromer, Dufour, & Laube, 2014; Miyazawa, Nakahashi, Usami, Matsuda, & 2016; Trailović, Marjanović, Nedeljković Trailović, Robertson, & Martin, 2015). Alkaloids and coumarins effect result from both competitive and non-competitive inhibition of parasitic acetylcholine receptors (Basumatary *et al*., 2020; Dubois *et al*., 2019). Flavonoids cause oxidative stress by increasing the production of the reactive oxygen species (ROS), thus affecting the normal physiology of parasites (Wang, Tidrick, Haque, & Stuehr, 2013). Terpenoids inhibit the motility and egg-hatching ability of worms (Ferreira *et al*., 2016; Katiki *et al*., 2017). Anthelmintic effects of saponins are due to their interaction with cell membranes causing changes in cell permeability (Doligalska *et al*., 2011; Tava & Avato, 2006; Vo, Fukushima, & Muranaka, 2017).

Methods

Research questions

i. Which plant species have their tuber parts culturally used as anthelmintics?

ii. What quantitative analysis methods were used in carrying out ethnobotanical surveys?

iii. Which plants and plant families are most represented in ethnobotanical studies?

iv. What are the growth habits of these tuberous plants and which is the most represented growth habit?

v. Which active compounds were commonly found in most of the identified plants exhibiting anthelmintic properties?

vi. What is the anthelmintic activity of these tuberous plants and which gastrointestinal parasite have they been tested against?

vi. What is the toxicological status of these plants, what are the toxic compounds found in these plants, and the toxic compounds belong to which classes of phytochemicals?

vii. Which tuberous plant with anthelmintics properties are endangered and which ones are not?

Materials

Extensive online literature surveys were done to retrieve relevant data from October 2020 up to July 2022. The identification of tuberous plants used to control helminths was restricted in respecting the following inclusion criterion: i. only ethnobotanical studies articles were considered; ii. articles that indicated a plant with its tuber or tuberous roots used as an anthelmintic, wormicidal vermifuge, and/or vermicide; and iii. only articles that were written in English or that had English translations were considered.

Key search words used were Ethnobotanical survey or study or observations AND tuber or tuberous roots AND anthelmintic or wormicidal or vermifuge or vermicide. Search engines used included Google Scholar, NISCAIR Online Periodicals Repository, NCBI, Taylor and Francis Online, Wiley Online Libraries, Science Direct, ResearchGate, and Springer Link. Data recorded included the scientific name of a plant, vernacular name, voucher number, botanical family, plant growth habit, area of study, country of study, sampling method used, method of data collection, qualitative analysis index, other uses of the plant, and the reference. The identification of the plant's anthelmintic activity was restricted to literature that reported: i. tests against gastrointestinal worms that affect animals but not humans, ii. literature that showed a positive anthelmintic effect of the tuberous plant, and iii. Keywords plant name AND anthelmintic eg. *Curcuma longa* AND anthelmintic. The toxicological statuses of the plants were researched with the following keywords, plant name eg, *Curcuma longa* AND toxicity. The endangerment status of the plants was determined by using the keywords, plant name eg. *Curcuma longa* AND endangered. To determine phytochemicals found in the culturally important plants, the search involved all articles that indicated phytochemical analysis of the plants with keywords Plant name AND phytochemicals or phytochemistry eg. *Curcuma longa* AND Phytochemistry. Quali-quantitative analysis was performed on the gathered data. Tables, radars, and bar graphs were used to display the results.

Data extraction

Data were gathered employing a standardized data collection as follows:

i. General data on studies: number of studies, authors, and date of publication
ii. Data on the survey: country and area of study, sampling method, data collection method, and quantitative analysis index technique used
iii. Data on medicinal plants: scientific name of the plant, vernacular name, voucher number, family, growth habit, phytochemistry, anthelmintic activity, parasites, toxicity, toxic compounds, and plant status

Quali-quantitative analysis:

Frequency cited (FC), Relative frequency of citation (RFC), and Family importance value (FIV).

Methods used for these calculations were adopted from (Tardío & Pardo-de-Santayana, 2008), (Vitalini *et al.*, 2013), and (Fakchich & Elachouri, 2021) with some modifications. FC was calculated as the frequency of mentioning for a single botanical species by studies. It is the number of times a plant was reported by different studies. RFC was obtained by dividing the FC by the total number of citations (N). FIV is calculated by counting the percentage of studies mentioning a specific family using the formula: FIV= (FC (family)/N). However, this was modified by calculating the number of plants representing each botanical family and dividing it by the total number of plants identified in this study.

Comparative analysis

The Jaccard Index (JI) was used to calculate similarities between studies carried out in the top mentioned countries of study. JI may be expressed as follows:

$$JI = C / (A+B-C)$$

Where A is the number of plants recorded in country A, B is the number of plants recorded in country B, and C is the number of plants common to A and B.

Results and discussion

Matrix of general data

The data included in this study were acquired through ethnobotanical surveys, as shown in Table 1. The data were compiled using 48 ethnobotanical studies in total. The tubers of 43 plant species from 24 botanical families have been documented to be used as anthelmintics.

Table 2.1: Tuberous plants with anthelmintic properties cited in various publications across the world.

Plant	Vernacular Name/ Voucher Number	Family/ Habit	Number of participants	Country/ Area	Quantitative Analysis Technique	Other Uses reported	Reference
Dioscorea deltoidea	Kanees	*Dioscoraeceae*/ Herb	-	Pakistan/ Dir, Kohistan valley	-	expectorant, diuretic, uterine sedative and homeostatic	(Hazrat, Nisar, Shah, & Ahmad, 2011)
Dioscorea deltoidea	Krish	*Dioscoraeceae*/ Herb	-	India/ Bangus Valley, Kashmir Himalaya	-	Treat ophthalmic infections	(Ishtiyak & Hussain, 2017)
Dioscorea deltoidea	Bhayakur	*Dioscoraeceae*/ Herb	50	Nepal/ Puranchaur VDC, Kaski District	-	kill lice and bush poison	(Khatri, 2012)
Dioscorea deltoidea	Kill Dhari	*Dioscoraeceae*/ Herb	-	India/ District Kathua (J&K)	-	To alleviate constipation and tubers used for washing hairs to kill lice.	(Kumar & Bhagat, 2012)
Dioscorea deltoidea	Kanees	*Dioscoraeceae*/ Herb	-	Pakistan/ Swat Valley	-	Uterine sedative, homeostatic, diuretic and expectorant. Tubers are also used as fish poison.	(Hamayun, 2007)
Dioscorea deltoidea Wall. ex Griseb	Ban goi	*Dioscoraeceae*/ Herb	90	Nepal/ Chepang community	-	fish poisoning	(Tamang, Thakur, Koirala, & Chapagain, 2017)
Dioscorea deltoidea Wall. ex Kunth	Yams	*Dioscoraeceae*/ Herb	-	Pakistan/ Chitral District, Malakand Division, NWFP	-	Kill lice, fish poison	(Ahmad, 2001)
Aconitum heterophylum m Wall	Sarba wali	*Ranunculaceae*/ Herb	-	Pakistan/ Dir, Kohistan valley	-	treat fever, gout, rheumatism and pain in body tonic, antiperiodic, vomiting, appetizer, astringent, diarrhea, gastric pain, stomach ache and cure cold.	(Hazrat *et al.*, 2011)

Stephania glabra Roxb	Nepali-Gurjagano, Lepcha-Burkil-Kunthek-rik	*Menispermaceae*/ Shrubby climber	-	India/ North Sikkim	-	Treatment of diabetes, fever, gastric problem, amoebic dysentery, rheumatic body ache, blood dysentery, leprosy, anticancer	(Maity, Pradhan, & Chauhan, 2004)
Rumex usambarensis Dammer.	Enkaisijoi/ JK05	*Polyganaceae*/ Herb	30	Kenya/ Loitoktok District	UVs, FUV, FIC	Treat constipation	(Muthee *et al*., 2011)
Dioscorea alata L.	Chupri Alu	*Dioscoraeceae*/ Herbaceous climber	-	India/ Tripura	-	Not reported	(Dey *et al*., 2012)
Dioscorea alata L.	Achuchu	*Dioscoraeceae*/ Herbaceous climber	40	India/ Sumi Nagas in Zunheboto District, Nagaland, Northeast India	-	aphrodisiac, Diuretic, it is useful in treating diabetes, piles, leprosy, gonorrhea	(Sumi & Shohe, 2018)
Dioscorea alata L.	Pangnang	*Dioscoraeceae*/ Herbaceous climber	90	Nepal/ chepang community	-	fish poisoning	(Tamang *et al*., 2017)
Dioscorea alata L	Not reported	*Dioscoraeceae*/ Herbaceous climber	-	India/ Chalsa forest range under Jalpaiguri division, West Bengal	F, RF, D, RD, RV, RDo, A, IVI, RH	Used as Diuretic, contraceptive and also useful in diabetes, Leprosy, gonorrhea	(Sarkar, Dey, & Mazumder, 2017)
Dioscorea bulbifera L.	Lak	*Dioscoraeceae*/ Herbaceous climber	90	Nepal/ chepang community	-	piles, dysentery	(Tamang *et al*., 2017)
Dioscorea bulbifera	Metealu, Ram bara	*Dioscoraeceae*/ Herbaceous climber	-	Bangladesh/ Bilaichari Upazilla, Rangamati District	Factor of informant consensus (FIC) Jaccard index (JI)	Not reported	(Faruque *et al*., 2019)
Dioscorea bulbifera	Varahikanda	Dioscoraeceae/ Herbaceous	-	India/ Jatasankar region of Girnar	-	Diabetes, skin disease,	(Nita & Haresh, 2013)

		climber		forest, Gujarat			
Dioscorea bulbifera	Githa/230-91 VN	*Dioscoraeceae*/ Herbaceous climber	130	Nepal/ Myagdi District	-	Not reported	(Manandhar, 1995)
Dioscorea bulbifera	Kitthee, Vansittha	*Dioscoraeceae*/ Herbaceous climber	-	India/ district Samba of Jammu Province, Jammu & Kashmir	-	Tonic, alterative, aphrodisiac, stomachic, expectorant, and astringent	(Pandita, Pandita, & Pandita, 2013)
Zingiber zerumbet (L) Roscoe ex sm.	Bura uth	*Zingiberaceae*/ Herb	42	India/ Udalguri district of Assam	FC, RFC, FIV	Not reported	(Swargiary, Daimari, & Roy, 2020)
Zingiber officinale Roscoe	Haijeng	*Zingiberaceae*/ Herb	42	India/ Udalguri district of Assam	FC, RFC, FIV	Not reported	(Swargiary *et al.*, 2020)
Curcuma longa L.	Haldi	*Zingiberaceae*/ Herb	42	India/ Udalguri district of Assam	FC, RFC, FIV	Not reported	(Swargiary *et a*l., 2020)
Curcuma longa L.	Haldi/ BUBH2018002	*Zingiberaceae*/ Herb	27	India/ Udalguri district of Assam	-	Not reported	(Swargiary, Roy, & Daimari, 2019a)
Curcuma longa L.	Holud	*Zingiberaceae*/ Herb	5	Bangladesh/ villages of Natore and Rajshahi districts	-	Gonorrhea, sore throat, hepatitis, appetizer, allergy, eye disorders.	(Hossain, Khatun, & Miajee, 2010)
Oroxylum indicum (L) Kurz	Kharong	*Bignoniaceae*/ Tree	42	India/ Udalguri district of Assam	FC, RFC, FIV	Not reported	(Swargiary *et al.*, 2020)
Allium sativum (L.)	Sambram gufur	*Amaryllidaceae* / Herb	42	India/ Udalguri district of Assam	FC, RFC, FIV	Not reported	(Swargiary *et al.*, 2020)
Kaempferia galanga L.	Sompera	*Zingiberaceae*/ Shrub	42	India/ Udalguri district of Assam	FC, RFC, FIV	Not reported	(Swargiary *et al.*, 2020)
Neorautanenia brachypus (Harms) C.A.SM	zhombwe	Fabaceae/ Shrub	83	Zimbabwe/ Sengwe, Chiredzi	-	Feeding animals, Treating bad wounds, Harvesting fish, feed dogs to improve on tracking abilities	(Murungweni *et al*, 2012)
Cyperus rotundus	Deela	Cyperaceae/ Herb	250	Pakistan/ District Bahawalpur, Southern Punjab	-	Astringent, appetizer, stomachic, and leprosy	(Muhammad Farrukh Nisar *et al.*, 2014)

				province			
Cyperus rotundus	Dellia ghas	*Cyperaceae*/ Herb	90	India/ Panna District, Central India	-	stimulant, diuretic	(Gwalwanshi, Salunkhe, Shukla, Bishwas, & Vyas, 2014)
Cyperus rotundus	Seida	*Cyperaceae*/ Herb	-	Sudan/ Southern Blue Nile district	-	treat stomach troubles	(El-Kamali & El-Khalifa, 1999)
Cyperus rotundus	Deela	*Cyperaceae*/ Herb	-	Pakistan/ Bahawalnagar, Punjab	-	Appetizer, biliousness, pruritis, pain, vomiting, epilepsy, diuretic, diaphoretic, vulnerary ulcers, sores, fevers and dyspepsia	(Nisar *et al*., 2014)
Cyperus rotundus	Motha	*Cyperaceae*/ Herb	-	India/ Bundelkhand region, Uttar Pradesh	-	Tonic and stimulant effect, demulcent, diuretic, diaphoretic, fever, dyspepsia, vomiting cholera, diarrhea, dysentery	(Unial, Singh, Singh, Kumar, & da Silva, 2011)
Elephantorrhiza elephantina	Intolwane/ MSAN02/2015	*Fabaceae*/ Shrub	53	South Africa/ Eastern Cape Province	FL	Not reported	(Sanhokwe, 2015)
Flemingia vestita Benth and Hooker	Soh-phlang	*Fabaceae*/ Shrub	-	India/ Meghalaya	-	Not reported	(Rao, 1981)
Bulbine asphodeloides (L.) Wild.	Uyakayakane	*Asphodelaceae*/ Herb	80	South Africa/ Amathole district municipality of the Eastern Cape province	-	Rashes, dysentery, diarrhea	(Wintola & Afolayan, 2015)
Bulbine asphodeloides	Not reported	*Asphodelaceae*/ Herb	30	South Africa/ Nkonkobe Municipality, Eastern Cape Province	-	Not reported	(Wintola & Afolayan, 2010)
Azadirachta indica	Not reported	*Meliaceae*/ Tree	-	India/ Paderu division of Visakhapatnam District, AP	-	Not reported	(Padal, Murty, Rao, & Venkaiah, 2010)

Pelargonium reniforme	Uvendle/ VMAP20/2006	*Geraniaceae*/ Shrub	30	South Africa/Eastern Cape Province	-	Not reported	(Maphosa & Masika, 2010)
Gunnera perpensa	Iphuzi (River pumpkin)/ VMAP10/2006	*Gunneraceae*/ Herb	30	South Africa/Eastern Cape Province	-	Not reported	(Maphosa & Masika, 2010)
Gunnera perpensa	Iphuzi/ MSAN08/2015	*Gunneraceae*/ Herb	53	South Africa/Eastern Cape Province	FL	Not reported	(Sanhokwe, 2015)
Hypoxis argentea	Inongwe yehashi (Yellow stars)/ VMAP12/2006	*Hypoxidaceae*/ Herb	30	South Africa/Eastern Cape Province	-	Not reported	(Maphosa & Masika, 2010)
Albuca setosa	Ingwebeba/ MSAN03/2015	*Hyacinthaceae*/ Herb	53	South Africa/Eastern Cape Province	FL	Not reported	(Sanhokwe, 2015)
Rhoicissus tridentate	Omumara	*Vitaceae*/ Shrubby climber	160	Uganda/Nakasongola District	_	Not reported	(Nalule, Mbaria, Olila, & Kimenju, 2011)
Rhoicissus tridentate	ntagaraga	*Vitaceae*/ Shrubby climber	32	South Africa/ Madikwe area of the North West Province of South Africa	-	heart water red water general ailments abortion	(Van der Merwe, Swan, & Botha, 2001)
Pueraria tuberosa	Ghora ro bel/ FB 04	*Fabaceae*/ Shrubby climber	-	India/ Aravalli hill range	-	relieves pain	(Bhardwaj, Bharadwaj, Trigunayat, & Trigunayat, 2011)
Curcuma amada	Jangli haldi/ ZN 04	*Zingiberaceae*/ Herb	710	India/ Aravalli hill range	-	Not reported	(Bhardwaj *et al*., 2011)

Curcuma aromatica	Haldi/ ZN 06	*Zingiberaceae*/ Herb	710	India/ Aravalli hill range	-	Not reported	(Bhardwaj *et al*., 2011)
Curcuma caesia Roxb.	haldi gaswm/ BUBH0000 008	*Zingiberaceae*/ Herb	710	India/ Chirang District of Assam	-	Not reported	(Swargiary *et al*., 2019a)
Peliosanthes bakeri Hook. f.	sikho bifang/ BUBH2018 039	*Liliaceae*/ Shrub	27	India/ Chirang District of Assam	-	Not reported	(Swargiary *et al*., 2019a)
Melastoma malabatricum L.	tinkur bedor/BUB H0000130	*Melastomatace ae*/ Herb	27	India/ Chirang District of Assam	-	Not reported	(Swargiary *et al*., 2019a)
Asparagus racemosus Willd.	Sansarpali	*Asparagaceae*/ Shrubby climber	27	India/ Murari Devi and surrounding areas (Mandi district, Himachal Pradesh	-	aphrodisiac, rheumatism, cough, dysentery, febrifuge, gastric complaints gonorrhea, , headache, menstrual complaints, snake bite, stomachache, tonic, urine complaints	(Sharma, Agnihotry, & Sharma, 2015)
Asparagus racemosus Willd.	Sansarpali	*Asparagaceae*/ Shrubby climber	-	India/ Naina Devi Sacred Shrine Rewalsar, Himachal Pradesh, North Western Himalaya	-	Medicinal (Anthelmintic, aphrodisiac, rheumatism, bleeding from nose, cough, dysentery, febrifuge, gastric complaints, gonohorrea, headache, menustral complaints, snake bite, stomachache, tonic, urine complaints); Edible	(Marpa, Samant, Tewari, & Paul, 2020)
Costus speciosus (Koen) Sm.	Bogachi	*Costaceae*/ Herb	-	India/ Visakhapatnam district, Andhra Pradesh	-	Not reported	(Padal, Satyavathi, & Sandhyadeepika, 2014)

Costus speciosus (J. König.) Sm.	Keaw	*Costaceae*/ Herb	5	Bangladesh/ Daudkandi sub-district of Comilla district	-	Dermatitis, appetizer, leucorrhea, impotency, glassiness of skin.	(Hossain *et al*., 2010)
Raphanus sativus L.	Karaturp	*Brassicaceae*/ Herb	43	Turkey/ Çamlıdere	FIC, UV, CI	For asthma, bronchitis, cancer, diabetes, urinary tract diseases, as anthelmintic, antitussive, appetizer, tonic	(Gunbatan, Gurbuz, & Ozkan, 2016)
Dioscorea pentaphylla	Rani bhyagur, Bhegur;/ Mld.CG. – 022	*Dioscoreaceae*/ Herbaceous climber	21	India/ Bamangola Block of Malda District, West Bengal	-	Contraceptive, anthelmintic, stomach problems, gastric disorders, pains, allergic fever, veterinary problems	(Ghosh, 2017)
Dioscorea pentaphylla L.	Not reported	*Dioscoreaceae*/ Herbaceous climber	-	Nepal/ Kaski District	FIC, UV, FL	Not reported	(Subedi, 2017)
Dioscorea pentaphylla L.	Not reported	*Dioscoreaceae*/ Herbaceous climber	-	India/ Satpuda Hills	-	Not reported	(Kosalge & Fursule, 2009a)
Gloriosa superba	Kalappankiz hangu	*Colchicaceae*/ Herbaceous climber	-	India/ Kolli Malayalis of Nammakkal district, Eastern Ghats, Tamil Nadu	-	anti-inflammatory, alterative, antileprotic. Used for piles, swollen joints, parasitical affections of skin, Uterine stimulant	(Muthuraja, Nandagopalan, Thomas, & Marimuthu, 2014)
Gloriosa superba	Kalihari/ LL 04	*Colchicaceae*/ Herbaceous climber	710	India/ Aravalli hill range	-	Not reported	(Bhardwaj *et al*., 2011)
Gloriosa superba	kalihari	*Colchicaceae*/ Herbaceous climber	135	India/ Tarai region of Kumaun, Uttarakhand	-	kill head lice	(Mathur & Joshi, 2013)
Gloriosa superba L.	Kukadsira Kadiya-nag	*Colchicaceae*/ Herbaceous climber	-	India/ district Samba of Jammu Province, Jammu & Kashmir	-	Tonic, stomachic, remedy of gout, neuralgia, colic, chronic ulcers, piles, fever and thirst	(Pandita et al., 2013)

Flemingia procumbens Roxb.	sohphlang	*Fabaceae*/ Herb	-	India/ Meghalaya	-	Not reported	(Hynniewta & Kumar, 2008)
Dioscorea halmiltonii Hook. F Ban	Not reported	*Dioscoreaceae*/ Herbaceous climber	-	Nepal/ Kaski District	FIC, UV, FL	Not reported	(Subedi, 2017)
Sauromatum venosum (Ait) Schott	Pebada/ JBA-249	Araceae/ Herb	8	India/Jhabua District of Madhya Pradesh	-	applied on the pimple and blemishes	(Wagh & Jain, 2014)
Dryopteris setosa (Thunb.) Akas	Pak mo/Mxy72	*Dryopteridaceae*/ Herb	83	China/ Southwest Guizhou	FIC, UR	Not reported	(Xiong & Long, 2020)
Arisaema jacuemontii	Sappe didhaud, Sarp	*Araceae*/ Herb	-	India/ district Kathua (J&K)	-	Remedy for colic	(Kumar *et al*., 2012)
Alpinia galangal	Pannodara/ Lengkuas	*Zingiberaceae*/ Herb	-	Indonesia/ South Kalimantan	-	Not reported	(Hatta, 2020)
Arisaema consanguineum Schott	Banku	*Araceae*/Herb	12	Nepal/ Chepang community	-	Not reported	(Rijal, 2011)
Dioscorea alata L.	Pangnang	*Dioscoreaceae*/ Herbaceous climber	12	Nepal/ Chepang community	-	edible	(Rijal, 2011)
Dioscorea bulbifera L.	Pas	*Dioscoreaceae*/ Herbaceous climber	12	Nepal/ Chepang community	-	edible	(Rijal, 2011)
Dioscorea deltoidea Wall. ex Griseb.	Goi	*Dioscoreaceae*/ Herbaceous climber	12	Nepal/ Chepang community	-	edible	(Rijal, 2011)
Dioscorea prazeri Prain & Burkill	Jyar	*Dioscoreaceae*/ Herbaceous climber	12	Nepal/ Chepang community	-	edible	(Rijal, 2011)
Cyphostemma adenocaule	Ekimara	*Vitaceae*/ Herbaceous	32	Uganda/ Nakasongola District	_	Not reported	(Nalule *et al*., 2011)

(A.rich.)willd Drummond	climber

As shown in Figure 2.1, the publications cited were undertaken in India, Nepal, Pakistan, China, Kenya, Turkey, Indonesia, Sudan, Zimbabwe, South Africa, Bangladesh, Ethiopia, and Uganda. The highest numbers of citations were from India followed by Nepal, South Africa, and Pakistan. The reason for high citations in these countries could be that they are developing countries and over 80% of people in developing countries, for example, continue to rely on traditional plant-based medicines for primary health care (Bhat *et al.*, 2021; Chauhan, 2020). India and Nepal have been reported to be amongst the countries with greatest number of plant species by country in the world (Butler, 2020).

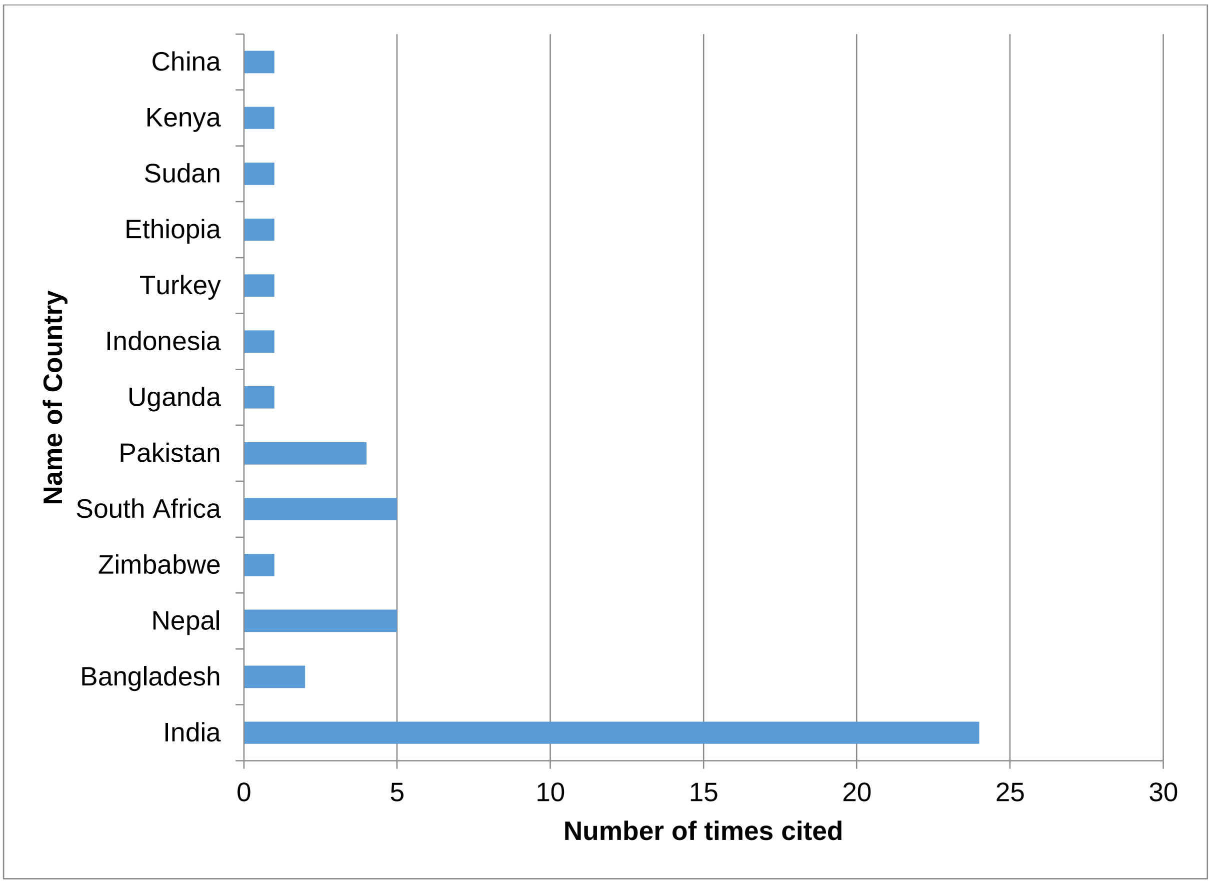

Figure 2.1: Countries cited to have tuberous plants with anthelmintic properties.

Species number of medicinal plants (families, genera, species, and growth habits)

A total of 24 plant families were recorded with tuber plants that are effective against helminths, results displayed in Figure 2.2. *Zingiberaceae* family was most represented with eight plants (33%), *Dioscoreaceae* six plants (25%), *Fabaceae* five plants (21%), *Araceae* three plants 13%), and *Polygonaceae* two plants (8%). The results on plant families recorded for this study differed from those by Swargiary, Roy, and Daimari (2019b) where *Apiaceae*, *Araliaceae*, *Bromeliaceae*, *Apocynaceae*, and *Maliaceae* families were represented by plants with anthelmintic effects. The results were similar to those by Ali *et al*. (2019), who found plants having veterinary effects to belong to the *Fabaceae* family. This was also similar to those by Sanhokwe, Mupangwa, Masika, Maphosa, and Muchenje (2016) where *Asphodelaceae*, *Hyacinthaceae*, *Fabaceae*, and *Gunneraceae* were represented by plants with anthelmintic effects. However, they reported *Apocynaceae*, *Apiaceae*, *Araliaceae*, and *Agapapanthaceae* to represent some anthelmintic plants that were not mentioned in the current study. Furthermore, Maphosa and Masika (2010), indicated *Anacardiaceae*, *Capparidaceae*, *Geraniaceae*, *Lamiaceae*, *Loganiaceae*, *Pittosporaceae*, *Ptaeroxylaceae*, *Rhamnaceae*, *Rutaceae*, *Sterculiaceae*, and *Titiaceae* families to also represent plants with anthelmintic action.

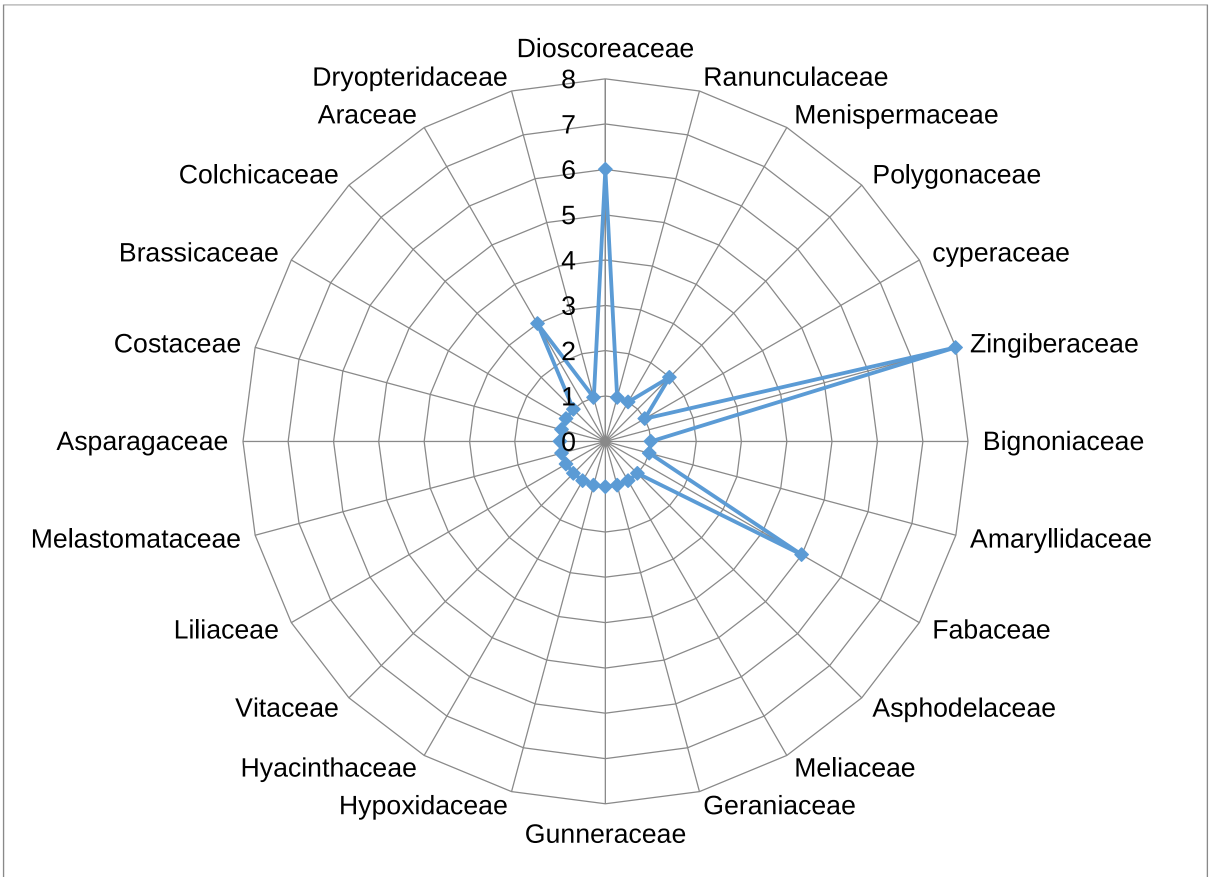

Figure 2.2: Botanical families of tuberous plants with anthelmintic properties.

Figure 2.3 depicts the proportions of the plant species' recorded growth behaviors. Twenty-two (51%) of the plants had a herbaceous growth habit, eight plants (19 %) herbaceous climbers, six shrubs (14%), four (9%) shrubby climbers, and three (7%) trees. However, other publications have cited trees and shrubs to represent plants with anthelmintic effects (Gemechu, 2021; Mutie *et al*., 2020). The research by Tefera and Kim (2019) cited herbs and trees to be the dominant growth habits of medicinal plants.

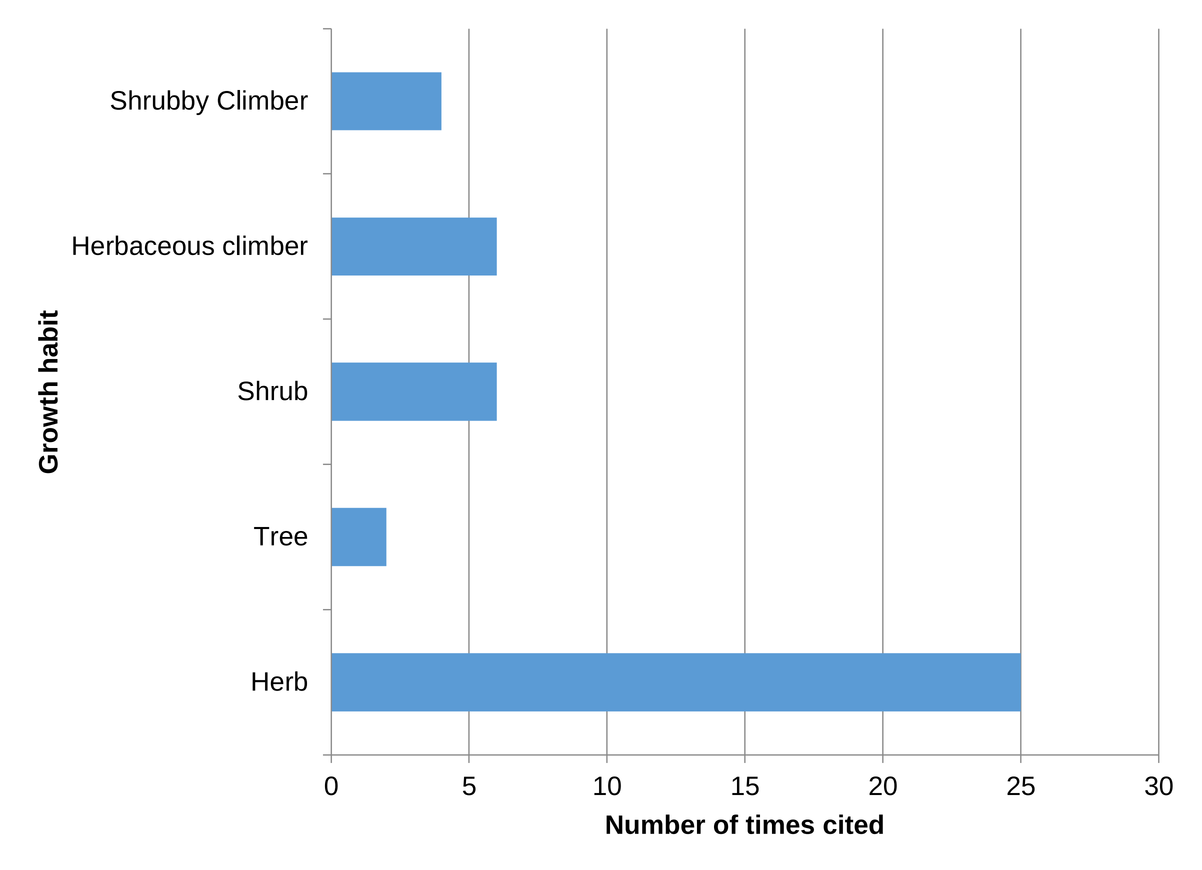

Figure 2.3: Distribution of growth habits of tuberous plants with anthelmintic properties.

Species Frequency of Citation (FC)

Species Frequency of Citation (FC) was used to identify plants that were frequently mentioned in ethnobotanical research as having tuber portions used as anthelmintics. It represented the most commonly utilized plants. The top seven plants with the highest FC value were as follows: *Dioscorea deltoidea* (eight citations) (Ahmad, 2001; Hamayun, 2007; Hazrat *et al*., 2011; Ishtiyak *et al*., 2017; Khatri, 2012; Kumar *et al*., 2012; Rijal, 2011; Tamang *et al*., 2017), *Dioscorea bulbifera* (six citations) (Faruque *et al*., 2019; Manandhar, 1995; Nita *et al*., 2013; Pandita *et al*., 2013; Rijal, 2011; Tamang *et al*., 2017), *Dioscorea alata* (five citations) (Dey *et al*., 2012; Rijal, 2011; Sarkar *et al*., 2017; Sumi *et al*., 2018; Tamang *et al*., 2017), *Cyperus rotundus* (four citations) (El-Kamali *et al*., 1999; Gwalwanshi *et al*., 2014; Nisar *et al*., 2014; Unial *et al*., 2011), *Gloriosa superba* (four citations)

(Bhardwaj *et al*., 2011; Mathur *et al*., 2013; Muthuraja *et al*., 2014; Pandita *et al*., 2013), *Curcuma longa* (three citations) (Hossain *et al*., 2010; Swargiary *et al*., 2020; Swargiary *et al*., 2019a), *and Dioscorea pentaphylla* (three citations) (Ghosh, 2017; Kosalge *et al*., 2009a; Subedi, 2017). A high FC score indicated that their tuber parts were known to be efficient against helminths in their culture. These findings, however, differed from those of Maphosa and Masika (2010). The most commonly reported plants were *Aloe ferox*, *Teucrium trifidum*, *Leonotis leonurus*, and *Strychnos henningsii*. Some common anthelmintic plants indicated in literature are *Carica papaya*, *Butea monosperma*, *Terminalia arjuna*, *Z. officinale*, *Nigella sativa*, *Fumaria parviflora*, *Flemingia vestita*, *Allium sativum*, *Melia azedarach*, *Cucurbita maxima*, *Ocimum sanctum*, *Achyranthes aspera*, *Azadirachta indica*, *Calotropis procera*, and *Artemisia annua* (Nirala, 2019). The research by Wintola & Afolayan (2015) found the most common anthelmintic plants to be *Hypoxis hererocallidea*, *Strychonos henningsii*, *Rumex lanceolatus*, *Ozoroa mucronata*, and *Acacia karoo*. The other tuberous plants with anthelmintic effects not indicated in this study are *Strychnos henningsii*, *Corallocarpus epigaeus*, and *Hypoxis hemerocallidea* (Ishnava & Konar, 2020; Matyanga, Morse, Gundidza, & Nhachi, 2020).

Species Relative Frequency of Citation (RFC)

Relative Frequency of Citation **(**RFC) ranges from zero (no citations indicating that the plant is essential) to one (when all the citations consider a certain plant important). As shown in Figure 2.4, the greatest RFC value was determined for *Dioscorea deltoidea*, *Dioscorea bulbifera* (0.080), *Dioscorea alata* L., *Cyperus rotundus* (0.067), *Gloriosa superba* (0.053), *Curcuma longa* L., and *Dioscorea pentaphylla* (0.040). The lowest RFC value of 0.013 was found in 31 species and 5 species had a value of 0.027. It was also revealed that plants with a high FC value also had a high RFC value. The plants' high RFC value may be attributed to their abundance in the area, as well as the fact that their tubers were known to have anthelmintic characteristics. Plants with high RFC values, on the other hand, are endangered and should be prioritized for conservation and long-term usage (Amjad *et al*., 2020). The results of this study were different from those by Swargiary, Daimari, and Roy (2021) who reported *Andrographis paniculata*, *Alstonia scholaris*, *Ananas comosus*, and *Azadirachta indica* to be the dominant anthelmintic plants. Despite having a low RFC value of 0.013 during the investigation, *Flemingia vestita* (Das, Tandon, Lyndem, Gray, & Ferro, 2009; Toner, Brennan, Wells, McGeown, & Fairweather, 2008), *Zingiber offficinale* (Ghafar, Arbabi, Mosayebi, Hooshyar, & Nickfarjam, 2021; Kiambom, Kouam, Ngangoum, Kate, & Teguia, 2021; Toulah, Ashoor, Wakid, & Alshathly, 2019), *Azadirachta indica* (Ibekwe, 2019; Salma *et al*., 2021; Yamson, Tubalinal, Viloria, & Mingala, 2019), and *Allium sativum* (Azra et al., 2019; Luce, 2019; Shirgholami, Borji, Mohebalian, & Heidarpour, 2021) plants' tubers have been widely explored as a source of anthelmintic chemicals against helminths and their important proteins (enzymes). Genistein, the active component derived from *Flemingia vestita* tubers, has been extensively studied for its efficacy against several forms of helminths (Moharm, Oshiba, & Ammar, 2020; Singla & Kaur, 2021)

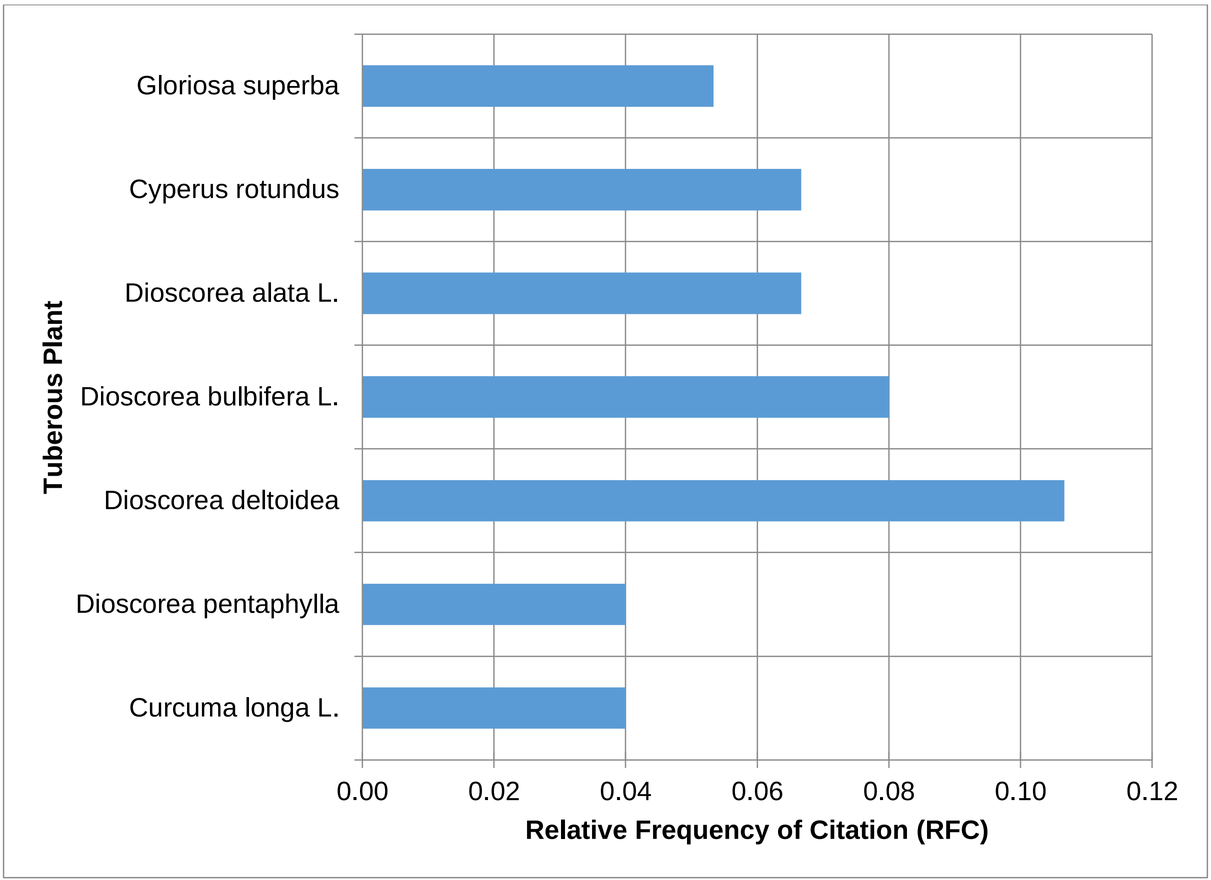

Figure 2.4: Relative Frequency of Citation (RFC) for tuberous plants with anthelmintic properties.

Phytochemicals found in plants with the highest RFC values

Table 2.2 below shows the presence of phytochemicals such as essential oils, flavonoids, tannin, saponins, unsaturated triterpenoides, resins, sterols, quinones, coumarins, and alkaloids in *Dioscorea deltoidea*, *Dioscorea bulbifera*, *Dioscorea alata L.*, *Cyperus rotundus*, *Gloriosa superba*, *Curcuma longa L.*, and *Dioscorea pentaphylla.* The classes of phytochemicals that are common in all these plants are phytosterols, tannins, alkaloids, saponins, essential oils, flavonoids, and terpenoids. Tannins, alkaloids, flavonoids, terpenoids, phenols, saponins, and essential oils have all been shown to have anthelmintic properties (Busari, Soetan, Aiyelaagbe, & Babayemi, 2021; Ishnava & Konar, 2020; Selvaraju & Dhanraj, 2019). The tuber extract of *Corallocarpus epigaeus* showed the presence of alkaloids, flavonoids, saponins, phenols, tannins, and steroids (Ishnava & Konar, 2020) The bark and stem of *Salsola imbicata* showed the presence of the following anthelmintic phytochemicals, anthraquinones, reducing sugar, tannins, saponins, flavonoids, alkaloids, and cardiac glycosides (Ajaib, Farooq, Khan, Perveen, & Shah, 2019). The leaves of *Termia catappa* revealed the presence of carbohydrates, cardiac glycosides, reducing sugars, alkaloids, triterpenes, saponins, tannins, phenols, and flavonoids (Olukotun, Bello, & Oyewale, 2018).

Table 2.2: Phytochemical constituents of tuberous plants with anthelmintic properties that are culturally important in the control of helminths.

PLANT	PHYTOCHEMICALS
Dioscorea deltoidea	diosgenin, corticosterone, 25-D-spirostan-3,5 diene, smilagenone, stigmasterol, B-sitosterol, dioscorin, dioscin and campastrol, Phytosterols, tannins, starch, alkaloids steroidal glycosides, ascorbic acid, beta-carotene, riboflavin (Tahir et al., 2016)
	flavonoids,0 saponins, unsaturated triterpinoides, resins, sterol (Subhash, Sarla, & Mridul, 2012)
	antroquinone, proteins, carbohydrates (Akalya & Subasri, 2016; Chandra, Saklani, & Dimari, 2012; Karnick, 1971)
	quercetin, cyanidin, kaempferol, caffeic, p-coumaric, synaptic, ferulic acids (Karnick, 1971; Semwal, Painuli, & Cruz-Martins, 2021)
	Deltonine, deltoside, diosgenine-3-β-d-glucopyranosyl (1→4)-β-d-glucopyranoside (Paseshnichenko & Guseva, 1975)
	Deltostim (Vasil'eva & Paseshnichenko, 1996)
	3-O-β-d-glucopyranosyl-ergost-5-ene-3β,26-diol-26-O-β-d-glucopyranosyl (1→3)-[β-d-glucopyranosyl(1→2)-β-d-glucupyranosyl(1→6)]-β-d-gluco pyranoside; isonarthogenin-3-O-α-l-rhamnopyranosyl-(1→2)-[α-l-rhamno pyranosyl-(1→4)]-β-d-glucopyranoside; methyl protobioside, protobioside (Shen et al., 2002)
Dioscorea alata L	diosgenin, dioscorin, dioscin, phytosterols, alkaloids, tannin, starch, ascorbic acid, beta-carotene, protein, riboflavin (Dutta, 2015)
	hexadecanoic acid, methyl stearate, cinnamyl cinnamate, and squalene (Dey, Roy Chowdhuri, Sarkar, & Chaudhuri, 2016)

	phenols, reducing sugars, flavonoids, glycoside, saponins, triterpenes, coumarins phytosterols, steroids, anthraquinones, proteins, cholin, mucin, allantoin, crude fat, crude fiber, catechins, chlorogenic acids, proanthocyanidins, myricetin, diosbulbin, sapogenin.(Kaur, Khatun, & Suttee, 2021; Poornima & Ravishankar, 2007; Zhang, Zhang, Jacob, Li, & Yang, 2008)
Gloriosa superba	Alkaloids such as colchicines and colchicosides (Padmapriya, Rajamani, & Sathiyamurthy, 2015)
	gloriosine, lumicolchicine, 3-demethyl-N-deformyl-Ndeacetylcolchicine, 3-demethylcolchicine and N-formyl deacetylcolchcine (Maroyi & Van der Maesen, 2011b; Suri, Gupta, Suri, Sharma, & Satti, 2001)
	benzoic acid, salicylic acid, sterols, resinous substances like as 3-demethyl colchicine, 1,2-didemethyl colchicine, 2,3-didemethyl colchicine, N-formyl, Ndeacetyl colchicines, tannins, superbine (Capraro, 1984)
	carbohydrates, flavonoids, vitamin C, vitamin E, phenols, glycosides, saponins (Jagtap & Satpute, 2014; Muthukrishnan & Annapoorani, 2012; Rehana & Nagarajan, 2012)
	xanthoproteins, triterpenoids, amino acids, carbohydrate, reducing sugar (Jebamalar, Gajalakshmi, & Sivakumar, 2019)
	terpenoids, coumarins (Nikhila, Sangeetha, Preetha, & Swapna, 2016)
	3-demethyl-N-deformyl-N-deacetylcolchicine, 3-demethylcolchicine, N-formyl deacetylcolchicine, salicylic acid, (Jana & Shekhawat, 2011)
Dioscorea pentaphylla	phenols/polyphenols, flavonoids, terpenoids, tannins, alkaloids, saponins (Prakash & Hosetti, 2010)
	glycosides, phenol, reducing sugars, steroids (Vivek & Prakash, 2018)
	gum protein (Sidde *et al.*, 2021)
Dioscorea bulbifera	Kaempferol-3,5-dimethyl ether, Quercetin-3-O galactopyranosid, Myricetin-3-O galactopyranoside, Myricetin-3-O glucopyranoside (Gao *et al.*, 2002).
	8-epidiosbulbin E acetate (Shriram *et al.*, 2008).
	Bafoudiosbulbin (Kuete *et al.*, 2012; Teponno *et al.*, 2006)
	Diosbulbiside (Liu *et al.*, 2009)
	Daucostero, Palmatic acid, Succinic acid, Shikimic acid, 3, 5-dimethoxykaempfero, 3, 5, 3′-trimethoxyquercetin, Caryatin, Myricetin-3-O-β-D galactopyranoside, Myricetin-3-O-β-D glucopyranoside, Hyperoside, Myricetin, Kaempferol-3-O-β-D galactopyrano, Kaempferol-3-O-β-D glucopyranoside Diosbulbin B is a demethyl diterpenoid (Gao, Hou, Kuroyanagi, & Wu, 2007)
	β-Sitosterol (Teponno *et al.*, 2006)
	(+)Catechin, Kaempfero, Dioscoreanoside (Tapondjou, Jenett-Siems, Böttger, & Melzig, 2013)
	Protocatechuic acid (Wang, Lin, Liu, & Wang, 2009)
	Vanillic acid (Tang *et al.*, 2006)
	Quercetin-3-O galactopyranoside, 2,7-dihydroxy-4- methoxyphenanthrene, 3-O-α-L-rhamnopyranosyl- (1→2)-[α-L-rhamnopyranosyl- (1→3)]-β-D-g1ucopyranosyl pennogenin

	(spiroconazol A), Quercetin-3-O-β-D glucopyranosid (Teponno *et al*., 2006) Demethyl batatasin IV (Wang, Liu, Lin, Wang, & Liu, 2009) 3-hydroxy-5-methoxybenzoic acid, Batatasin III, 1,6-dihydroxy-2,5,7-trimethoxyphenanthrene 2,4,6,7-tetrahydroxy-9,10- dihydrophenanthrene, 2,5,2',5'-tetrahydroxy-3'- methoxybibenzyl, Thunalbene, Flavanthrinin, Isorhamnetin (Liu *et al*., 2011) Pennogenin, Pennogenin-3-O-α-L rhamnopyranosyl-(1→3)-[α L-rhamnopyranosyl-(1→2)]-β-D- glucopyranoside, 26 4-hydroxy-[2-trans-3',7'- dimethyl octa-2',6'-dienyl]-6-methoxy acetophenone, 4,6-dihydroxy-2-O-(4'- hydroxybutyl) acetophenone (Gupta & Singh, 1989) Stigmasterol (Wang, Lin, *et al*., 2009) Lutein, Neoxanthin, Violaxanthin, Zeaxanthin, Auroxanthin, Cryptoxanthin (Ghosh, Parihar, More, Dhavale, & Chopade, 2015)
Cyperus rotundus	Sesquiterpene, (Chen *et al*., 2011) terpenoids, sesquiterpenes, sitosterol, cyperene, cyperol, nootkatone and valencene (Sonwa & König, 2001; Tsoyi *et al*., 2011) α-cyperone. (Jung *et al*., 2013) phenolic acids, ascorbic acids, tannins, alkaloids, essential oils (α-longipinane, β-selinene, cyperene, and caryophyllene oxide), and flavonoids (anthocyanidins, catechins, flavans, flavones, flavanonols, and isoflavane) alkaloids, cyperol, flavonoids, fatty oils, furochromones, glycerol, linolenic acid, myristic acid, nootkatone, starch, saponins, sesquiterpenes, sitosterol, stearic acid, terpenoids, polyphenol, and valencene (Sharma, Verma, & Ramteke, 2014; Sivapalan, 2013) cyanins, quinones, coumarins, glycosides, steroids terpenoids, (Jeyasheela, Chairman, Padmalatha, & Ranjit Singh, 2014; Madhulika & Varsha, 2015; Peerzada *et al.*, 2015) alpha-cyperone, betaselinene, cyperene, cyperotundone, patchoulenone, sugeonol, kobusone, and isokobusone (Lawal & Oyedeji, 2009) vitamin C, cardiac glycosides (Nagulendran, Velavan, Mahesh, & Begum, 2007) Cyproterone, cypera-2, 4-diene, a-copaene, cyperene, aselinene, rotundene, valencene, ylanga-2, 4-diene, g-gurjunene, trans-calamenene, d-cadinene, g-calacorene, epi-a-selinene, a-muurolene, g-muurolene, cadalene, nootkatene, cyperotundone mustakone, cyperol, isocyperol,a-cyperone (Pal, 2015)
Curcuma longa L	Turmeronol-A (1), turmeronol-B (2), 3,4-dimethoxycinnamic acid (3), 4-hydroxy3-methoxycinnamic acid (4), 4-hydroxybenzaldehyde (5), 2,3,5,6-tetrahydroxyarturmerone (6) and 4-hydroxybisabola-2,10-diene-9-one (7) (Khan, Nahar, Rahman, Hasan, & Rashid, 2009) Turmerin (Hatcher, Planalp, Cho, Torti, & Torti, 2008) Wenyujinlactone A, neolitamone A, zedoarondiol, isozedoarondiol, aerugidiol, curcumol, curdione, (1R,10R)-epoxy-(-)-1, 10-dihydrocurdine (Wang, Zhang, Guo, Song, & Zhao, 2007) parviflorene F4, curcuminoids (Pozharitskaya, Ivanova, Shikov, & Makarov, 2008) Alkaloids, Flavonoids, Cardiac glycosides, Saponins, Tannins, Balsams, Terpenes, Phenol,

	Resins, Carbohydrate, Proteins, Starch, Amino acids, Steroid, Glycoside, (Mohammed et al., 2019; Saxena & Sahu, 2012)
	Diarylheptanoids and diarylpentanoids, phenylpropene, monoterpenes, sesquiterpenes, diterpenes, triterpenoids, sterols, Ferulic acid (Sabale, Modi, & Sabale, 2013)
	Essential oils (Li *et al*., 2009)
	8-cineole, 2-bornanol, 2-hydroxymethyl-anthraquinone, 4-hydroxybisabola-2, 10-diene-9-one; 4-methoxy-5-hydroxybiosabola; 4-hydroxy-cinnamoyl-(Feruloyl)-methane, Alpha-atlantone, Alphapinene, Alphaterpineol, Ar-turmerone, Arabinose, Eugenol, Epiprocurcumenol; Eucalyptol; Eugenol; Feruloyl-p-coumaroyl-methane, Gamma-atlantone, Germacrone, Germacrone13-al;Guaiacol, Isoborneol, L-alphacurcumene(Chanda & Ramachandra, 2019)

Family Importance Value (FIV)

The Family Importance Value (Figure 2.5) was utilized in this study to highlight the importance of plant families. The *Dioscoreaeceae* family has the highest FIV value of 0.320, followed by *Zingiberaceae* 0.133, *Cyperaceae* 0.067, *Fabaceae* 0.067, *Cochicaceae* 0.0053, and *Araceae* 0.040. The lowest FIV value of 0.013 was recorded in 12 families and 0.027 in 6 families. A high FIV value showed that the families had plants that were often cited in ethnobotanical studies, whereas a low FIV value suggested that the families contained species with few citations.

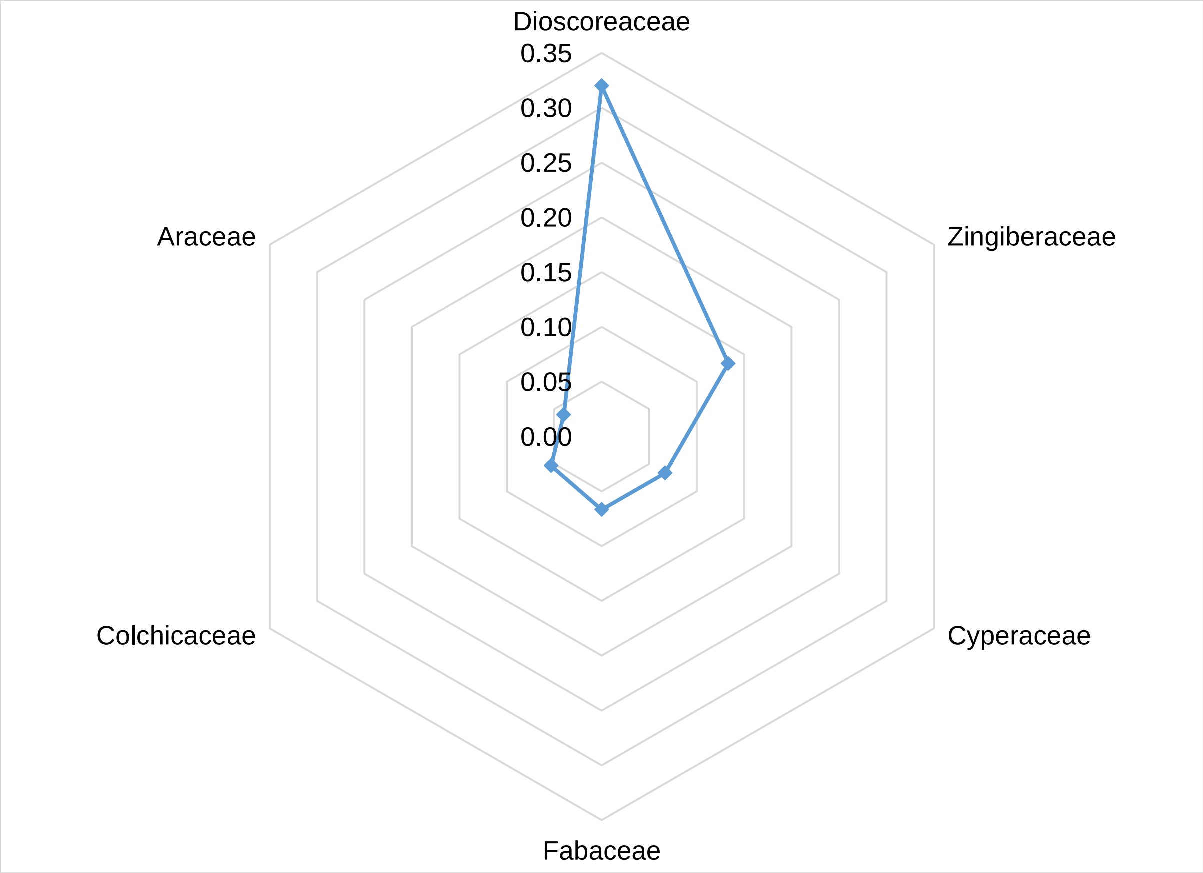

Figure 2.5: Family Importance Value (FIV) of tuberous plants with anthelmintic properties.

Comparative analysis (Jaccard Index JI)

This was computed between India, South Africa, and Nepal, which had the highest number of plants cited. India and Nepal had the highest JI value (0.15), and there was no relationship between South Africa and the other two countries. Figure 2.6 depicts the relationship between these three countries using a Venn diagram. Four plants are common in India and Nepal which are *D. deltoidea*, *D. alata*, *D. bulbifera*, and *D. pentaphylla* according to the findings. There was no floristic relationship between South Africa and the two Asian countries. A high similarity rating indicates that the countries share comparable culture, traditions, and vegetation, whilst a low number indicates that the countries do not share any shared cultural values. Ethnobotanical knowledge, on the other hand, is frequently influenced by origin, culture, sample size, vegetation variation, and microclimatic variables (Amjad *et al*., 2020; Kebede, Ayalew, Mesfin, & Mulualem, 2016).

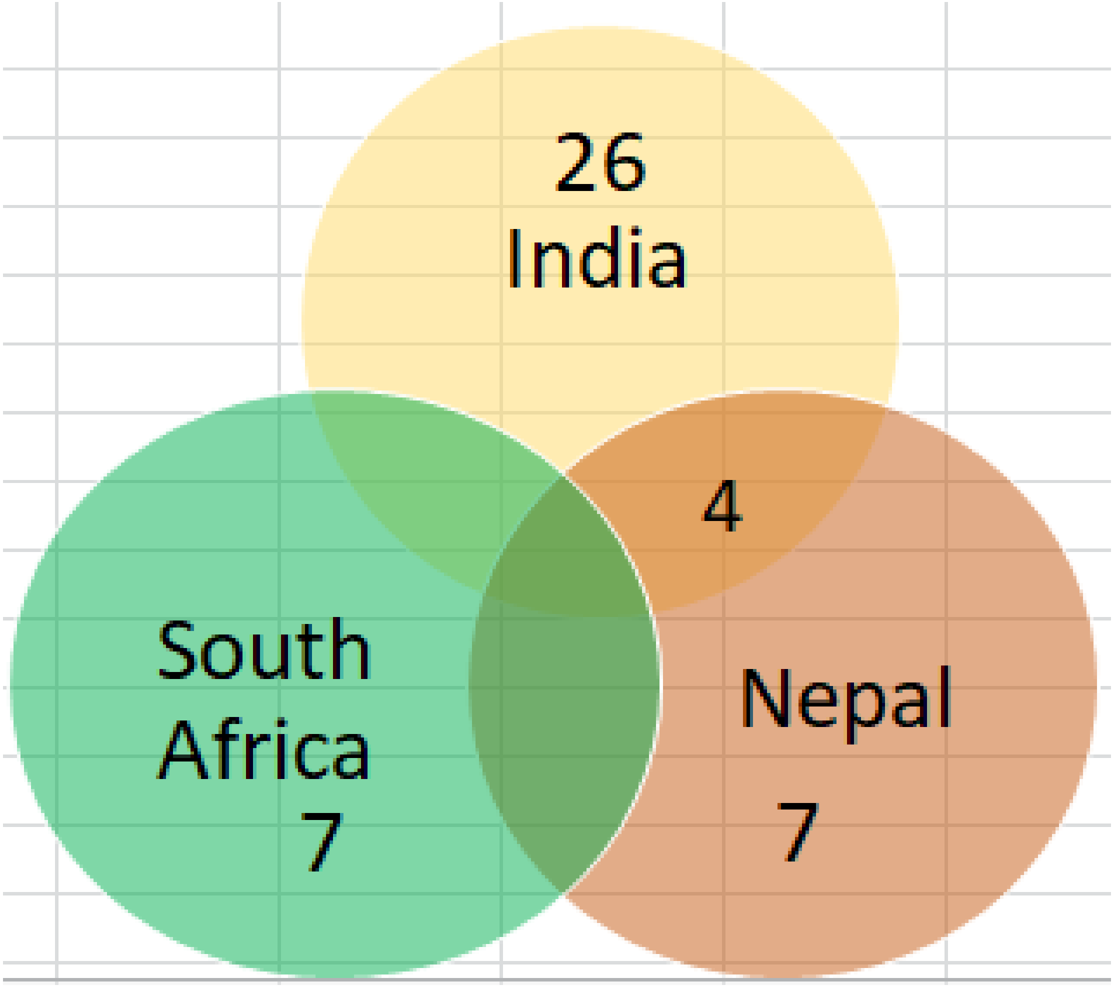

Figure 2.6: Different types of anthelmintic factors recorded in tuberous plants in different countries.

Anthelmintic activity of the tuberous plants

Table 2.3 below shows that 26 of the tuberous plants had their anthelmintic activity reported and 17 have not been reported. *Zingiber officinale, Azadirachta indica, Flemingia vestita, Allium sativum*, and *Curcuma longa* were the most studied plants for their anthelmintic activity. However, common anthelmintic plants indicated in literature are *Carica papaya*, *Butea monosperma*, *Terminalia arjuna*, *Z. officinale*, *Nigella sativa*, *Fumaria parviflora*, *Flemingia vestita*, *Allium sativum*, *Melia azedarach*, *Cucurbita maxima*, *Ocimum sanctum*, *Achyranthes aspera*, *Azadirachta indica*, *Calotropis procera*, and *Artemisia annua* (Nirala, 2019). The other tuberous plants with anthelmintic effects not indicated in this study are *Strychnos henningsii*, *Corallocarpus epigaeus*, and *Hypoxis hemerocallidea* (Ishnava & Konar, 2020; Matyanga *et al*., 2020).

Table 2.3: Anthelmintic activity of the tuberous plants with anthelmintic properties cited in various publications.

Tuberous Plant	Anthelmintic Activity
Dioscorea deltoidea	Not reported
Aconitum heterophylum Wall	*Pheretima postuma* (Pattewar, Pandharkar, Yerawar, & Patawar, 2012)
Stephania glabra Roxb	*Raillietina echinobothrida* (Das *et al*., 2009; Das, Tandon, & Saha, 2004a; Das, Tandon, Saxena, Joshi, & Singh, 2013)
Rumex usambarensis Dammer.	Not reported
Dioscorea alata L.	*Dactyogyrus intermedius* (Wang, Han, *et al*., 2010)
Dioscorea bulbifera L.	earthworms and liver flukes (Adedapo & Mubo, 2013), *Eisenia foetida*, *Raillietina spiralis* and *Ascardia galli* (Kosalge & Fursule, 2009c), *Fasciola gigantica* and *Pheretima posthuma* (Patel & Galani, 2017)
Zingiber zerumbet (L) Roscoe ex sm.	*Pheretima posthuma* (Goswami, Pandey, Tripathi, Singh, & Rai, 2011; Pandey, Goswami, Tripathi, & Singh, 2011; Raul, Padhy, Charly, & Kumar, 2012; Sahu, Panda, & Nayak, 2018),
Zingiber officinale Roscoe	*Dirofilaria immitis* (Datta & Sukul, 1987), Anisakis larvae (Goto, Kasuya, Koga, Ohtomo, & Kagei, 1990), *Fasciola gigantica* (Jeyathilakan et al., 2010; Sunita & Singh, 2011; Toulah *et al*., 2019), *Setaria cervi* (Ghosh, Ghosh, Sinha Babu, & Sukul, 1992), *Pheretima posthuma* (Dubey, Verma, *et al*., 2010; Korukola, Medisetti, Sravanam, & Sanga, 2014; Nirmal, Gupta, Ghogare, & Christian, 2009; Raul *et al*., 2012), *Toxocara vitulorum* (Shalaby, El Namaky, Kamel, Ashry, & Farag, 2017), *Haemonchus contortus* (Iqbal, Nadeem, Khan, Akhtar, & Waraich, 2001), nematodes (Iqbal, Lateef, Akhtar, Ghayur, & Gilani, 2006), *Angiostrongylus cantonensis* (Lin, Chen, Chung, & Yen, 2010), *Lumbricus rubellus* (Dhiman, 2017), *Strongyloides ransomi*, *Hyostrongylus rubidus*, *Trichostrongylus axei* and *Globocephalus urosubulatus* (Kiambom *et al*., 2021), *Raillietina cesticillus* (El-Bahy & Bazh, 2015), Fasciola miracidia (Ghafar *et al*., 2021), *Echinococcus granulosus* (Almalki, Al-Shaebi, Al-Quarishy, El-Matbouli, & Abdel-Baki, 2017), gastrointestinal worms (Adeniji, Adediran, Ososanya, & Uwalaka, 2017), *Toxocara canis* (El-Sayed, 2017), *Fasciola hepatica* (Moazeni & Khademolhoseini, 2016), Eimeria species (Ashraf *et al*., 2020).
Curcuma longa L.	*Echinococcus granulosus* (Almalki *et al*., 2017), *Pheretima posthuma* (Nirmal *et al*., 2009; Raul *et al*., 2012), *Toxacara canis* (Kiuchi *et al*., 1989), gastrointestinal worms (Bannerjee, Nigam, & 1978; Sivashanmugapillai, 2016), *Raillietina cesticillus* (El-Bahy et al., 2015), Eimeria species (Ashraf *et al*., 2020; Cervantes-Valencia *et al*., 2015), Haemonchus spp. (Nasai *et al*., 2016; Pandey, Mishra, & Jaiswal, 2018), *F. gigantica* (Ullah *et al*., 2017), *Neoechinorhynchus buttnerae* (de Oliveira *et al*., 2021), nematodes (Amin,

	Mostofa, Awal, & Sultana, 2008; Nath *et al.*, 2019), *Ascaridia galli* (Bazh & El-Bahy, 2013), Strongyles (Nasai, 2012; Ramdani, Budinuryanto, & Julaeha, 2021)
Oroxylum indicum (L) Kurz	*Hymenolepis diminuta* (Deori & Yadav, 2016), strongyle (Downing, 2000), *Pheretima posthuma* (Islam *et al.*, 2016).
Allium sativum (L.)	nematodes (Ahmed, Laing, & Nsahlai, 2014; Amin, Mostofa, Awal, & Sultana, 2008; Kanojiya, Shanker, Sudan, Jaiswal, & Parashar, 2015), *Giardia lamblia* (Mirelman, Monheit, & Varon, 1987), gastrointestinal worms (Nadkarni, 1954; Sunada *et al*., 2011), *H.gallinarum*, A. *galli* (Kavindra & Shalini, 2000; Raza *et al*., 2016), *H. contortus* (Ahmed, Laing, & Nsahlai, 2013; Azra *et al*., 2019; Iqbal *et al*., 2001; Luce, 2019; Navaneetha & Veerakumari, 2009; Palacios-Landín *et al*., 2015; Perry, 1980; Shalaby & Farag, 2014; Veerakumari & Lakshmi, 2006; Worku, Franco, & Baldwin, 2009) , *A. suum* (Chybowski, 1997; Urban, Kokoska, Langrova, & Matejkova, 2008), strongyloids (Lakshmi, Veerakumari, & Raman, 2011; Sutton & Haik, 1999; Tavassoli, Jalilzadeh-Amin, Fard, & Esfandiarpour, 2018), *Fasciola gigantica* (Jeyathilakan, Murali, Anandaraj, & Abdul Basith, 2012; Kumar, Sunita, & Singh, 2016; Singh, Kumar, Gupta, & Tandan, 2007; Singh, Kumar, Tandan, & Mishra, 2009), *Gigantocotyle explanatum* (Singh *et al*., 2007; Singh, Kumar, & Tandan, 2008), *Neoechinorhynchus buttnerae* (de Oliveira *et al*., 2021), *Pheretima posthuma* (Dubey, Paroha, *et al*., 2010; Ittiyavirah, Jumimol, Krishnapriya, Krishnaja, & Manjusha, 2012; Kadam *et al*., 2015), *Trichostrongylus colubriformis* (Urban et al., 2008), *T. canis* and *A. caninum* (Orengo, Maitho, Mbaria, Maingi, & Kitaa, 2016), Coccidia (Worku *et a*l., 2009), *Echinococcus granulosus* (Mohammadi, Heidarpour, & Borji, 2018; Shirgholami *et al*., 2021), *Aspiculuris tetraptera* (Ayaz, Turel, Gul, & Yilmaz, 2008), whipworm (Lucido, 2014), *Moniezia expansa* (Shalaby et al., 2014), hookworm (Ambriani, 2012), *Dipylidium camnum* and *Taenia hydatigena* (Sblivanova-yartseva, 1959), Trichostrongylous (Hayajneh, Titi, Alnimer, & Irshaid, 2019).
Kaempferia galanga L.	*Pheretima posthuma* (Dash *et al*., 2017)
Neorautanenia brachypus (Harms) C.A.SM	strongyloid (Murungweni *et al*., 2012)
Cyperus rotundus	tapeworms and earthworms (Mishra, Gaud, Sharma, & Chaturvedi, 1979), *Pheretima posthuma* (Kasala, Ramanjaneyulu, Himabindhu, Alluri, & Babu, 2016)
Elephantorrhiza elephantina	*Haemonchus contortus* (Maphosa & Masika, 2012a; Maphosa, Masika, Bizimenyera, & Eloff, 2010), *Paramphistomum cervi* (Mazhangara, Masika, *et al*., 2020), Trichuris spp.(Maphosa & Masika, 2012b)
Flemingia vestita Benth and Hooker	*Raillietina echinobothrida* (Das *et al*., 2009; Das *et al*., 2004a; Das,

	Tandon, & Saha, 2004b; Das, Tandon, & Saha, 2006; Das, Tandon, & Saha, 2007; Pal & Tandon, 1998a; Tandon & Das, 2007; Tandon, Das, & Saha, 2003; Tandon, Pal, Roy, Rao, & Reddy, 1997), *Fasiolopsis buski* (Kar & Tandon, 2000; Kar, Tandon, & Saha, 2002; Kar, Tandon, & Saha, 2004; Pal & Tandon, 1998b; Roy & Tandon, 1996), *Fasciola hepatica* (Toner *et al*., 2008), *E. multiloculoris* and *E. granulous* (Naguleswaran *et al*., 2006), *Artyfechinostomum sufrartyfex* (Kar *et al*., 2000; Roy *et al*., 1996),
Bulbine asphodeloides (L.) Wild.	Not reported
Azadirachta indica	*H. contortus* (Akhter, Dey, Hossain, Dey, & Begum, 2015; Azra *et al*., 2019; Costa *et al*., 2008; Iqbal, Asim, Ahmad, Abbas, & Aslam, 2014; Iqbal, Babar, Abbas, & Sajid, 2012; Iqbal, Lateef, Jabbar, & Gilani, 2010; Nawaz *et al*., 2014; Perry, 1980; Radhakrishnan, Gomathinayagam, & Balakrishnan, 2010; Rahman, Lee, & Sulaiman, 2011; Sakti, Kustantinah, & Nurcahyo, 2018; Swarnkar, Singh, Khan, & Bhagwan, 2008; Tomar & Preet, 2017; Zahoor-ul-Hassan *et al*., 2012), gastrointestinal worms (Ajabe *et al*., 2018; Chandrawathani, Adnan, & Zaini, 2000; Prasad, Sai, & Srilekha, 2011; Premaalatha *et al*., 2013; Sarker, Khan, Rashid, & Islam, 2016; Sivashanmugapillai, 2016; Vikram, Mishra, Vishwakarma, & Shukla, 2019), *Pheretima posthuma* (Naidu, Ramu, & Kumar, 2016; Nazneen, Muddassir, Meshram, Umekar, & Lohiya, 2017; Rabiu & Subhasish, 2011; Salma *et al*., 2021; Singh, Chandrudu, & Parmar, 2018), *Raillietina spiralis* (Rabiu *et al*., 2011), *Ascaridia galli* (Ali, Beguh, Rahman, & Shanta, 2006; Hellawi & Ibrahim, 2021; Hogade, Jalalpure, Bhinge, Kuthar, & Kosgi, 2013; Rabiu *et al*., 2011), *Fasciola gigantica* (Kushwaha, Kumar, Tripati, & Tandan, 2004), *Setaria cervi* (Kausar, 2017; Mishra, Parveen, Singhal, & Khan, 2005) , Trichostrongylus (Iqbal *et al*., 2010), strongyle (Das, Dongre, Nath, Dixit, & Agrawal, 2015; Jamra, Das, Singh, & Haque, 2015; Suhaimi & Mossadeq, 2016), *Gastrothylax indicus* (Aggarwal & Bagai, 2014; Aggarwal, Kaur, Suri, & Bagai, 2016), *Paramphistomum cervi* and *Fasciola hepactica* (Ibekwe, 2019), Fasciola spp. (Sunita, Kumar, Singh, & Singh, 2013; Yamson *et al*., 2019), *Eudrilus eugeniae* (Hogade *et al*., 2013; Priya & Santhi, 2015), nematodes (Alam, Alam, Begum, & Amin, 2014; Amin, Mostofa, Awal, & Hossain, 2008; Bhattacharjee et al., 2021; Jamnah, Khadijah, & Vincent, 2006; Priscilla, Amin, & Rahman, 2014; Yakubu, Saleh, & Abdullahi, 2006; Zapata Salas et al., 2013), *Eisenia foetida* (Priya *et al*., 2015), *Heligmosomoides polygyrus* (Githiori, Höglund, Waller, & Baker, 2003), *Teladorsagia (Ostertagia) circumcincta* (Al-Rofaai, Rahman, Sulaiman, & Yahaya, 2012), *Toxocara vitulorum* (Chamuah, Mech, Perumal, & Dutta, 2014),

Pelargonium reniforme	not reported
Gunnera perpensa	*Heterakis gallinarum* (Mwale & Masika, 2015), nematodes (Fomum & Nsahlai, 2017), gastrointestinal worms (Mhlongo, 2018)
Rhoicissus tridentate	*Ascaris suum* (Innocent & Deogracious, 2006; Nalule, Mbaria, Kimenju, & Olila, 2012b), *Haemonchus contortus* (Mdletshe, 2018),
Curcuma amada Roxb.	*Pheretima posthuma* (Rakh, Pawar, & Khedkar, 2014), Eisenia foetida (Gill, Kalsi, & Singh, 2011)
Curcuma aromatic Salisb.	Not reported
Curcuma caesia Roxb.	Earthworms (Chadalavada & Budala, 2017), *Eisenia foetida* (Randeep, Vandna, & Amandeep, 2011), *Pheretima posthuma* (Chadalavada *et al*., 2017; Karim, Singh, Khan, & Chourasia, 2017)
Melastoma malabatricum L.	*Haemonchus contortus* (Suteky, 2019).
Asparagus racemosus Willd.	*F. gigantica* (Vishwakarma & Kumar, 2021), *Gastrothylax crumenifer* (Soren & Yadav, 2021), *Pheretima posthuma* (Kiranmayi, Ravishankar, & Priyabandhavi, 2012)
Costus speciosus (Koen) Sm.	*Pheretima posthuma* (Srivastava *et al.*, 2011). *Eisenia foetida* and *Taenia saginata* (Kosalge & Fursule, 2009b)
Raphanus sativus L.	*Pheretima posthuma* (Robertson & Thamizharasi, 2016; Shetty, Kamath, Bhat, Hegde, & Shabaraya, 2011), *Raillietina spiralis* and *Ascaridia galli* (Shetty *et al*., 2011)
Dioscorea pentaphylla L.	Not reported
Gloriosa superba L.	*Pheretima posthuma* (Pawar *et al*., 2010), *Eisenia foetida* (Suryavanshi *et al*., 2012)
Alpinia galanga	*Pheretima posthuma* (Babu *et al*., 2017; Subash, Rao, Cheriyan, Bhaarati, & Kumar, 2012), *Ascardia galli* (Subash *et al*., 2012), earthworms (Patil, Bhapkar, *et al*., 2014)
Hypoxis argentea, *Albuca setosa*, *Pueraria tuberosa*, *Peliosanthes bakeri* Hook. f., *Flemingia procumbens* Roxb, *Dioscorea hamiltonii* Hook. F Ban, *Sauromatum venosum* (Aiton) Schott, *Dryopteris setosa* (Thunb.) Akas, *Arisaema jacuemontii*, *Arisaema consanguineum* Schott, *Dioscorea prazeri* Prain & Burkill	Not reported
Cyphostemma adenocaule (A.rich.) willd Drummond	Gastrointestinal worms (Tumwesigye, 2011)

Parasites tested against during anthelmintic activity studies

The reported tuberous plants with anthelmintic properties have been tested against nematodes, trematodes, cestodes, protozoa, eoacanthacephala, and coccidia that affect animals and fish as shown in Table 2.4 below. The tuberous plants have been tested against 41 different parasites that were indicated in literature and are subdivided into 21 nematodes, nine cestodes, nine trematodes, one protozoan, and one eoacanthacephala. The top five parasites to be tested against to determine the anthelmintic activity of the tuberous plants were *Pheretima posthuma* (33 citations), *Haemonchus contortus* (31 citations), *Raillietina echinobothrida* (12 citations), *Fasciola gigantica* (11 citations), and *Ascardia galli* (10 citations). This was in agreement with Patil, Bagade, Sharma, and Hatware (2019) who reported *Haemonchus contortus* to be the most commonly employed test agent for anthelmintic potential. Twenty parasites had more than one citation (number of times it has been indicated to be used as a model during research using the tuberous plants). Of the 41 parasites indicated four are non-parasitic earthworms which are *Lumbricus rubellus*, *Eudrilus eugeniae*, *Pheretima posthuma*, and *Eisenia foetida*. They are used as test worms because of their anatomical and physiological resemblance to gastrointestinal tapeworms and because they are easily available (Choudhary, Khatik, Choudhary, Singh, & Suttee, 2021; Salma *et al*., 2021).

Table 2.4: List of parasites that were used as helminths models in anthelmintic studies of tuberous plants with anthelmintic properties.

Parasite Name	**Number of citations**	**Parasite Class**
Pheretima posthuma	33	Nematode
Haemonchus contortus	31	Nematode
Raillietina echinobothrida	12	Cestode
Fasciola gigantica	11	Trematode
Ascardia galli	10	Nematode
Eisenia foetida	6	Nematode
Fasciolopsis buski	5	Trematode
Echinococcus granulosus	4	Cestode
Ascaris suum	4	Nematode

Raillietina spiralis	3	Cestode
Setaria cerviz, Toxocara canis	3	Nematode
Fasciola hepatica	3	Trematode
Neoechinorhynchus buttnerae	2	Eoacanthacephala
Heterakis gallinarum, Toxocara vitulorum	2	Nematode
Artyfechinostomum sufrartyfex, Gastrothylax indicus, Gigantocotyle explanatum, Paramphistomum cervi	2	Trematode
E. multiloculoris, Hymenolepis diminuta, Moniezia expansa, Raillietina cesticillus, Taenia hydatigena, Taenia saginata	1	Cestode
Ancylostoma caninum, Angiostrongylus cantonensis, Aspiculuris tetraptera, Eudrilus eugeniae, Globocephalus urosubulatus, Heligmosomoides polygyrus, Hyostrongylus rubidus, Lumbricus rubellus, Strongyloides ransomi, Teladorsagia circumcincta, Trichostrongylus axei, Trichostrongylus colubriformis	1	Nematode
Giardia lamblia	1	Protozoa
Dactyogyrus intermedius, Gastrothylax crumenifer	1	Trematode

Toxicological indications of tuberous plants and their active compounds

The results displayed in Table 2.5 showed that 11 (26%) of the anthelmintic tuberous plants indicated have not been subjected to any toxicological studies. The other 32 were reported to be cytotoxic, hepatotoxic, mutagenic, carcinogenic, embryogenic, teratogenic, hemotoxic, cardio toxic, and cause mortality, pathological changes of organs, paralysis, diarrhea, and vomiting. The differences in toxic effects exhibited by these plants might have been because of differences in animal species tested on, the dosage of plant extract, and duration of exposure. One study mentioned the following plants to be poisonous to livestock *Manihot spp. 78.3%, S. coriaceum 55%, Brachiaria spp. 43.3%, E. contortisiliquum 41.7%, M. pseudoglaziovii 25%, D. mollis Benth 14.3%, M. indica 7.1% e D. ecastophyllum* (de Sousa *et al*., 2019). *Tribulus terrestris*, *Narthecium ossifragum*, *Agave lecheguilla*, *Trifolium hybridum*, and *Lantana camara* have been reported to cause hepatotoxicity in livestock (Clayton, Davis, Knoppel, & Stegelmeier, 2020). Mainly larger doses are responsible for major deleterious effects (Maroyi & Van der Maesen, 2011a). This information shows the therapeutic safety of the cited anthelmintic tuberous plants before conducting clinical studies.

The results in Table 2.5 show that 22 compounds produced by the cited anthelmintic tuberous plants have been reported to exhibit toxic effects. These compounds belong to the following classes of phytochemicals essential oils (12 compounds), alkaloids (four compounds), flavonoids (two compounds), terpenoids (two compounds, phenols (one compound), and organic acids (one compound). These results showed the majority of toxic compounds in tuberous plants are essential oils. However, alkaloids are cited as the most important plant-derived toxins in livestock (Clayton *et al*., 2020).

Endangerement status of tuberous plants

Since many of the plants used in ethnoveterinary systems are native to an area they may be endangered. Medicinal plants turn to be endangered because of overexploitation, dwindling natural habitat, unselective harvesting, and less production (Umavathi, Gopinath, Manjula, Chinnasamy, & Ayyakannu, 2020). Seven plants indicated as the most culturally important plants in this study are potentially endangered plants. These are *Dioscorea deltoidea, Dioscorea bulbifera*, *Dioscorea alata L., Curcuma longa L., Dioscorea pentaphylla, Gloriosa superba*, and *Cyperus rotundus*. However, Table 2.5 below lists 12 plants (28%) that have been reported to be endangered and 31 tuberous anthelmintic plants are not endangered. Other anthelmintic plants that have been reported to be endangered are *IIek khasiana* (Lalnunfela, Lalthanpuii, Lalhriatpuii, & Lalchhandama, 2020), *Picrorhiza kurroa* (Mehta, Sharma, & Singh, 2021), *Potentilla fulgens* (Kumar, Sunita, Singh, & Singh, 2020), *Embelia ribes* (Choudhary, Kaurav, & Chaudhary, 2021).

There is an urgent need for conservation and rapid scientific studies of endangered plants before they become highly endangered or totally extinct (Singh & Geetanjali, 2016). The use of leaves is less damaging when compared to the use of roots, tubers, and bark, which negatively affects the conservation of medicinal plants (Odongo *et al*., 2018). However, roots, tubers, and bark are sometimes preferred by traditional healers due to their easy storage and transport when compared to leaves.

Table 2.5: Toxicological indications, toxic compounds, and endangerement status of tuberous plants with anthelmintic properties.

Plant	**Positive Toxicology results**	**Negative Toxicology results**	**Toxic compound**	**Class of toxic compound**	**Status**
Dioscorea deltoidea	cytotoxicity effects (Mohammad, Fazili, Bhat, & Ara, 2017; Shen, 2002)	No acute toxicity (Ali *et al*., 2020; Povydysh *et al*., 2021)	oxalate (Bhandari & Kawabata, 2005)	-	Endangered (Mandal & Dixit-Sharma, 2007; Nazir *et al*., 2021)
Aconitum heterophylum Wall	Aconitine (Wani, Kaloo, & Dangroo, 2022)	non-toxic (Kumar & Chauhan, 2016; Prasad, Jain, Patel, Sahu, & Hemalatha, 2014)	Aconitine (Wani *et al*., 2022)	Alkaloid	Endangered (Beigh, Nawchoo, & Iqbal, 2006)
Stephania glabra Roxb	Gindarine (1) shows some toxic effects on pregnant rats (Arzamastsev, Mironova, Krepkova, Bortnikova, & IuV, 1983)	No acute toxicity (Semwal, Rawat, Badoni, Semwal, & Singh, 2010; Semwal, iSemwal, Semwal, Jacob, & Gurjaspreet, 2011)	Gindarine (1) (Arzamastsev *et al*., 1983)	Alkaloid	Endangered (Chhetri, Parajuli, & Subba, 2005)
Rumex usambarensis Dammer.	Not reported	Not reported	Not reported	-	Not endangered
Dioscorea alata L.	Cytotoxicity (Bhandari *et al*., 2005; Raju & Mehta, 2008; Wallace, Asemota, & Gray, 2021)	Not reported	Not reported	-	Not endangered
Dioscorea bulbifera L.	Hepatotoxicity (Guan *et al*., 2017; Tan, Ruan, Chen, & Wang, 2003; Wang, Ji, Liu, & Wang, 2010), Cytotoxicity (Nur & Nugroho, 2018; Yu, Liu, Mcculloch, & Gao, 2004)	no form of blood toxicity (Princewill-Ogbonna, Abagha, & Ijioma, 2015), No acute toxicity (Webster, Beck, & Ternai, 1984)	Diosbulbin B and D	Terpenoid	Not endangered
Zingiber zerumbet (L) Roscoe ex sm.	Zerumbone showed selective cytotoxicity (Latif *et al*., 2019; Matthes, Luu, & Ourisson, 1980; Sharifah Sakinah, Tri	No acute toxicity, no chronic toxicity (Chang, Tzeng, Liou, Chang, & Liu, 2012b; Rahman *et al*., 2014), poses no risk of	Zerumbone	Terpenoid	Not endangered

	Handayani, & Azimahtol Hawariah, 2007)	genotoxicity (Chang, Tzeng, Liou, Chang, & Liu, 2012a)			
Zingiber officinale Roscoe	toxic by causing severe hypotension and bradycardia with induction of pre-necrotic changes in cardiac tissue (Elkhishin & Awwad, 2009), Findings suggest caution on chronic use of ginger oils (Idang *et al*., 2019), cytotoxicity (Plengsuriyakarn *et al*., 2012), β-phellandrene showed genetic toxicity in spleen cells evaluated by comet assay (Cheng *et a*l., 2017), 6-gingerol induces DNA strand breaks in Hepatoma G2 cells evaluated by comet assay (Yang *et al*., 2010)	no sub-acute toxicity, no acute toxicity, no chronic toxicity (Elkhishin *et al*., 2009; Plengsuriyakarn & Na-Bangchang, 2020)	β-phellandrene, 6-gingerol	Essential oils, Alkaloid	Not endangered
Curcuma longa L.	Out of 200 *C. longa* compounds, 184 compounds were predicted as toxigenic, 136 compounds were mutagenic, 153 compounds Were carcinogenic and 64 compounds were hepatotoxic, curcumin and its derivatives may cause dose-dependent hepatotoxicity (Balaji & Chempakam, 2010; Deshpande *et al*., 1998), cytotoxicity and apoptotic effects of ar-turmerone, R-turmerone, and β-turmerone (Aratanechemuge *et*	No acute, sub-acute, oral toxicity, and chronic toxicity (Ibukun & Oluwadare, 2021; Qureshi, Shah, & Ageel, 1992), No acute, subchronic and genotoxicity of turmeric essential oil Ar-turmerone (Liju, Jeena, & Kuttan, 2013), nonmutagenic (Soleimani, Sahebkar, & Hosseinzadeh, 2018) curzerene has limited toxicity and side effects *in vivo* and cytotoxicity (Wang *et al*., 2017)	Curcumin, ar-turmerone, R-turmerone, and β-turmerone	Phenol, Essential oils	Endangered (Patel, 2015)

	al., 2002; Ji, Choi, Lee, & Lee, 2004) Embryotoxicity, and Teratogenic (Alafiatayo, Lai, Syahida, Mahmood, & Shaharuddin, 2019)				
Oroxylum indicum (L) Kurz	toxic for the brine shrimp (Chowdhury, Karim, & Rana, 2005), Mortality was observed for 72 hours after administration to mice (Tripathy, Panda, Sahoo, Mishra, & Nayak, 2011), fruit extracts caused hepatotoxicity in rats (Konsue & Katisart, 2021), cytotoxicity (Buranrat, Noiwetch, Suksar, Ta-ut, & Boontha, 2018)	No acute oral toxicity and sub-acute oral toxicity (Joshi, Vyas, *et al*., 2011; Reduan, Hamid, *et al*., 2020; Tamboli, Karpe, Shaikh, Manikrao, & Kature, 2011) not toxic to humans and experimental animals even up to high doses (Siddiqui *et al*., 2012), stem bark extracts showed non-mutagenic, non-cytotoxic, and non-genotoxic (Singh, Chattopadhyay, Borthakur, & Policegoudra, 2017)	Not reported	-	Endangered (Tiwari, Singh, & Shah, 2007)
Allium sativum (L.)	Mortality occurred in rabbits given the extract at 3200 and 4200 mg/kg with other behavioral signs like loss of appetite and partial paralysis (Mikail, 2010), Garlic-derived di allyl sulfide (DAS) caused death at 1600 mg/kg (1/5 male) and 1920 mg/kg (2/5 female and 3/5 male) doses. DAS also induced marked pathological changes in the lungs, liver, and reproductive organs. DAS highest dose had genotoxicity (Dutta, Dahiya, Prakash, &	No acute and sub-acute toxicity (Lawal *et al*., 2016; Njue, Ombui, Kanja, Gathumbi, & Nduhiu, 2015), no hepatotoxicity (Samson, Olasunkanmi, Joel, & Alfred, 2012)	Garlic derived di allyl sulfide (DAS)	Essential oil	Not endangered

	Agrawala, 2021) toxic at high doses to the liver, heart, kidney, spleen, and lungs, cause loss of appetite and anemic conditions (Fowotade, Fowotade, Enaibe, & Avwioro, 2017; Gatsing *et al*., 2005)				
Kaempferia galanga L.	Essential oil toxic to brine shrimp (AlSalhi *et al*., 2020) cytotoxic (Dash, Nasrin, & Ali, 2014; Omar *et al*., 2017) Ethanolic extracts cause central nervous system depression, decreased motor activity and respiratory rate, loss of screen grip and analgesia in rats (Kanjanapothi *et al*., 2004; Koh, Tan, & Chua, 2009),	No oral acute and sub-acute toxicity (Amuamuta, Plengsuriyakarn, & Na-Bangchang, 2017; Kanjanapothi *et al*., 2004), its essential oils namely ethyl p-methoxycinnamate, trans-ethyl cinnamate and trans-cinnamaldehyde were safe to aquatic fauna (AlSalhi *et al*., 2020) 1,8-cineole showed weak acute toxicity (Liu *et al*., 2014), Hexane fraction when applied on skin of rabbits, showed no sign of dermal irritation (Kanjanapothi *et al*., 2004)	Not reported	Essential oils	Endangered (Kalpana & Anbazhagan, 2009)
Neorautanenia brachypus (Harms) C.A.SM	Not reported	Not reported	Not reported	-	Not endangered
Cyperus rotundus	Cytotoxic (Susianti, Yanwirasti, & Darwin, 2018), the hematological parameters showed an increase in white blood cells count and Hemoglobin level after administration of ethanolic	No sign of toxicity at 10, 100 and 1000mg/kg doses of ethanolic extract (Ahmad, Mahayrookh, Rehman, & Jahan, 2012), Acute and Subacute toxicities tests showed no cause changes in	humulene epoxide,, caryophyllene oxide, Cyperene, α-cyperone, isolongifolen-5-one, rotundene, and cyperorotundene	Essential oils	Not endangered

	extracts to rats. The kidney function and liver function didn't change even after long term exposure (Jebasingh, Jackson, Venkataraman, & Emerald, 2012). humulene epoxide and caryophyllene oxide exhibited moderate cytotoxicity (Samra *et a*l., 2020) Cyperene, α-cyperone, isolongifolen-5-one, rotundene, and cyperorotundene had cytotoxicity effects (Kilani *et al*., 2008)	terms of general behaviors, mortality, weight gain, Hematological and clinical blood chemistry parameters. The results of gross and pathological examinations showed a normal appearance of the internal organs as compared to those of the control group (Thanabhorn, Jaijoy, Thamaree, Ingkaninan, & Panthong, 2005) Sub-chronic toxicity study revealed that food, water consumption, and body weight of animals didn't vary significantly. The kidney function and liver function didn't change even after long-term exposure (Jebasingh *et al*., 2012).			
Elephantorrhiza elephantina	Is harmful when used at an excessive dosage (Gelfland, Mavi, Drummond, & Ndemera, 1985; Hutchings, 1996; Watt & Breyer-Brandwijk, 1962), root infusions have constipating effects seeds are strongly irritant and have been suspected of causing human death when used as herbal medicine (Hutchings, 1996). Seeds are toxic to sheep with a lethal dose 250 g and	Root extract showed no physiological and behavioral changes in the animals and also no mortalities were recorded (Maphosa, Masika, & Moyo, 2009)	Not reported	-	Not endangered

	rabbits (lethal dose 5–7.50 g/kg) causing gastroenteritis and pulmonary edema (Jansen & Cardon, 2005) Root extracts caused changes in body weight and hematological and serum biochemical parameters between the control and treated animals were observed. In acute tests, decreased respiratory rate was observed at higher doses and in sub-acute tests, the root extract caused an increase in white blood cells, monocytes, and serum levels of creatinine at higher doses. In chronic toxicity, caused increase in lymphocytes and platelets and changes were also noted in the body and organ weights in both sub-acute and chronic toxicities (Maphosa, Masika, & Moyo, 2010) showed cytotoxicity effects (Mpofu, Msagati, & Krause, 2014)				
Flemingia vestita Benth and Hooker	the administration of high doses of isoflavones could induce potentially adverse effects (Sirtori, 2001). Genistein causes cell death by inducing apoptosis and other cytotoxic processes. (Klein & King, 2007). Genistein showed significant negative impacts on ovarian	No toxicity to was observed in postmenopausal women after a single dose that exceeded normal dietary intakes of purified unconjugated isoflavones (Bloedon *et al*., 2002)	Genistein	Isoflavone, Flavonoid	Not endangered

	differentiation, estrous cyclicity, and fertility in the rodent model (Jefferson & Williams, 2011; Spagnuolo *et al*., 2015)				
Bulbine asphodeloides (L.) Wild.	Not reported	No cytotoxicity (Otang-Mbeng & Sagbo, 2021)	Not reported	-	Not endangered
Azadirachta indica	Acute toxicity, mortality after 24hr (Saravanan, Ramesh, Malarvizhi, & Petkam, 2011) root bark aqueous extract was considered moderately toxic using the Brine shrimp lethality test (Mwangi, Wagacha, Nguta, & Mbaria, 2015) chronic toxicity of BioneemTM (Botelho *et al*., 2010; Maranho *et al*., 2014) genotoxicity (Chandra & Khuda-Bukhsh, 2004) oil neem oil showed sub-chronic toxicity (Wang, Cao, *et al*., 2013)	no acute toxicity and sub-acute toxicity in mammals (Dorababu, Joshi, Kumar, Chaturvedi, & Goel, 2006; Kingsley, Lateef, Olga, Stephen, & Mavis, 2012), no reproduction and Teratogenicity(Babalola & Areola, 2010; Da Silva *et al*., 2015)		Essential oil	Not endangered
Pelargonium reniforme	Not reported	aqueous root extract is not toxic (Adewusi & Afolayan, 2009)	Not reported	-	Not endangered
Gunnera perpensa	Cytotoxicity to brine shrimp (McGaw, Gehring, Katsoulis, & Eloff, 2005; Simelane, Lawal, Djarova, & Opoku, 2010) 200 mg/kg dose of chronic test was 20% potentially toxic when used consecutively for a long period (Mwale & Masika, 2011)	Neither rat mortality nor changes in behavior were noted for acute test and rat mortality for 400 mg/kg dose of sub-acute (Mwale *et al*., 2011) nonmutagenic (Ndhlala, Finnie, & Van Staden, 2011)	Not reported	-	Not endangered
Hypoxis argentea	Not reported	Not reported	Not reported	Not reported	Not endangered
Albuca setosa	Albuca setosa silver	aqueous extracts not cytotoxic	Not reported	-	Not endangered

	nanoparticles are cytotoxic (Odeyemi & Afolayan, 2019)	(Odeyemi, Koekemoer, van de Venter, Afolayan, & Bradley, 2015)			
Rhoicissus tridentate	Cytotoxicity (Tshikalange, Mamba, & Adebayo, 2016)	Not reported	Not reported	-	Not endangered
Pueraria tuberosa	Sub-chronic toxicity (Santosh, Mohan, Royana, & Yamini, 2010) Puerarin and Genistein have potent inhibitory effects on the metabolic activities of cytochrome enzymes (Burnett, Pillai, Bitto, Squadrito, & Levy, 2011; Kim, Kim, Jung, Chun, & Rhew, 2014)	Acute and sub-acute test of tuber extract was found to be safe in rats (Pal & Mishra, 2019; Pandey, Srivastava, Kumar, & Tripathi, 2018; Shukla, 1995)	Puerarin, Genistein	Isoflavone-Flavonoids	Not endangered
Curcuma amada Roxb.	showed brine shrimp lethal activity (Krishnaraju *et al*., 2006)	non-toxic by cytotoxicity tests (Nag, Banerjee, Goswami, Bandyopadhyay, & Mukherjee, 2021; Policegoudra, Rehna, Jaganmohan Rao, & Aradhya, 2010; Prema, Kamaraj, Achiraman, & Udayakumar, 2014)	Not reported	-	Not endangered
Curcuma aromatic Salisb.	Not reported	Not reported	Not reported	-	Not endangered
Curcuma caesia Roxb.	Not reported	non-toxic by cytotoxicity tests (Nag *et al*., 2021)	Not reported	-	Endangered (Borah, Kumar, Paw, Begum, & Lal, 2020)
Peliosanthes bakeri Hook. f.	Not reported	Not reported	Not reported	-	Not endangered
Melastoma malabatricum L.	Cytotoxicity (Kamsani, Zakaria, Md Nasir, Mohtarrudin, & Mohamad Alitheen, 2019; Kumar, Ahmed, Gupta, Anwar, & Mujeeb, 2013)	extract is safe even at a high dose of 5,000 mg/kg and has no oral toxicity (Alnajar, Abdulla, Ali, Alshawsh, & Hadi, 2012) no acute, sub-	Not reported	-	Not endangered

		acute, and sub-chronic toxicity (Kumar *et al*., 2013; Reduan, Shaari, *et al*., 2020)			
Asparagus racemosus Willd.	partial teratogenic effects have been observed in pre- and post natal studies with methanol extract (Goel, Prabha, Kumar, Dorababu, & Singh, 2006), alcoholic extract of the root produces positive ionotropic and chronotropic effects on frog's heart with lower doses, and cardiac arrest with higher doses (Goyal, Singh, & Lal, 2003), cytotoxicity (Karmakar *et al*., 2012; Singh, Kumar, Choudhary, & Singh, 2018)	No acute toxicity (Kumar, Udupa, *et al*., 2010; Ngeny, Magiri, Mutai, Mwikwabe, & Bii, 2013), no reproductive, sub-acute, and sub-chronic toxicity (Bhandary, Sharmila, Kumari, Bhat, & Fernandes, 2017; Goel *et al*., 2006; Goyal *et al*., 2003)	Not reported	-	Endangered (Bopana & Saxena, 2008)
Costus speciosus (Koen) Sm.	Cytotoxicity (Jha, Alam, Hossain, & Islam, 2010)	No sub-acute toxicity (Sari & Nurrochmad, 2016)	Not reported	-	Endangered (Pandey, Gupta, & Yadav, 2011)
Raphanus sativus L.	Not reported	Not reported	Not reported	-	Not endangered
Dioscorea pentaphylla L.	Not reported	toxicity test demonstrated that the starch was safe and can be classified as non-toxic (Lazim *et al*., 2021)	Not reported	-	Not endangered
Gloriosa superba L.	Poisoning is indistinguishable from alkaloid colchicine overdose (Mendis, 1989), Causes gastrointestinal and haematological abnormalities, hepatic and renal insufficiency, cardiotoxicity and hair loss (Khanam *et al*., 2015), acute	Not reported	colchicine	alkaloid	Endangered (Sivakumar & Krishnamurthy, 2000)

	respiratory distress syndrome and sustained multiple organ dysfunction following ingestion of tubers (Peranantham, Manigandan, & Shanmugam, 2014), fatal (Joshi, 1993), The study of colchicine on rats and monkeys has been shown to induce epileptic foci in rats, causing generalized seizures and death in animals (Eddleston, 2000), causes diarrhea, depressant action on bone marrow and alopecia (Gooneratne, 1966), tubers are extremely poisonous (Aleem, 1992; Angunawela & Fernando, 1971), causes vomiting, purging, stomachache and burning sensation (Roberts, Liang, & Stern, 1987), use of colchicine has been shown to induce epileptic foci in rats, causing generalized seizures and death (Dasheiff & Ramirez, 1985; Sechi et al., 2003), Causes acute renal failure (Badwaik, Giri, Tripathi, Singh, & Khan, 2011)				
Flemingia procumbens Roxb.	Not reported	Not reported	Not reported	-	Not endangered
Dioscorea halmiltonii Hook. F	Not reported	Not reported	Not reported	-	Not endangered

Ban					
Sauromatum venosum (Aiton) Schott	Not reported	Not reported	Not reported	-	Not endangered
Dryopteris setosa (Thunb.) Akas	Not reported	Not reported	Not reported	-	Not endangered
Arisaema jacuemontii	classified as poisonous plant (Ali & Yaqoob, 2021)	Not reported	Not reported	-	Not endangered
Alpinia galanga	2000 mg/kg of extract was highly toxic to Wistar rats when administered intraperitoneally (Karunarathne, Thammitiyagodage, & Weerakkody, 2018), causes cytotoxicity, Apoptosis and DNA Damage (Muangnoi *et a*l., 2007), caused an increase in the relative weight of the heart, liver, spleen, and kidney. Hematological studies revealed a fall in the red blood cells and white blood cells level as well as hemoglobin and platelets (Alajmi, Mothana, Al-Rehaily, & Khaled, 2018)	No acute toxicity and mortality observed (Qureshi *et al*., 1992; Unnisa & Thahera, 2011) did not produce significant changes in the general behavior, body weights, feed intake (Karunarathne *et al*., 2018)	Not reported	-	Endangered (Shetty & Monisha, 2015)
Arisaema consanguineum Schott	Not reported	Not reported	Not reported	-	Not endangered
Dioscorea prazeri Prain & Burkill	Not reported	Not reported	Not reported	-	Endangered (Thankappan & Morawala-Patell, 2011)
Cyphostemma adenocaule	Not reported	Not reported	Not reported	-	Not endangered

(A.rich.)willd Drummond

Conclusions

Forty-two plants have been recorded to have their tuber parts used in the control of helminths. Analysis indicated seven plants to be the most culturally important plants in the control of helminths. These were *Dioscorea deltoidea, D. bulbifera, D. alata L., D. pentaphylla, Curcuma longa L., Gloriosa superba,* and *Cyperus rotundus*. The classes of phytochemicals that are common in these plants are phytosterols, tannins, alkaloids, saponins, essential oils, flavonoids, and terpenoids. These plants are mostly found in India and Nepal. Twenty-six of these tuberous plants have been tested for their anthelmintic effect and 17 have not. These plants have been tested against trematodes, cestodes, nematodes, protozoa, coccidian, and eoacanthacephala. The most used helminth modes were *Pheretima posthuma, Haemonchus contortus, Raillietina echinobothrida, Fasciola gigantica*, and *Ascardia galli*. Eleven of the anthelmintic tuberous plants indicated have not been subjected to any toxicological studies. The other 32 were reported to be cytotoxic, hepatotoxic, mutagenic, carcinogenic, embryogenic, teratogenic, hemotoxic, cardiotoxic, and cause mortality, pathological changes of organs, paralysis, diarrhea, and vomiting. Twenty-two compounds produced by the cited anthelmintic tuberous plants have been reported to exhibit toxic effects. Twelve tuberous plants have been indicated to be endangered.

Therefore, there is a need for conservation programs for these high culturally important plants to prevent extinction. In addition, there is a need to investigate other tuberous plants, especially those found in Africa, and to identify unique compounds that are active against helminths and combine them to develop more robust anthelmintic drugs. These drugs will have the potential to effectively control helminths and reduce the rate at which anthelmintic resistance occurs. The results of the present investigation add more information about the therapeutic safety of these popular folklore drugs and provide grounds for an assessment of possible measures to be introduced before conducting clinical studies. It is hoped that this research will lead to wider public and Government recognition of endangered plants species and spur conservation efforts toward saving both plants and folk medicinal knowledge.

CHAPTER 3: Phytochemical Profile of *Neourautanenia brachypus* (Harms) C.A.Sm. Tubers.

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Abstract

Phytochemicals are non-nutritive plant compounds with disease-preventive or protective qualities. In livestock production, increased use of antibiotics from live or attenuated bacteria is increasingly becoming a major concern to farmers and the general population due to antimicrobial resistance and environmental pollution. In this study, phytochemicals in *Neorautanenia brachypus* (Harms) C.A. Sm., a recently discovered plant in Zimbabwe were identified. Air-drying, sun-drying, and oven-drying methods were used to prepare samples. Extraction was done using the Soxhlet and maceration methods with methanol, distilled water, ethyl acetate, and chloroform as solvents. Standard techniques were used for phytochemical screening. The Total Phenol Content and Total Tannins Content in extracts were determined using a spectrophotometric merthods. The compounds present in *N. brachypus* tuber extracts were identified using a GC-MS machine. *Neorautanenia brachypus* extracts contained essential oils, terpenoids, quinones, saponins, coumarins, phenols, flavonoids, alkaloids, and tannins, according to phytochemical analysis. The Total Phenol content of methanol and distilled water extracts was 365.18mg GAE/g and 89.43mg GAE/g, respectively. The Total Tannin Content of methanol and distilled water extracts was determined to be 2.33 mg TAE/g and 1.42 mg TAE/g respectively. The compounds in *N. brachypus* tuber extracts were reported to have several therapeutic properties such as antifungal, antibacterial, anthelmintic, antiviral, anti-tumor, antioxidant, anti-inflammatory, etc. It was concluded that white *N. brachypus* tubers were the most significant to use for extraction of phytochemicals giving a high extract yield when air-dried and Soxhlet extracted using chloroform 50%: ethyl acetate 50% as a solvent. While Methanol was the most significant solvent when extracting the greatest number of different classes of phytochemicals and also gave an acceptable extract yield. Methanolic samples exhibited a high phenol and tannin content compared to distilled water extracts.

Keywords: solvents, extraction, phytochemicals, *Neorautanenia brachypus,* tubers, anthelmintic, anthelmintic resistance

Abbreviations: Chl, chloroform; EA, ethyl acetate; Meth, methanol; DW, distilled water; Chl-Meth, chloroform and methanol; Chl-EA, chloroform and ethyl acetate; Meth-EA, methanol and ethyl acetate; GC-MS, Gas Chromatography-Mass Spectrometry; GAE, Gallic Acid Equivalent; TAE, Tannic Acid Equivalent

3.1 Background

Helminthiases have emerged as a global threat to livestock production, resulting in significant losses in ruminant production due to reduced weight gain, anemia, diarrhea, reduced reproductive performance, reduced growth rate, low live mass, dull rough coat, organ condemnation, and mortality (Johansson, 2017; León *et al*., 2019; Morgan *et al*., 2013). Conventional medications such as ivermectin, albendazoles, levamisole, salicylanilides, and praziquantel are used to control helminths (Vercruysse *et al.*, 2014). Anthelmintic resistance to these kinds of medications, on the other hand, has been increasing in prevalence and severity (Muthee, Gakuya, Mbaria, & Mulei, 2016). Other drawbacks to using conventional medications include environmental and product pollution from drug residues, high expense, and limited accessibility particularly in remote areas (Lem *et al.*, 2014). As a result, innovative anthelmintic agents, such as plant extracts and their components, are required. Controlling helminths using plant extracts have proven to be an effective strategy. This is due to plants' extracts ease of use and the reduced cost compared to conventional drugs, and acceptability (plant extracts are considered safer for the environment and animal products) (Poné, Bilong, & Mpoame, 2010).

Plants are a major source of medications, with at least one plant-derived chemical included in around 25% of pharmaceutical prescriptions in the United States (Wachtel-Galor & Benzie, 2011). Over the previous century, over 121 plant based medicines have been developed based on indigenous knowledge from a variety of sources (Pandey, Debnath, Gupta, & Chikara, 2011). Health benefits of plants are ascribed to phytochemicals that occur naturally in the plants.

Commercially, phytochemical analysis is important, and pharmaceutical companies are interested in using the phytochemicals to develop novel medications (Manisha, Chandrashekhar, & Raghunath, 2018). Phytochemical screening is also useful for establishing the efficacy of plant use. The phytochemicals in the ethanolic extract of the root peel of *F. vestita,* for example, were extracted and identified to confirm the use of the root tuber of *F. vestita* against helminths (Tandon & Das, 2018).

The preparation of samples is the initial stage in ethnoveterinary plant research. Because fresh samples are delicate and decay quickly, most researchers prefer dried samples. Fresh and dried *Moringa oleifera* leaves were studied by Vongsak et al. (2013), who found that the two procedures had a significant effect on total phenolics, while dried samples had a significant amount of flavonoids. The dried sample had a high significant amount of flavonoids probably because they were more extractable as the surface area was reduced and the solvent easily penetrated the sample. After drying, the particle size is reduced to powder to maximize surface contact between the samples and the extraction solvents. Borhan, Ahmad, Rusop, and Abdullah (2013), investigated nanoparticles powder of *Centella asiatica* produced by Planetary Ball Mill (PBM) and found that it yielded 82.09% more than micro powder using the maceration procedure in 90% methanol for 3 days.

There are two types of extraction methods: classic and novel. The classic ectraction methods are maceration, percolation, and Soxhlet techniques are frequently utilized. Supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and accelerated solvent extraction are examples of advanced procedures (De Silva, Abeysundara, & Aponso, 2017).

In most cases, it is best to test two distinct extraction procedures, such as Soxhlet extraction and maceration, to determine which one yields more, and then employ that approach when studying that particular plant. In comparison to maceration or percolation, other researchers suggest that Soxhlet extraction is the best method for continuous extraction with maximum efficiency, requiring the least time, and solvent usage (Altemimi, Lakhssassi, Baharlouei, Watson, & Lightfoot, 2017; Ingle *et al*., 2017). Soxhlet extraction method is cost-effective in terms of time, energy, and financial inputs, but it has drawbacks, such as exposure to dangerous and combustible liquid organic solvents.

There's also a requirement to pick solvents that can extract the most bioactive substances with the least amount of effort. The principle of “like dissolves like” is applied in the selection of an optimum solvent for extraction. Methanol was found to have the highest % extraction potential and anthelmintic action when compared to acetone, chloroform, ethyl acetate, and hexane in a study by Chandran and colleagues in 2018. Another research of the same experiment found that methanol extract had the highest percent phytochemical yield (85.36%), followed by distilled water (78%), ethyl acetate (62.44%), and acetone (62.48%). Extraction with hexane and chloroform yielded minimum percent yields of 19.4 and 13.68 percent, respectively (Dhawan & Gupta, 2017). Advanced techniques such as Gas Chromatography, Liquid Chromatography, High-Performance Liquid Chromatography, and High-Performance Thin-Layer Chromatography are used to do a qualitative analysis of phytochemicals (Altemimi *et al*., 2017).

To our knowledge, nothing has been reported on the phytochemical constitution of *N. brachypus*. The goals of this study were to compare the number of phytochemicals extracted from three different *Neorautanenia brachypus* accessions (brown, cream, and white) using different extraction methods (Soxhlet and maceration), solvents (methanol, ethyl acetate, Chloroform, and distilled water), and drying methods (sun-drying, air-drying, and oven-drying).

Neorautanenia brachypus (Harms) C.A. was discovered in Zimbabwe's South Eastern Lowveld. The plant produces purple flowers which develop into dehiscent pods densely covered with hairs. It belongs to the family *Leguminosae-Papilionaceae*. *Neorautanenia brachypus* tubers exhibit three distinct color differences in the flesh; white, light brown (cream), and dark brown (Nyarumbu *et al*., 2019). The white tubers are soft and exude a milky white substance and the brown tubers are more fibrous. *Neorautanenia brachypus* tubers are used for cattle feeding, dosing animals, curing wounds, and catching fish, according to an ethnobotanical study by (Murungweni *et al*., 2012). Its tubers were also reported to have anthelmintic properties during an *in vivo* trial against Strongyloides in cattle and goats (Murungweni *et al.*, 2012).

3.2 Materials and methods

Sample collection

Neorautanenia brachypus tubers were collected from Zanamwi farm in Chikombezi, Zimbabwe, south of Gonarezhou National Park; GPS coordinates 21°45'0" S and 31°19'0" E. For each tuber accession, there were five tubers (brown, cream, and white).

Sample preparation

The tubers were cleaned to remove soil and other debris and then the skins were peeled off. The tubers were then cut into smaller pieces with a maximum volume of 1000 cubic centimeters. Each tuber color category was divided into three samples: 1) dried in the laboratory at room temperature, 2) dried at 65 degrees Celsius in an oven for 12 hours, and 3) sun-dried for 4 days. The dried materials were then ground using a portable electric grinder into a powder. The ground powder samples were packed in inert plastic sample bags and stored at room temperature for analysis.

3.2.1 Extraction

A 10 gram dried powder of the tubers was extracted successively in 60 ml each of seven solvents listed below in a Soxhlet Apparatus for 6 hours. After 6 hours the extract was concentrated in rotatry evaporator at 40 degrees Celsius. A 10 gram powder of the tubers was macerated in 60 ml each of seven solvents listed below at room temperature for 72 hours. After 72 hours of maceration extracts were filtered using the Whatman filter paper No.1 and the resultant filtrate was concentrated in a rotary evaporator at 40 degrees Celsius. Extract yield was determined as the weight of extract after removing the solvent (g). Two hundred and fifty-two (252) samples were obtained after forming 126 treatment combinations replicated twice. After extraction, the extract weight was calculated and each sample was screened for phytochemicals.

Solvents were prepared as follows:

1) Methanol 100% (Meth) (10 L)
2) Methanol 50% (5 L) : Ethyl acetate 50% (Meth-EA) (5 L)
3) Methanol 50% (5 L) : Chloroform 50% (Chl-Meth) (5 L)
4) Ethyl acetate 100% (EA) (10 L)
5) Chloroform 100% (Chl) 10L
6) Chloroform 50 % 5L: Ethyl acetate 50% (Chl-EA) 5L
7) Distilled water 100% (DW) 5 L

3.2.2 Phytochemical screening

Screening of phytochemicals was done using standard methods described by Morgan *et al*. (2013) and Edeoga, Okwu, and Mbaebie (2005). The tests used were as in Table 1 below.

Table 3.1: Tests used to screen *N. brachypus* extracts for phytochemicals.

PHYTOCHEMICAL	METHODS OF SCREENING
Essential Oils	Emulsion test
Alkaloids	Wagner’s Test
Flavonoids	Lead acetate test
Glycosides	Borntrager’s Test,
Saponins	Foam Test
Tannins	Gelatin Test
Terpenoids	Concentrated sulphuric acid test
Phenols	Ferric chloride Test
Coumarin	Sodium Chloride test
Quinone	Sodium Chloride/Chloroform test

Data analysis

The data was tested for normality using the Shapiro-Wilk statistic in SPSS version 20. Four-way ANOVA (Analysis of Variance) by Tukey Studentized Range (HSD) was used for statistical evaluation (significance level 5%) in SAS version 9.4 in a 3×3×2×7 factorial arrangement. Rao-Scott Chi-square test was done to test the association between solvents used and the presence of certain phytochemicals in the extract.

3.2.3 Determination of Total Phenolic Content and Total Tannin Content

Extraction

Since, the white tubers that were air-dried were found to exhibit a significant extract yield they were selected for use in this experiement. Methanol was found to exhibit a significant extract yield and also yielded the greatest number of different phytochemicals after screening. 10 g of white tuber air-dried powder sample was extracted using methanol and distilled water in a Soxhlet extractor for 6 hours. The samples were concentrated using a rotary evaporator at 40 degrees Celsius and stored.

Determining Total Phenolic Content

Total phenols were measured according to the method by Shirazi, Khattak, Shukri, and Nasyriq (2014). A standard Gallic acid curve was created by diluting a standard 1 solution of Gallic acid (10 mg/mL) in methanol to obtain dilutions of (0.1, 0.5, 1.0, 2.5, and 5.0 mg/mL). Each of these dilutions was mixed with 0.5 mL distilled water, then added 0.1ml Folin–Ciocalteu reagent and let to stand for 6 minutes. After that, 1 mL sodium carbonate (7%) and 0.5 mL distilled water were added to the reaction mixture. After 90 minutes, the absorbance was spectrophotometrically determined at 760 nm. The methanol and distilled water tuber extracts were treated in the same way as that of the standard. All of the experiments were carried out in triplicates. From a calibration curve with Gallic acid, the total phenol content of plant parts was reported as milligrams of Gallic acid equivalents per gram of dry weight (mg GAE/g DW). The following formula was used to compute total phenolic content in mg GAE/g:

$C = c\ (V/m)$

Where **C** represents the total phenolic content in mg GAE/g dry extract, **c** represents the Gallic acid concentration in mg/g determined from the calibration curve, **V** represents the volume of extract in ml, and **m** represents the mass of extract in gram.

Determining Total tannin content

The Total Tannins Content was determined using the Folin-Ciocalteu method, which was developed by Makkar, Blümmel, Borowy, and Becker (1993) and Haile and Kang (2019). The dilutions of (0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL) in distilled water from the tannic acid stock (0.1 mg/mL) were used to create a standard tannic acid curve. Each dilution received 0.5ml of Folin-Ciocalteu reagent, 0.25 mL of Folin-Ciocalteu reagent, and 1.25 mL of 20 percent Sodium Carbonate solution. After vortexing the tubes for 40 minutes, the absorbance was measured at 725 nm. The methanol and distilled water tuber extracts were treated in the same way. All of the experiments were carried out in triplicates. The tannin content of the dried sample was measured in milligrams of tannic acid equivalents per gram of dried sample. The total tannin concentration was determined as mg TAE/g as follows:

$C = c \times (V/m)$

Where **C** is the total tannin content mg TAE/g dry extract, **c** is the concentration of Tannic acid obtained from the calibration curve in mg/g, **V** is the volume of extract in ml and **m** is the mass of extract in gram.

Data analysis

Data on absorbance was recorded on an excel sheet. Excel was used to formulate calibration curves; calculate the concentration of methanol and distilled water samples, and calculated the total phenol and tannins in the samples. Data were tested for normality using the Shapiro-Wilk statistic in SPSS version 20. SAS version 9.4 was used to compute the analysis of variance and separation of means (significance level 5%).

3.2.4 GC-MS analysis

Sample preparation

Since, the white tubers that were air-dried were found to exhibit a significant extract yield they were selected for use in this experiement. Methanol was found to exhibit a significant extract yield and also yielded the greatest number of different phytochemicals after screening. Samples extracted using Chloroform 50%: ethyl acetate 50% as a solvent had an acceptable amount extract yield during interaction analysis. In this reaserach 10 g of white tuber air-dried powder sample was extracted using methanol and chloroform 50%: ethyl acetate 50% in a Soxhlet extractor for 6 hours.

GC-MS procedure

GC-MS analysis of the extracts was performed using an Agilent Technologies 7890A GC system coupled with an Agilent Technologies 5975C VL MSD with a Triple-Axis Detector at the Scientific and Industrial Research and Development Centre (SIRDC) in Harare, Zimbabwe. The GC-MS conditions used to carry out the experiment are displayed in Figure 3. The GC instrument column was coated with poly-methyl silicon (30 mm x 250 µm x 0.25 µm). The injection volume was 1µL using a syringe size of 10µL. The initial column temperature was 50 ^{o}C then held for 2 minutes and increased afterward at 5 ^{o}C per minute up to 310 ^{o}C and held for 60 minutes. The auxiliary temperature was 280 ^{o}C at the GC-MS interface. The total run time for each sample was 60 minutes. The operating system software for the instrument was ChemStation. The sample spectrum was compared to the compound spectrum in the NIST 2011 edition with a 250,000 compounds library. The identification of bioactive chemical compounds was based on the peak area (%), RT (Retention Time), molecular weight, and molecular formula.

3.3 Results and discussion

3.3.1 Comparison of extraction efficiency on extract weight and number of phytochemicals

The 2-way interactions, 3-way interactions, and 4-way interactions had significant effects ($\boldsymbol{P}< 0.05$) on the extract weight recorded (Appendix 1). Main effects were not discussed because interactions were significant.

The results in Figure 3.1 showed that white tubers had the highest extract weight of 0.91 g (9.1 %), followed by brown tubers with 0.72 g (7.2 %) and cream tubers with 0.70 g (7.0 %), respectively. The genetic diversity within the *N. brachypus* plant community, as described by Nyarumbu et al. (2019), was most likely the cause of these variances. The data points to the occurrence of many *N. brachypus* biotypes, each with potentially distinct characteristics. Because white tubers are soft compared to brown tubers, which are more fibrous, they may have yielded larger extract yields (Nyarumbu *et al*., 2019). As a result, solvents easily penetrated the white tubers, extracting the bioactive chemicals, whereas bioactive compounds are covered by the fibrous tissues in brown and cream tubers.

Drying is a critical process that has a big impact on bioactive compound retention and degradation, as well as manufacturing costs in terms of drying time and energy use (Bernard *et al*., 2014). Oven-dried samples had the highest extract weight of 0.81 g (8.1 %), followed by air-dried samples of 0.77 g (7.7 %), and sun-dried tubers had the lowest extract weight of 0.75 g (7.5 %). The faster inactivation of enzymes that can lead to metabolic deterioration of materials could explain the increased extract yield of oven-dried samples (Lim & Murtijaya, 2007). Plant phenolics can also be attached to the cell membrane or cell wall or be free, and processing at high temperatures, such as in an oven-dried, can trigger the release of these chemicals due to matrix disintegration (Dewanto, Wu, & Liu, 2002; Jeong *et al*., 2004). Lower extract yields in air-dried and sun-dried samples could be due to slower processes and longer metabolic processes, resulting in quality degradation (Keinänen & Julkunen-Tiitto, 1996; Pirbalouti, Oraie, Pouriamehr, & Babadi, 2013). Because of chemical modifications that may have occurred as a result of interaction with ultra-violet radiation, sun-dried-dried samples had the lowest extract yield (Osinubi, Banjoko, Anselm, Akinrinola, & Osofodunrin, 2020). However, there was a paper that claimed that oven-dried drying caused certain phytochemicals to degrade (Mohd Zainol, Abdul-Hamid, Abu Bakar, & Pak Dek, 2009).

Several authors have reported on the impact of different extraction procedures on chemical composition and extract yield (Kaur, Gupta, Dey, & Pandey, 2019; Paz, Contreras, Munguía, Aguilar, & Inungaray, 2018; Pudziuvelyte *et al*., 2018). The extract yield of Soxhlet extracted and macerated samples were compared in this experiment. Soxhlet extracted samples had the highest extract weight of 0.89 g (8.9 %), whereas maceration samples had the lowest extract weight of 0.66 g (6.6 %). According to Bhokare, Khadke, Kuchekar, and Kulkarni (2018), a comparison of extraction techniques of different portions of the plant revealed that the Soxhlet extraction technique yields the highest percentage of yield with the highest presence of phytochemical elements. When compared to the maceration procedure, this was most likely due to the high temperatures utilized during the extraction process, which caused the cell wall and cell membrane to break down, releasing the phytoconstituents. These findings contradict a publication that suggests that in Soxhlet extraction high temperatures and a long extraction period increase the risk of thermal degradation of bioactive chemicals (Li *et al*., 2008).

The influence of the drying procedure, extraction process, and solvent on extract weight across different tuber accessions was statistically significant. White air-dried tubers Soxhlet extracted with Chl-EA 1.91 g (19.1 %), white air-dried macerated with Chl-EA 1.85 g (18.5 %), white oven-dried Soxhlet extracted with Meth, white sun-dried macerated with Chl 1.60 g (16 %), and white oven-dried Soxhlet extracted with Chl-EA 1.58 g (15 %) were the top five samples with the highest yield. Cream air-dried macerated with DW 0.13 g (1.3 %), brown air-dried macerated with Chl-Meth 0.18 g (1.8 %), cream oven-dried Soxhlet extracted with Chl-Meth 0.24 g (2.4 %), white oven-dried macerated with Chl-EA 0.26 g (2.6 %), and brown oven-dried macerated with Chl-Meth 0.29 g (2.9 %) were the five samples with the lowest extract yields (2.9 %).

Even in interactions with other parameters, white tubers had the highest extract weights. In interactions with other parameters, the cream and brown tuber varieties demonstrated the lowest extract weights. Because white tubers are soft compared to brown tubers, which are more fibrous, they may have yielded larger extract yields (Nyarumbu *et al*., 2019). As a result, solvents easily penetrated the cells of white tubers, extracting the bioactive chemicals, whereas bioactive compounds are covered by the fibrous substance in brown and cream tubers.

When comparing the effects of tuber type, the extraction process, and solvent on extract weight in various drying methods, in this interaction, air-dried samples yielded the most significant extract yields, followed by oven-dried and sun-dried samples. The extract yields of sun-dried samples were not among the bottom five samples with the least significant extract weights. This probably because the lower temperatures in the room protected the bioactive ingredients from degradation, resulting in increased extract yields in the air-dried samples (Andrean, Prasetyo, Kristijarti, & Hudaya, 2014; Bernard *et al*., 2014; Keinänen *et al*., 1996). The method of air-drying was found to have the highest phytochemical content (Adebayo, Olasehinde, Lajide, & Oloruntoba, 2019). When air-dried materials were extracted using maceration, they too had low extract yields. When compared to Soxhlet extraction, the maceration approach yielded lower extract yields. Oven-dried samples had low significant extract weights, which was likely due to their interaction with brown and cream accessions and maceration procedure, which had low extract weights in the results of the main effect. It has been claimed that thermal processing in the oven-dried and sun-dried drying break the cell structure, causing bioactive components to migrate and be lost (Bernard *et al*., 2014).

When comparing the effects of different tuber varieties, drying techniques, and solvents on extract weight across various extraction procedures. In comparison to macerated samples, Soxhlet extracted samples showed the highest extract yields of 0.89g in combination with other parameters. Macerated samples had the lowest extract weights of 0.66g when compared to Soxhlet extracted samples when other parameters were taken into account. This was most likely because heating improved the extractability of phytoconstituents by rupturing the cell wall and membrane during Soxhlet extraction. In comparison to maceration, polyphenols bound to the wall and membrane-bound could have been freed more easily by this method (Dewanto *et al*., 2002; Jeong *et al.*, 2004).

When comparing the effects of different tuber varieties, drying processes, and extraction procedures on extract weight in different solvents. Amongst the top five highest extract yields, Chl-EA extracted samples had the highest extract (1.01 g) yields followed by Meth and lastly Chl. The least extract yields were recorded for DW extracted sample followed by Chl-EA and Chl-Meth respectively in interaction with other parameters. The heterogeneity of the phytoconstituents in *N. brachypus* in terms of polarity was shown by the changes in extract yield across the different solvents utilized for extraction. This concept was explained by Nawaz and colleagues 2019. The varied solvents caused differences in phytochemical concentrations in extracts (Oliveira *et al*., 2017). The bulk of phytochemicals in *N. brachypus* are mid-polar to non-polar, which could explain the high extract weight for Chl-EA. Methanol (12.23%) samples had the highest extract yield, followed by aqueous (9.27%), chloroform (4.1%), hexane (2.8%), and ethyl acetate (1.8%), according to Thooyavan and Karthikeyan (2016).

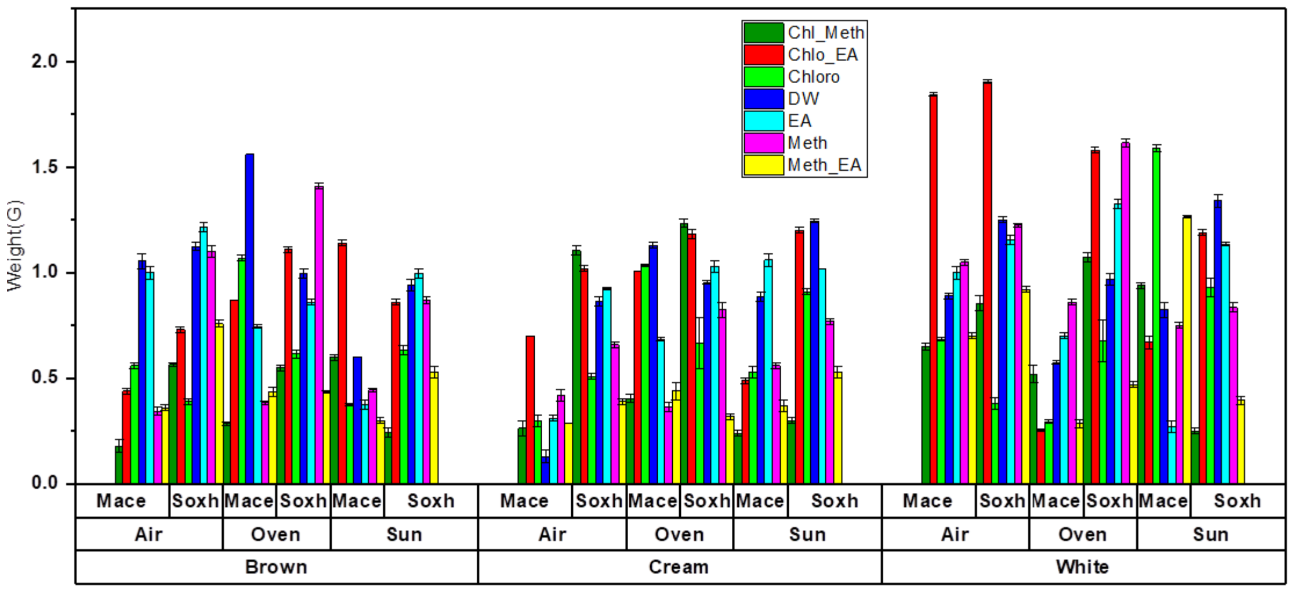

Figure 3.1: 4-way interaction of tuber color (brown, cream, and white), drying method (air, sun, and oven), extraction method (maceration and Soxhlet), and solvent (Chl-Meth, Chl-EA, Chl, DW, EA, Meth, and Meth-EA) on extract weight (g). Mace, maceration; Soxh, Soxhlet

The quantity, quality, extraction velocity, inhibitory substances, toxicity, other biological activities, and biosafety of extracts are all affected by the solvent used for extraction (Do *et al*., 2014; Rafińska *et al*., 2019; Zhang *et al*., 2019). Throughout the experiment, each solvent was used 36 times. The solvent means for extract weight are statistically different, as shown in the Table 3.2 below. Results showed that extract weight was in the following order for the different solvents: Chl-EA 1.01 g (10.1%), DW 0.96 g (9.6%), EA 0.88 g (8.8%), Meth 0.81 g (8.1%), Chl 0.68 g (6.8%), Chl-Meth 0.57 g (5.7%), and Meth-EA 0.51 g (5.1%). The heterogeneity of the phytoconstituents in *N. brachypus* in terms of polarity was shown by the changes in extract yield across the different solvents utilized for extraction (Nawaz, Aslam, & Muntaha, 2019). The varied solvents caused differences in phytochemical concentrations in extracts (Oliveira *et al*., 2017). The bulk of phytochemicals in *N. brachypus* are mid-polar to non-polar, which could explain the high extract weight for Chl-EA.

Table 3.2: Results for the influence of each solvent on extract weight across all accessions, drying methods, and extraction methods used.

Solvent	**Mean (extract weight grams)**
Meth	0.81^{d}
Meth-EA	0.51^{g}
Chl-Meth	0.57^{f}
DW	0.96^{b}
EA	0.88^{c}
Chl-EA	1.01^{a}
Chl	0.68^{e}

[1]Data with the same superscripts in the same column were statistically similar (***P***<0.05)

Phytochemical screening revealed the presence of essential oils, terpenoids, quinones, saponins, coumarins, phenols, flavonoids, alkaloids, and tannins, as shown in Table 3.3. Glycosides, flavonoids, alkaloids, saponins, coumarins, terpenoids, steroids, and tannins were found in tuber extracts (Ishnava & Konar, 2020; Nikhila *et al*., 2016; Saravanakumar, 2014), and essential oils (De, Dey, & Ghosh, 2010). Flavonoids, pterocarpans, and coumarins are abundant in the tubers of *Neorautanenia mitis* (Joseph, Ndoile, Malima, & Nkunya, 2004; Sakurai *et al*., 2006).

Table 3.3: Results for phytochemical screening of *N. brachypus* extracts. The plus sign (+) shows positive results for the phytochemical screened.

Phytochemical	**Method of screening**	**Screening results**
Essential Oils	Emulsion test	+
Alkaloids	Wagner's Test	+
Flavonoids	Lead acetate test	+
Glycosides	Borntrager's Test,	+
Saponins	Foam Test	+
Tannins	Gelatin Test	+
Terpenoids	Concentrated sulphuric acid test	+
Phenols	Ferric chloride Test	+
Coumarin	Sodium Chloride test	+
Quinone	Chloroform test	+

The Rao-Scott Chi-square test was used to examine the data for phytochemical counts against each solvent to see if there was a link between the solvent employed and the presence of phytochemicals in the extract. Because the F-value was greater than the 0.05 significance level, the solvents were not significantly linked with the presence of phytochemicals in the extracts for quinones only, as shown in Table 4.3.

Table 3.4: Rao-Scott (F-based) test statistics and F-Values, for the association of solvents and presence of phytochemicals in the extracts.

Phytochemical	**F-value**	**Pr > F**
Essential oils	4.3077	0.0003
Terpenoids	3.6733	0.0012
Quinones	1.5534	0.1572
Saponin	4.3077	0.0003
Glycosides	7.4870	<0.0001
Coumarins	2.2312	0.0379
Phenols	23.8519	<0.0001
Flavonoids	7.2160	<0.0001
Alkaloids	11.5554	<0.0001
Tannins	2.2312	0.0379

The data for extract weight and phytochemical count for each solvent were combined in Table 3.5 below. The three highest counts for each phytochemical type across the different solvents were highlighted using blue color, tallied the number of highlighted spots for each solvent, and recorded the total in the table. The results revealed that Meth had the most highlighted points (8), followed by Meth-EA, Chl-Meth, DW, EA, Chl-EA, and Chl. The results indicated that phytochemical counts were consistently high when methanol was employed, whereas phytochemical counts were lowest when chloroform was used. When it came to the extract weight, however, the trend was reversed, with EA, DW, and Chl-EA having the highest extract weights compared to the others, highlighted in yellow color. A second Table 3.6, containing the top three solvents with the greatest phytochemical counts, was created.

Table 3.5: Phytochemical counts and mean extract weight for each solvent used during extraction in this experiment.

Solvent	Extract Weight (g)	Essential oils	Saponin	Terpenoids	Quinones	Glycosides	Tannins	Coumarins	Phenols	Flavonoids	Alkaloids	Total
Meth	**0.81**	**36**	**22**	**34**	**20**	**14**	**32**	**34**	**32**	**20**	**24**	**8**
Meth-EA	**0.51**	**36**	**22**	**34**	**24**	**24**	**28**	**28**	**30**	**26**	**26**	**6**
Chl-Meth	**0.57**	**34**	**10**	**30**	**24**	**22**	**34**	**26**	**32**	**18**	**30**	**6**
DW	**0.96**	**36**	**12**	**32**	**22**	**6**	**34**	**32**	**34**	**8**	**6**	**5**
EA	**0.88**	**36**	**8**	**32**	**20**	**12**	**2**	**34**	**10**	**18**	**34**	**5**
Chl-EA	**1.01**	**36**	**14**	**30**	**26**	**12**	**6**	**28**	**4**	**16**	**26**	**3**
Chl	**0.68**	**32**	**8**	**22**	**30**	**2**	**0**	**32**	**2**	**2**	**32**	**3**

Table 3.6 was used to determine the most effective solvent to employ in the extraction of *N. brachypus* for optimum extract weight and phytochemical counts between Meth, Meth-EA, and Chl-Meth. In terms of phytochemical counts, the table showed that Meth-EA had the most highlighted points in blue, followed by Meth and Chl-Meth respectively. Meth had the highest mean extract weight, followed by Chl-Meth, and finally Meth-EA in terms of extract weight. Overall, Meth was the most effective solvent for extraction because it exhibited a high mean extract weight and phytochemical count. Under Meth, the counts for quinones and tannins were close to the highest ever recorded for the phytochemicals. The counts of glycosides, flavonoids, and alkaloids, on the other hand, significantly differed from the highest recorded count.

The results were similar to those reported by Thooyavan *et al*. (2016), where methanol extracts contained the greatest quantity of phytoconstituents. This had already been reported by Sheikh, Kumar, Misra, and Pfoze (2013) before. The reason why methanol was the most effective solvent could be because the phytoconstituents in *N. brachypus* extracts were predominantly polar. Methanol had the highest extraction yield of the solvents evaluated for *S. buxifolia* extraction, followed by distilled water, ethanol, acetone, chloroform, and dichloromethane (Truong *et al*., 2019). Methanol, acetone, and ethanol have also been reported to be the most effective solvents for extracting flavonoids, tannin, steroids, diterpenes, terpenoids, coumarin, cardiac glycoside, saponins, and reducing sugars, with positive results for tests such as flavonoids, tannin, steroid, diterpenes, terpenoids, coumarin, cardiac glycoside, saponins, and reducing sugars (Labar, Sarkar, Sen, & Bhattacharya, 2019).

Table 3.6: Phytochemical counts and mean extract weight for solvents Meth, Meth-EA, and Chl-Meth used during extraction in this experiment.

Solvent	Extract Weight (g)	Essential oils	Saponin	Terpenoids	Quinones	Glycosides	Tannins	Coumarins	Phenols	Flavonoids	Alkaloids	Total
Meth	**0.81**	**36**	**22**	**34**	**20**	**14**	**32**	**34**	**32**	**20**	**24**	**5**
Meth-EA	**0.51**	**36**	**22**	**34**	**24**	**24**	**28**	**28**	**30**	**26**	**26**	**6**
Chl-Meth	**0.57**	**34**	**10**	**30**	**24**	**22**	**34**	**26**	**32**	**18**	**30**	**4**

3.3.2 The Total Phenol Content and Total Tannin Content of *N. brachypus* extracts

Data was analyzed for normality and found to be normally distributed at ***P***>0.05 using the Shapiro Wilk test in SPSS version 20.

The graphs in Figure 3.2 and Figure 3.3 were used to compute the total phenolic content and total tannin content, respectively. The Total Phenol Content was calculated using the standard curve equation y=0.122x+0.554, with R2 of 0.7883, while the Total Tannin Content was calculated using the standard curve equation y=5.7767x-0.003, with R2 of 0.9763. The generation of molybdenum-tungsten blue, which was evaluated spectrophotometrically, rose linearly with the concentration of phenolics and tannins in the reaction mediums, as shown in Figure 3.3 and Figure 3.4.

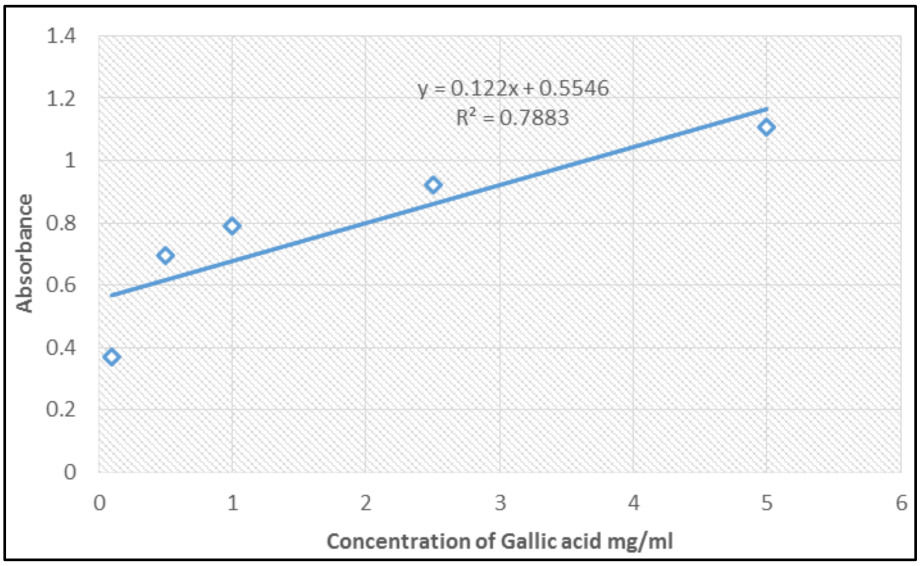

Figure 3.2: Standard calibration curve of Gallic acid and equation used to determine the Gallic acid concentration of methanol and distilled water *N. brachypus* extracts.

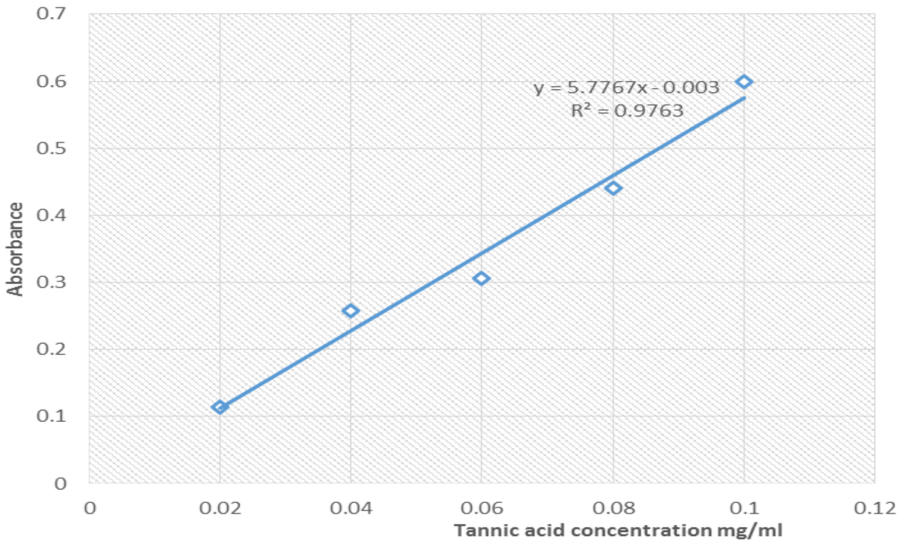

Figure 3.3: Standard calibration curve of Tannic acid and equation used to determine the Tannic acid concentration of methanol and distilled water *N. brachypus* extracts.

The Total Phenol and Total Tannin Content results are presented in Table 3.7 below. In terms of GAE, the total phenolic content of methanol and distilled water extracts was determined to be 365.18mg GAE/g and 89.43mg GAE/g, respectively. The Total Tannin Content of methanol and distilled water extracts was determined to be 2.33 mg TAE/g and 1.42 mg TAE/g, respectively. Methanol extracts had the highest Total Phenol and Tannin content compared to distilled water extracts.

Due to the presence of a hydroxyl group, phenolic compounds are more soluble in polar organic solvents, therefore methanol is a suitable solvent for extraction (Aryal *et al*., 2019; Karim *et al*., 2020; Mahomoodally *et al*., 2021). Even though distilled water is a polar solvent it exhibited a reduced phenolic concentration extraction power. This was probably because when extracting *N. brachypus* tubers, the samples that were extracted using distilled water had a reduced extract yield when in interaction with tuber color, extraction method and drying method. Sur, Hazra, Hazra, and Bhattacharyya (2016) found that the phenolic content of *B. gymnorrhiza* leaves was low (2.34 ± 0.039 µg GAE/mg) when compared to methanol (30.07mg GAE/g) (Nurjanah, Jacoeb, Hidayat, Hazar, & Nugraha, 2016; Sur *et al*., 2016). The total phenolic content of *Helianthus tuberosus* L. tubers was reported to range from 7.06 ± 0.27 to 20.43 ± 0.69 mg GAE/g (Sreekanth & Devi, 2019).

Table 3.7: Comparison of Total Phenolic Content (mg GAE/g) and Total Tannins Content (mg TAE/g) between methanol and distilled water *N. brachypus* extracts.

Tube	**mg GAE/g**	**mg TAE/g**
Methanol	289.43[a]	2.33[a]
Distilled water	89.43[b]	1.42[b]

[1]Data with the different superscripts in the same column were statistically different (***P***<0.05)

3.3.3 Phytochemical constituent of *N. brachypus* and its therapeutic properties

The chemical constituents' analysis results for Meth and Chl-EA extracts from *N. brachypus* tuber were recorded in Table 3.8 and Table 3.9 and their GC-MS chromatograms are presented in Figure 3.4 and Figure 3.5. The GC-MS analysis of methanol and Chloroform 50%: Ethyl acetate 50% extracts of *N. brachypus* confirmed the presence of 38 and 80 compounds respectively. The identification of bioactive chemical compounds was based on the peak area (%), retention time, molecular weight, and molecular formula. The time from when the injection was made (initial time) to when elution occurred is referred to as the retention time (RT).

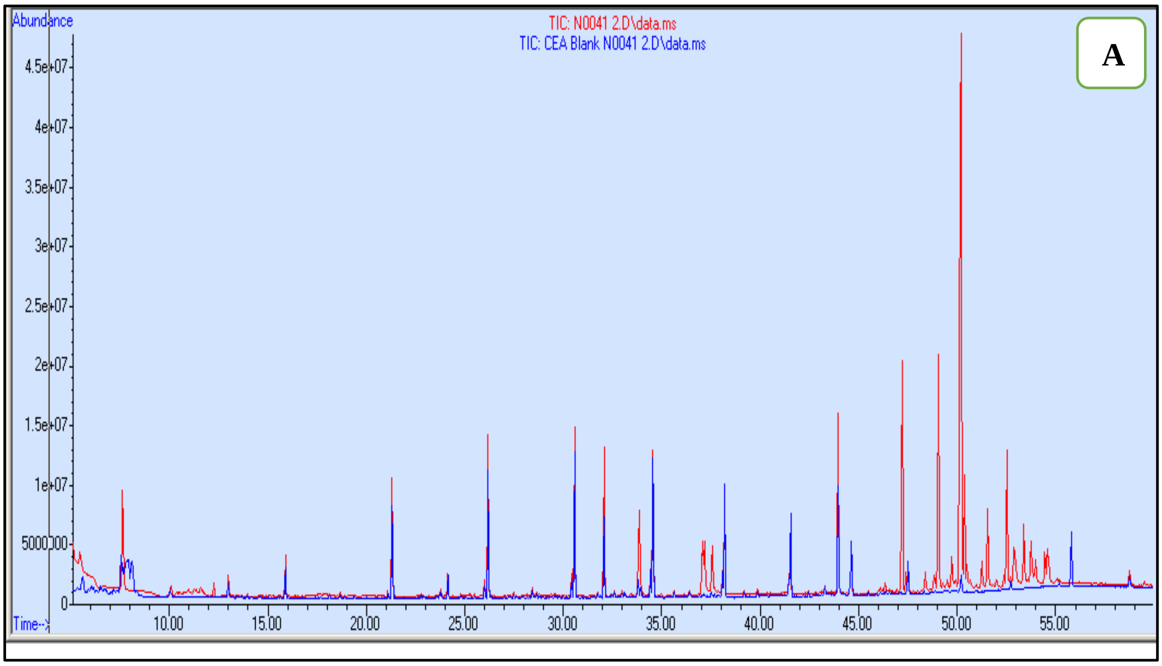

Figure 3.4: Spectrum for GCMS analysis of *N. brachypus* extracted using Chloroform 50%: Ethyl acetate 50%. The blue line indicated the blank used and red line was for the *N. brachypus* sample.

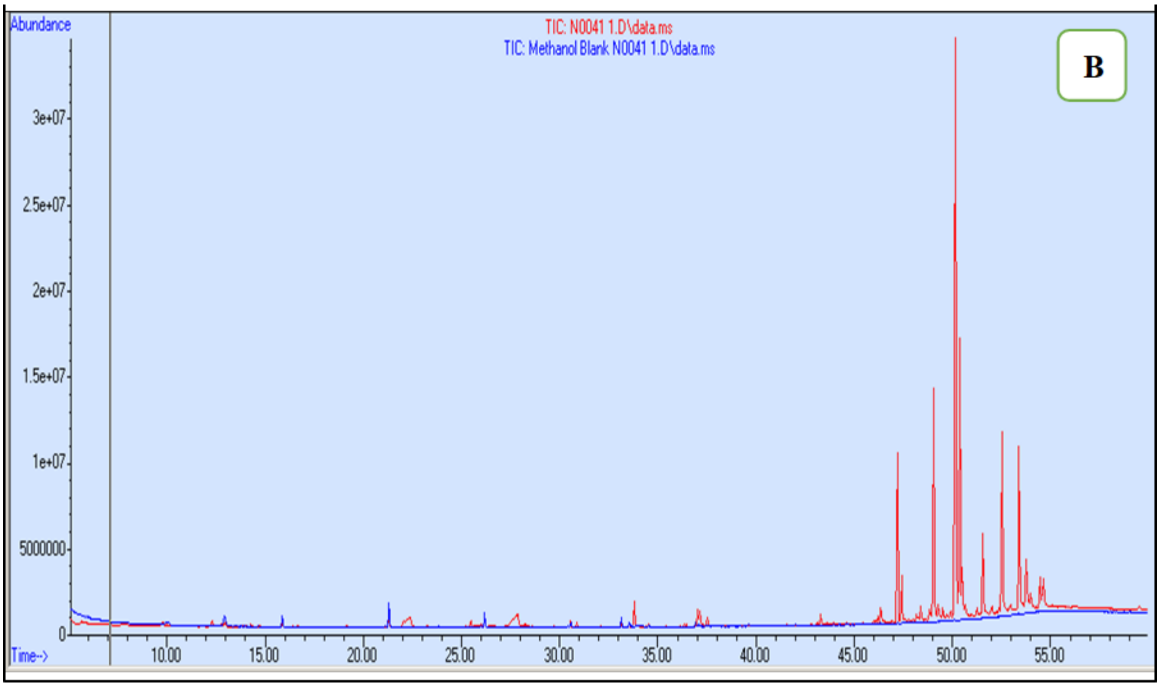

Figure 3.5: Spectrum for GCMS analysis of *N. brachypus* extracted using Methanol. The blue line indicated the blank used and red line was for the *N. brachypus* sample.

Each of the peaks in the chromatograms represented the signal created when a compound eluted from the GC column into the detector. The X-axis showed the RT and the Y-axis measured the intensity of the signal to quantify the component in the sample injected. Chromatogram A for methanol extract showed that the 1st compound identified RT (12.313 minutes) was 1-Ethylamino-1-propylclohexane whereas; Rotenone was the last compound to be identified RT (54.650 minutes). Chromatogram B for chloroform 50%: Ethyl acetate 50% showed that the 1st compound identified was Trichloromethane (5.470 minutes) whereas 1,4-Phthalazinedine, 2,3-dihydro-6-nitro was the last compound to be identified (58.753 minutes). The compounds with the longest peak in Chl-EA extract were 3-(1-methyl-1-silacyclobutyl) benzoic acid and 2-Benzothiazolamine,5,6-dimethyl (20.08%) at RT 50.188 minutes. The compound with the longest peak in methanol extract was Methyleugenol (28.73%) at RT 50.164 minutes.

The chemical constituents in methanol and Chl-EA were classified as esters, alkanes, alkenes, fatty acids, phenol, ketones, silicon-containing, sulfur-containing, coumarin, alkaloids, fluorine-containing, benzothiazoles, flavonoids, terpenoids, carbohydrates, benzimidazoles, cyclic carbonate, and aromatic piperidine hydrocarbons. This has been supported by literature where Abubakar and Majinda (2016), reported that the non-polar extracts are mainly composed of essential oils and their oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols, and oxides).

Out of the 38 compounds in methanol extract, 9 compounds had the highest abundance in the. These were Methyleugenol (28.73%), Benzene,1,1-(1,2-propadienylidene)bis (11.28%), Acetamide, N-(3,4,5-trimethoxyphenethyl) (9.13%), 11H-Cyclopenta(a)phenanthrene-15-carboxylic acid,12,13,16,17-tetrahydro-3-methoxy-13-methyl-17-oxo-methyl ester (7.72%), Indole,6-methyl-2-(4-pyridyl) (6.85), Pyridine,3-(3-nitro-5-phenoxyphenoxy) (6.78%), Coumarine,6-(7-hydroxycoumarin-8-yl)-7-methoxy (3.46%), Rotenone (3.16%), and Methyl (methyl,4-0-methyl-alpha-d-mannopyranoside)urinate (2.49%). The minor compounds represented with a peak area less than 0.25% were Octadecane (0.18%), Tris (tert-butylidimethylsilyloxy) arsane (0.18%), and Benzofuran,2-3-dihydro,2,2,5,6-tetramethyl (0.23%).

The most abundant 16 compounds in the Chl-EA extract of *N. brachypus* were 3-(1-methyl-1-silacyclobutyl)benzoic acid (20.08%), 2-benzothiazolamine,5,6-dimethyl (20.08%), Pyridine,3-(3-nitro-5-phenoxyphenoxy) (6.18%), 3-hydroxy-4-methoxycinnamic acid (5.82%), Bis(2-ethylhexyl)phthalate (3.95%), 2.4-Diamino-5-chloro-6-[(O-chlorophenyl)thio]quinazoline (3.45%), Octadecane (3.27%), n-Hexadecanoic acid (3.14%), Hexadecane (3.07%), 1,2-benzenedicarboxylic acid,bis(2-methylpropyl)ester (3.06%), 2-Ethoxyethyl acetate (2.93%), Eicosane (2.78%), Benzene,1,1-(1,2-propadienyldene)bis (2.67%), Tetradecane (2.25%), and Rhodium (.eta.5-2,4-cyclopentadien-1-yl)[(3,4-.eta.)-4,5-diethyl-1,2,2,3-tetramethyl-1-aza-2-sila-5-boracyclopent-3-ene-B5,N1] (2.21%). 19 minor compounds identified in the Chl-EA extract were Hexadecane (0.06%), Heptadecane,9-octyl (0.07%), Docosane (0.07%), 1,2-benzisothiol-3-amine tbdms (0.07%), 10-methylnonadecane (0.08%), Hexadecane,4-methyl (0.08%), Tridecane (0.09%), Octadecane (0.12%), Octadecane,4-methyl (0.12%), 2-Tetradecane,(E)(0.13%), Heneicosane (0.13%), 1-Octadecane (0.14%), 1,2-Benzenedicarboxylic acid,8-methylnonyl ester (0.15%), Pentadecane (0.15%), Silane, diethyl(3,5-dimethylphenoxy)nonyloxy (0.16%), 1,2-benzenedicarboxylic acid, bis(2-methyl propyl) ester (0.16%), Cyclotrisiloxane, hexamethyl (0.19%), and 3,3 diisopropxy,1,1,1,5,5,5-hexamethyl trisiloxane (0.19%) respectively.

11 compounds were found in both Methanol and Chl-EA extracts of *N. brachypus* tubers. These were Rotenone, Tetradecane, Hexadecane, Octadecane, n-Hexadecanoic acid, 9,12-

Octadecadienoic acid, Oleic acid, Acridin-9-yl-(2,4-difluoro-phenyl)-amine, Pyridine, 3-(3-nitro-5-phenoxyphenoxy), Benzene,1,1-(1.2-propadienylidene)bis, and Indole,6-methyl-2-(3-pyridyl).

Compounds identified in this study show that there are similarities with earlier studies on the chromatographic analysis of tubers, methanol, chloroform, and ethyl acetate extracts. Chloroform plant extracts have shown the presence of n-Hexadecanoic acid, Bis (2-ethylhexyl) phthalate, N-methyl-1-adamantaneacetamide, 2-Tetradecene, (E), 2,4-bis (1,1-dimethylethyl)phenol, Cetene, Heptadecane, 9,12-Octadecadienoic acid (Z,Z), Octadecanoic acid, Tetracosane, Octacosane, E-15-heptadecenal, Hexadecane, Oleic acid, and 1-docosene (Adeniran, Olowokudejo, & Kadiri, 2021; Ramadas & Chandralega, 2020; Yogeswari, Ramalakshmi, Neelavathy, & Muthumary, 2012). Compounds found in Ethyl acetate plant extracts were 2,4-bis (1,1dimethylethyl)phenol, Docosane, Oleic acid, Eicosane, Cetene, Dodecane, Heneicosane, n-Hexadecanoic acid, 1-docosene, E-15 heptadecenal, and Cyclotrisiloxane hexamethyl (Elsayed, Galil, Sedik, Hassan, & Sadik, 2020; Lata, 2015; Priyanka, Kumar, Bankar, & Karthik, 2015; Yogeswari *et al*., 2012). The compounds identified in tuber extracts were n-Hexadecanoic acid, 9,12-octadecadienoic acid (Z,Z), octadecanoic acid (Ishnava & Konar, 2020; Krishnamoorthy & Subramaniam, 2014).

The major compounds identified in the methanolic tuber extract of *Solena amplexicaulis* were 17-octadecadienal (Z)- (21.77%), n-hexadecanoic acid (21.75%) phthalic acid, di(2-propylpentyl) ester (9.48%), and 9,12-octadecadienoic acid (Z,Z)- (9.35%) (Krishnamoorthy et al., 2014). The analysis of gadung tuber *Dioscorea hispida* dennst revealed the presence of 7-Azabicyclo [4.1.0] heptane, 1-methgyl- (23.16%), n-Hexadecanoic acid (18.85%), 10E, 12(Z)- Conjugated linoleic acid (13.73%), 1, 4, 7, 10, 13, 16 - hexaoxacyclooctadecane (4.34%), and 5- Hydroxymethylfurfural (4.07%) (Suryowati, Sirait, Siagian, & Nursyam, 2020). GC-MS analysis of methanol/chloroform extract of *C. esculenta* tubers indicated the presence of hexadecanoic acid methyl ester (0.43%), octadecanoic acid (20.91%), 9,12-octadecadienoyl chloride (0.77%), 11-octadecenoic acid methyl ester (2.12%), 9-octadecenoic acid (64.37%),3-hexadecyloxycarbonyl-5-(2-hydroxylethyl)-4-methylimidazolium (1.36%), hexanedoic acid, bis(2-ethylhexyl)ester (1.36%) and 3,5-di-*t*-butyl phenol (3.27%) (Eleazu, 2016).

Chemical constituents of *N. brachypus* tubers have been reported to be involved in several pharmaceutical and biological activities as indicated in Table 3.7 and Table 3.8. Such as antifungal, antibacterial, anthelmintic, antiviral, anti-tumor, antioxidant, anti-inflammatory, anticancer, insecticidal, pesticide, anti-tuberculosis, analgesic, antipyretic, anti-androgenic, allelopathic, anti-asthmatic, hemolytic, larvicidal, hepatoprotective, anti-arthritic, anti-malarial, anti-obesity, hypercholesterolemic, anticonvulsive, antidepressant, antiparasitic, antidiabetic, antiaging, antileukemia, immunomodulatory, cardioprotective, carcinogenic, mutagenic, anesthetic, anti-allergic, and anticoagulant (Kumar, Kumaravel, & Lalitha, 2010; Manoj et al., 2012; Olubunmi, Gabriel, Stephen, & Scott, 2009). This affirms the therapeutic applications of *N. brachypus* tubers. Tuber plants and plants belonging to the Fabaceae family have been reported to exhibit the above-mentioned pharmaceutical and biological activities (Ishnava & Konar, 2020; Thooyavan *et al*., 2016).

Table 3.8: GC-MS results for Chloroform and Ethyl acetate *N. brachypus* extracts.

Retention Time	**Area %**	**Chemical Formula**	**Molecular Weight g/mol**	**Classification**	**Compound name**	**Biological Activity**
5.470	0.85	$CHCI_3$	119.38	chloromethane	Trichloromethane	Anesthetic (Heckel *et al*., 2019)
				ester	2-Ethoxyethyl acetate	Insecticide (Guest, Hamilton, Deisinger, & DiVincenzo, 1984) teratogenic, spermatotoxic, or hematotoxic effects (Söhnlein, Letzel, Weltle, Rüdiger, & Angerer, 1993)
10.087	0.38	$C_{10}H_{22}$	142.29	Alkane hydrocarbon	Decane	Not indicated
10.399, 10.961, 11.260, 11.607	0.12, 0.17, 0.16, 0.13	NH_3	17.03	Inorganic	Ammonia	pesticide
12.265	0.38	C_2CI_6	236.7	Halogenated hydrocarbon, organochlorine	Ethane, hexachloro	Fungicidal, insecticidal, anthelmintic (Snedecor, 1999)
13.006	0.49	$C_{11}H_{24}$	156.31	Alkane hydrocarbon	Undecane	prevention or treatment of skin inflammatory disorders, such as atopic dermatitis, and other

						allergic diseases (Choi, Kang, & Park, 2020)
15.890	0.79	$C_{12}H_{26}$	170.33	Alkane hydrocarbon	Dodecane	Antioxidant (Begum, Mohankumar, Jeevan, & Ramani, 2016), antimicrobial (Usha, Sangareshwari, & Kumari, 2015)
18.665	0.09	$C_{13}H_{28}$	184.37	Alkane	Tridecane	Antimicrobial, anti-inflammatory (Ashour *et al*., 2009)
21.105	0.13	$C_{14}H_{28}$	196.37	Alkene	2-Tetradecene (E)	Antimicrobial, anticancer, antioxidant (Manoj et al., 2012; Tiloke, Anand, Gengan, & Chuturgoon, 2018)
21.309	2.25	$C_{14}H_{30}$	196.39	Alkane	Tetradecane	Antifungal, antibacterial, nematicide (Arora & Kumar, 2018), diuretic, anti-tuberculosis (Girija, Duraipandiyan, Kuppusamy, Gajendran, & Rajagopal, 2014)
23.725	0.08	$C_{20}H_{42}$	282.5	Alkane	10-Methylnonadecane	Not indicated
23.797	0.15	$C_{15}H_{32}$	212.41	Alkane	Pentadecane	anti-inflammatory, analgesic, and antipyretic (Patrick, 2020), antimicrobial
24.411	0.48	$C_{17}H_{30}OSi$	278.5	Organosilico phenolic	Phenol,2,4-bis(1,1-dimethylethyl)	Antibacterial (Ajayi, Olagunju, Ademuyiwa, & Martins, 2011), antioxidant (Abdullah, Mirghani, & Jamal, 2011), anticancer, antifungal (Malek, Shin, Wahab, & Yaacob, 2009; Zhou *et al*., 2011)

25.998	0.30	$C_{16}H_{32}$	224.43	Alkene	Cetene	Antimicrobial and Antioxidant (Mou *et al*., 2013)
26.178	3.04	$C_{16}H_{34}$	226.44	Alkane	Hexadecane	antibacterial, antioxidant (Kumar, Bhatnagar, & Srivastava, 2011)
27.482	0.08	$C_{17}H_{36}$	240.5	Alkane	Hexadecane,4-methyl	
28.415	0.19	$C_{17}H_{36}$	240.5	Alkane	Heptadecane	Antimicrobial (Rahbar, Shafaghat, & Salimi, 2012)
28.678	0.06	$C_{16}H_{34}$	226.44	Alkane	Hexadecane	antibacterial, antioxidant (Kumar, Bhatnagar, *et al*., 2011)
30.425	0.47	$C_{18}H_{36}$	252.5	Alkene	1-Octadecene	Anticancer, antioxidant, antimicrobial (Lee, Kang, Cho, & Jeong, 2007; Mishra & Sree, 2007)
30.568	3.27	$C_{18}H_{38}$	254.49	Alkane	Octadecane	Antimicrobial (Girija *et al*., 2014), antifungal (Usha et al., 2015)
31.753	0.12	$C_{19}H_{40}$	268.52	Alkane	Octadecane,4-methyl	
32.064	3.06	$C_{16}H_{22}O_4$	278.35	Phthalic acid ester	1 2-benzenedicarboxylic acid bis (2-methylpropyl) ester	anti-androgenic (Borch, Axelstad, Vinggaard, & Dalgaard, 2006), allelopathic, antimicrobial, insecticidal (Huang *et al*., 2021)
32.602	0.17	$C_{19}H_{40}$	268.52	Alkane	Nonadecane	Antioxidant, antimicrobial (Usha *et al*., 2015)
32.985	0.16	$C_{16}H_{22}O_4$	278.35	Phthalic acid	1 2-benzenedicarboxylic	anti-androgenic (Borch *et al*., 2006),

				ester	acid bis(2-methylpropyl) ester	allelopathic, antimicrobial, insecticidal (Huang *et al*., 2021)
33.116	0.14	$C_{21}H_{44}$	296.57	Alkane	Heneicosane	Antiasthmatics urine acidifiers Antimicrobial (Begum *et al.*, 2016)
33.116	0.14	$C_{31}H_{64}$	436.8	Alkane	Hentriacontane	Antitubercular agent, antitumor and antimicrobial (Olubunmi, Gabriel, Stephen, & Scott, 2009), Anti-inflammatory (Kim, Chung, Kim, Ko, & Um, 2011)
33.870	3.14	$C_{16}H_{32}O_2$	256.42	Fatty acid	n-Hexadecanoic acid	Antimicrobial, antioxidant (Abdullah, Mehdi, Khan, & Pathan, 2020) Anti-inflammatory (Aparna *et al*., 2012), Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor (Kumar, Kumaravel, & Lalitha, 2010), potent mosquito larvicidal
34.432	0.59	$C_{17}H_{32}O$	252.43	Alkene	E-15-Heptadecenal	Antioxidant and antibacterial activity (Yogeswari *et al*., 2012)
34.552	2.78	$C_{20}H_{42}$	282.54	Alkane	Eicosane	Antimicrobial, anticancer, antioxidant (Dehpour, Babakhani, Khazaei, & Asadi, 2011), inflammatory, analgesic, and antipyretic (Patrick, 2020), antifungal (Usha

						et al., 2015)
35.629	0.15	$C_{18}H_{38}$	254.49	Alkane	Octadecane	Antimicrobial (Girija *et al*., 2014), antifungal (Usha *et al*., 2015)
36.406	0.13	$C_{21}H_{44}$	296.57	Alkane	Heneicosane	Antiasthmatics urine acidifiers Antimicrobial (Begum *et al*., 2016)
37.076	1.66	$C_{18}H_{32}O_2$	280.45	Fatty acid	9,12-octadecadienoic acid (Z,Z)	anti-inflammatory (Das, 2006), antibacterial (Zheng *et al*., 2005), anticancer, antiandrogenic, dermatitigenic, irritant, antileukoliene (Krishnamoorthy *et al*., 2014)
37.172	1.95	$C_{18}H_{34}O_2$	282.46	Fatty acid	Oleic acid	Antibacterial (Awa, Ibrahim, & Ameh, 2012), anti-androgenic, anti-cancer, antimicrobial (Novak *et al*., 1961), hypercholesterolemic, dermatitigenic, anti-inflammatory, and anti-tumor activity (Gideon, 2015)
37.567	1.66	$C_{18}H_{34}O_2$	282.46	Fatty acid	Octadecanoic acid	Antimicrobial, anti-inflammatory, hepatoprotective, nematicide (George, Radha, & Somasekariah, 2018), hypercholesterolemic, antiarthritic (Hussein, Hameed, & Hadi, 2017)
38.093	0.52	$C_{22}H_{44}$	308.58	Alkene	1-Dodecene	Antibacterial (Togashi *et al*., 2007), antifungal (Usha *et al*., 2015), Anti-

						inflammatory (Elsayed *et al*., 2020)
38.189	2.05	$C_{20}H_{42}$	282.54	Alkane fatty acid	Eicosane	Antimicrobial, anticancer, antioxidant (Dehpour *et al*., 2011), inflammatory, analgesic, antipyretic (Patrick, 2020), antifungal (Usha *et al*., 2015)
39.182	0.07	$C_{25}H_{52}$	352.64	Alkane	Heptadecane, 9-octyl	antifungal (Abubacker & Devi, 2014)
39.875	0.14	$C_{18}H_{36}$	252.48	Alkene	1-Octadecene	Anti-oxidant, lowering cholesterol, inhibiting lipid (Lakshmi & Nair, 2017)
41.455	0.43	$C_{24}H_{48}$	336.64	Alkane	Cyclotetracosane	anti-bacterial, antioxidant, and anticancer (Mongalo, Soyingbe, & Makhafola, 2019)
41.538	1.44	$C_{24}H_{50}$	338.65	Alkane	Tetracosane	Antioxidant and antimicrobial activity (Boussaada *et al*., 2008) cytotoxicity (Uddin, Grice, & Tiralongo, 2012), antimicrobial activity, anti-bacterial and anti-tumor activities, antiviral (Chathuranga *et al*., 2021)
42.459	0.07	$C_{22}H_{36}$	310.60	Alkane	Docosane	antimicrobial activity, antiviral (Chathuranga *et al*., 2021)
43.285	0.24	$C_{35}H_{68}O_5$	568.9	Fatty acid	1.6:3,4-Dianhydro-2-O-acetyl-.beta.-d-galactopyranose	Not indicated
43.955	3.94	$C_{24}H_{38}O_4$	391.56	Ester	Bis(2-	Antimicrobial (Usha *et al*., 2015),

					ethylhexyl)phthalate	fetotoxicity, hepatotoxicity, and testicular atrophy (Arcadi *et al*., 1998)
44.637	1.15	$C_{21}H_{44}$	296.57	Alkane	Heneicosane	Antibacterial (Usha *et al*., 2015)
44.637	1.15	$C_{20}H_{42}$	282.54	Alkane fatty acid	Eicosane	Antimicrobial, anticancer, antioxidant (Dehpour *et al*., 2011), inflammatory, analgesic, antipyretic (Patrick, 2020), antifungal (Usha *et al*., 2015)
46.096	0.12	$C_{18}H_{38}$	254.49	Alkane	Octadecane	Antimicrobial (Girija *et al*., 2014), antifungal (Usha *et al*., 2015)
46.347	0.36	$C_{18}H_{16}O_3$	280.3	Coumarin	Dibenz(a,h)anthracene,5, 6-dihydro	Not indicated
47.221	6.18	$C_{17}H_{12}N_2O_4$	308.29	Alkaloid	Pyridine,3-(3-nitro-5-phenoxyphenoxy)	analgesic, antifungal, antimalarial, anti-inflammatory, antibacterial, anti-HIV, antitumor, and antiviral (Rasekhi, Tajick, Rahimian, & Sharifimehr, 2014)
47.412	0.47	$C_{19}H_{18}N_2O_2S$	338.4	Sulfur containing Benzithiazol	N,N-Dimethylmalonyl-2-thia-10-11-diaza(3,2)metacyclophane	Antioxidant
47.520	0.62	$C_{28}H_{58}$	394.8	Alkane	Octacosane	antimicrobial, antioxidant, and anti-inflammatory (Khatua, Pandey, & Biswas,

						2016)
48.393	0.49	$C_{10}H_{12}O_2$	164.20	Phenol ester, Eugenol	7-benzofuranol 2,3-dihydro-2,2-dimethyl-	Antibacterial, antimicrobial (Kossakowski, Hejchman, & Wolska, 2002; Kossakowski, Ostrowska, Struga, & Stefańska, 2009), anthelmintic
48.393	0.49	$C_{10}H_{16}N_2$	164.25		1,4-Benzenediamine,N,N,N, N-tetramethyl	Not indicated
48.393	0.49	$C_{11}H_{16}O$	164.24	Aromatic ketone	3-methyl-2-pent-2-enyl-clyclopent-2-enone	Not indicated
48.836	0.59	$C_{12}H_{17}NO_2$	207.27	Ester	Benzoic acid, 4-(1-methylpropyl)amino-,methyl ester	Not indicated
49.051	5.82	$C_{10}H_{10}O_4$	194.18	Fatty acid	4-hydroxy-3-methoxycinnamic acid	anti-obesity and anti-hyperglycemic (Kinyua *et al.*, 2018), antioxidant, anti-inflammatory, antifungal (Hidalgo *et al.*, 2009), nematicide (Hölscher *et al.*, 2014)
49.278	0.24	$C_{18}H_{18}N_2OS_2$	342.5	Sulfur-containing Benzothiazole	2-(Benzothiazol-2-ylsulfanyl)-N-(4-isopropyl-phenyl)-acetamide	antitumor (Stojkovic *et al.*, 2006), antimicrobial (Basser & Mote, 2001), antibacterial, antifungal (Hothi, Makkar, Sharma, & Manrao, 2008), anti-inflammatory

						(Pontiki & Hadjipavlou-Litina, 2007), anticonvulsive, analgesic (Mruthyunjayaswamy & Shanthaveerappa, 2000)
49.422	0.15	$C_{22}H_{34}O_4$	362.50	Fatty acid	1, 2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	antibacterial and antifouling (Patil & Jadhov, 2014)
49.518	0.29	$C_{19}H_{12}F_2N_2$	306.3	Fluorine containing	Acridin-9-yl-(2 4-difluoro-phenyl0-amine	antibacterial, anti-viral, antiprotozoal, anti-viral, antitubercular, anti-fungal, anti-malarial and anti-cancer agents (Rupar, Dobričić, Aleksić, Brborić, & Čudina, 2018)
49.733	0.89	$C_{16}H_{12}F_2N_2O_2S$	334.3		1,4-Benzodiazepin-2-one,7-difluoromethylthio-1,3-dihydro-3-hydroxy-5-phenyl	Not indicated
49.841	0.16	$C_{21}H_3O_2Si$	350.61	Organosilico Alkane	Silane,diethyl(3,5-dimethylphenoxy)nonyloxy	Not indicated
49.924	0.34	C_4H_4BrNS	178.08	Hetero	Isothiazole,5-bromo-3-	Anti-inflammatory, antithrombotic,

				aromatic	methyl	and anticonvulsive agents, herbicides (Gupta, Kumar, & Gupta, 2013), antimicrobial, antiretroviral, antifungal, anticancer, antidiabetic, anti-Alzheimer, antihypertensive, antioxidant, and hepatoprotective activities (Pattan *et al*., 2009)
50.188	20.08	$C_{11}H_{14}O_2Si$	206.31	Organosilico	3-(1-methyl-1-silacyclobutyl) benzoic acid	Not indicated
50.188	20.08	$C_9H_{10}N_2S$	178.26	Benzithiazol	2-Benzothiazolamine,5,6-dimethyl	antitumor (Stojkovic *et al*., 2006), antimicrobial (Basser *et al*., 2001), antibacterial, antifungal (Hothi *et al*., 2008), anti-inflammatory (Pontiki *et al*., 2007), anticonvulsive, analgesic (Mruthyunjayaswamy *et al*., 2000)
50.379	2.67	$C_{15}H_{12}$	192.25	Alkene	Benzene,1,1(1,2-propadienylidene)bis	Not indicated
50.499	0.74	$C_{14}H_{12}N_2$	208.26	Member of benzimidazole alkaloid	1H-Indole,3-methyl-2-(2-pyridyl)	Not indicated
50.666	0.33	$C_{16}H_{14}N_4$	342.4		6-Amino-2-thioxo-4-	Not indicated

		O_3S			(3,4,5-trimethoxyphenyl)-1,2-dihydropyridine-3,5-dicarbonitrile	
51.252	0.68	$C_{14}H_{12}N_2$	208.26	Member of benzimidazole alkaloid	1,3-dihydroxy-6,7-dihydro-5H-cyclopenta(c)pyridine-4-carbonitrile	antitumor (Stojkovic *et al.*, 2006), antimicrobial (Basser *et al.*, 2001), antibacterial, antifungal (Hothi *et al.*, 2008), anti-inflammatory (Pontiki et al., 2007), anticonvulsive, analgesic (Mruthyunjayaswamy *et al.*, 2000)
51.252	0.68	$C_{10}H_8OS$	176.24	Sulfur containing	3-methylbenzo(b)thiophene-2-carboxaldehyde	Not indicated
51.252	0.68	$C_9H_8N_2S$	176.24	Benzithiazol	1H-cyclopenta (b)pyridine-3-carbonitrile,2,5,6,7-tetrahydro-2-thioxo	antitumor (Stojkovic *et al.*, 2006), antimicrobial (Basser *et al.*, 2001), antibacterial, antifungal (Hothi *et al.*, 2008), anti-inflammatory (Pontiki *et al.*, 2007), anticonvulsive, analgesic (Mruthyunjayaswamy *et al.*, 2000)
51.551	2.15	$C_{24}H_{20}N_2$	336.4	Alkaloid	1-Ally-2,4,5-triphenylimidazole	antimicrobial, anti-inflammatory, analgesic, antitubercular, anticancer (Burungale &

						Bhitre, 2013)
52.006	0.29	$C_{15}H_{13}N$	207.27	Alkaloid	2- Ethylacridine	Bacteriostatic (Babaiwa, Erharuyi, Falodun, & Akerele, 2017), antimicrobial, anticancer, antibiotic, anti-AchE, antileukemia, antimalarial, antipsychotic, antidepressant, antidementia, telomerase inhibition (Mishra, Kumar, Singh, Tripathi, & Tiwari, 2015)
52.389	0.26	$C_{16}H_{15}N_3O_3$	297.31	Mebendazole alkaloid	1-(4-Methoxy-benzyl)-7-methyl-3H,6H-pyrido (3,4-d)pyridazine-4,5-dion	Antiparasitic
52.532	3.45	$C_{14}H_{10}CI_2N_4S$	337.2	Aromatic heterocycle	2 4-diamino-5-chloro-6-(0-chlorophenyl)thio quinazoline	antibacterial, antifungal, anticonvulsant, anti-inflammatory, anti-HIV, anticancer, and analgesic activities (Jafari, Khajouei, Hassanzadeh, Hakimelahi, & Khodarahmi, 2016)
52.724	0.26	$C_{50}H_{102}$	703.3	Alkane	tetratriacontane,17-hexadecyl-	Antiasthmatics (Subramanian, Dowlath, Karuppannan, Saravanan, & Arunachalam, 2020)
52.903	2.21	$C_{15}H_{27}BN$			Rhodium,(.eta.5-2,4-	Not indicated

		RhSi			cyclopentadien-1-yl)[(3,4-.eta.)-4,5-diethyl-1,2,2,3-tetramethyl-1-aza-2-sila-5-boracyclopent-3-ene-B5,N1]	
53.394	1.72	$C_{14}H_{12}N_2$	208.26	Benzimidazole alkaloid	Indole,6-methyl-2-)3-pyridyl)	antitumor (Stojkovic *et al*., 2006), antimicrobial (Basser *et al*., 2001), antibacterial, antifungal (Hothi *et al*., 2008), anti-inflammatory (Pontiki *et al*., 2007), anticonvulsive, analgesic (Mruthyunjayaswamy *et al*., 2000)
53.561	0.19	$C_{12}H_{32}O_4Si_3$	324.63	organosilicon	3 3-diisopropoxy-1 1 1 5 5 5-hexamethyltrisiloxane	Antimicrobial (Chelvan *et al*., 2016)
53.561	0.19	$C_9H_{27}AsO_3Si_3$	342.49	organosilicon	Arsenous acid,tris(trimethylsilyl)ester	Not indicated
53.561	0.19	$C_6H_{18}O_3Si_3$	222.46	Organosilicon alkane	Cyclotrisiloxane, hexamethyl	Antimicrobial, antioxidant (Anjukrishna, Hafza, Poorna, Lekhya, & Bhaskara, 2015)
53.741	1.63	$C_{23}H_{22}O_6$	394.41	Flavonoid	Rotenone	antineoplastic agent, insecticide, anti-cancer,

						and fish poison (Beaulieu *et al*., 2021; Xiao *et al*., 2020)
53.992	0.91	$C_{22}H_{18}O_7$	394.4	Flavonoid pyrene	9H-Furo(2,3-H)chromene-2,8-dione,4-methyl-9-(3,4,5-trimethoxybenzylidene)	Not indicated
54.458	0.74	$C_{13}H_{21}NO$	207.32		N-Methyl-1-adamantaneacetamide	antidiabetic activity (Lekshmi, Sreekutty, & Mini, 2015)
54.578	0.90	$C_{26}H_{22}$	334.5	Alkene	Hexacene,1,2,3,4,7,14-hexahydro	Not indicated
54.638	0.77	$C_{23}H_{22}O_6$	394.41	Flavonoid	Rotenone	antineoplastic agent, insecticide, anti-cancer, and fish poison (Beaulieu *et al*., 2021; Xiao *et al*., 2020)
55.057	0.07	$C_{13}H_{20}N_2SSi$	264.46	Organosilicon benzisothiazole	1,2- benzisothiazol-3-amine tbdms	Antiviral, Antifungal (Annadurai, Cyril, & Narayanan, 2020)
58.753	0.49	$C_8H_5N_3O_4$	207.14	aromatic	1,4 phthalazinedione,2,3 dihydro-6-nitro	anticancer (Kim *et al*., 2008; Li, Zhao, Yuan, Xu, & Gong, 2006), anticonvulsant (Grasso *et al*., 2000), antimicrobial (El-Sakka, Soliman, & Imam, 2009), antifungal (Ryu, Park, Ma, & Nho, 2007) and anti-inflammatory (Rashdan, Gomha, El-Gendey, El-Hashash, & Soliman,

						2018) activities.

Table 3.9: GC-MS results for the methanol extract of *N. brachypus*

Retention Time	**Area %**	**Chemical Formula**	**Molecular Weight g/mol**	**Classification**	**Compound name**	**Biological Activity**
12.313	0.44	$C_{10}H_{20}$	140.27	Alkane	1-Methylamino-1-propylcyclohexane	Antimicrobial, anticancer activity, antioxidant activity, cytotoxic activity, analgesic activity, anti-inflammatory activity, and antithrombin activity (Shoaib, Israyilova, & Ganbarov, 2021) anti-Candida, antidiabetic (Kadhim, 2016)
21.309	0.40	$C_{14}H_{30}$	198.39	Alkane	Tetradecane	Antifungal, antibacterial, nematicide (Arora *et al*., 2018), diuretic, anti-tuberculosis (Girija *et al*., 2014)

22.074	0.41	$C_5H_{10}O$	86.13	Alkenyl	2-penten-1,ol (E)	pesticide
22.397	1.66	$C_7H_{14}O_3$	146.18	Cyclic carbonate	1,3-dioxane-5-methanol 5-ethyl	Not indicated
25.472	0.23	$C_{12}H_{16}O$	176.25	Coumarin	benzofuran 2 3 dihydro 2 2 5 6-tetramethyl-	Analgesic and anti-inflammatory (Idan, Al-Marzoqi, & Hameed, 2015), Antiarrhythmic, spasmolitic, and antiviral (Al-Tameme, Hadi, & Hameed, 2015)
26.178	0.35	$C_{16}H_{34}$	226.41	Alkane	Hexadecane	antibacterial, antioxidant (Kumar, Bhatnagar, *et al.*, 2011)
27.853	2.49	$C_9H_{16}O_7$	236.22	Helminthsporide sugar, carbohydrate	methyl(methyl 4-0-methyl-alpha-d-mannopyranoside)uronate	antimicrobial activities (Lu *et al.*, 2016)
30.568	0.18	$C_{18}H_{38}$	254.49	Alkane	Octadecane	Antimicrobial (Girija *et al.*, 2014), antifungal (Usha *et al.*, 2015)
33.810	1.12	$C_{16}H_{32}O_2$	256.42	Fatty acid	n- Hexadecanoic acid	Antimicrobial, antioxidant (Abdullah *et al.*, 2020) Anti-inflammatory (Aparna *et al.*, 2012), Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase

						inhibitor (Kumar, Kumaravel, *et al*., 2010), potent mosquito larvicide
37.040	0.69	$C_{18}H_{32}O_2$	280.45	Fatty acid	9,12-octadecadienoic acid (Z,Z)	Inflammatory (Das, 2006), antibacterial agent (Zheng *et al*., 2005), anticancer, antiandrogenic, dermatitigenic, irritant, antileukoliene (Krishnamoorthy *et al*., 2014)
37.136	0.82	$C_{18}H_{34}O_2$	282.47	Fatty acid	Oleic acid	Antibacterial (Awa *et al*., 2012), anti-androgenic, anti-cancer, hypocholesterolemic, antimicrobial, dermatitigenic, anti-inflammatory, and anti-tumor activity (Gideon, 2015)
37.531	0.36	$C_{18}H_{34}O_2$	282.47	Fatty acid	Octadecanoic acid	Antimicrobial, anti-inflammatory, hepatoprotective, nematicide (George *et al*., 2018), hypercholesterolemic, antiarthritic (Hussein *et al*., 2017)
43.285	0.37	$C_{35}H_{68}O_5$	568.9	Fatty acid	hexadecanoic acid 1-(hydroxymethyl)-1 2-ethanediyl ester	Antiandrogenic, hemolytic, antioxidant, hypocholesterolemic (Zayed, Wu, & Sallam, 2019), Anti-microbial, antifungal (Kadhim, Al-

						Rubaye, & Hameed, 2017)
46.216	0.26	$C_{16}H_{10}N_4O_4S$	354.3	Sulfur containing phenol	2-Ethyl-5-(4-nitro-1,8-naphthalimido)-1,3,4-thiadiazole	antibacterial (Zamani, Faghihi, Tofighi, & Shariatzadeh, 2004), antimycobacterial (Foroumadi, Mirzaei, & Shafiee, 2001), antifungal, and antidepressant (Clerici *et al*., 2001).
46.359	0.74	$C_{22}H_{16}$	280.36	Alkene	(9E)-Strylanthracene	Not indicated
47.209	6.78	$C_{17}H_{12}N_2O_4$	308.29	Carboxylic acid alkaloid	pyridine,3-(3-nitro-5-phenoxyphenoxy)	Analgesic, antifungal, antimalarial, anti-inflammatory, antibacterial, anti-HIV, antitumor, antiviral
47.412	1.58	$C_{22}H_{42}O_2$	338.57	Terpene	ethyl 3,7,11,15-tetramethyl-2-hexadecenoate	Antidiabetic, anti-inflammatory (IA Mgbeje, Abu, O Ugoanyanwu, & E Ebong, 2020)
48.178	0.31	$C_{16}H_{12}O_4$	268.26	Flavonoid lipid	4H-1-Benzopyran-4-one,7-hydroxy-3-(4-methoxyphenyl)	antioxidant, antiaging, anti-inflammatory, immunomodulatory, cardioprotective, antimicrobial, antiviral, antibacterial, antiparasitic, and antifungal (Jucá *et al*., 2020)
48.393	0.67	$C_{19}H_{33}CoGe$		Ester	Cobalt, (.eta.-3-trimethylgermylcyclooctenyl)	Not indicated

					-1,5-cyclooctadiene	
48.836	0.56	$C_{17}H_{19}NO_3$	285.34	Alkaloid, benzylisoquinoline	7-Isoquinolinol, 1, 2, 3, 4-tetrahydro-6-methoxy-1-salicyl	antimicrobial, antiviral, antitumor, antimalarial, and cytotoxicity, anti-HIV, antiprotozoal (Rinaldi, Díaz, Suffredini, & Moreno, 2017)
49.051	9.13	$C_{11}H_{15}NO_4$	225.24	Carboximic acid	Acetamide,N-(3,4,5-trimethoxyphenethyl)	Antimicrobial, carcinogenic, anti-inflammatory
49.278	0.72	$C_{14}H_{12}N_2$	208.26	Alkaloid	.gamma.-Cyano-3-methyl-5,10-dihydrobenzo(f)indolizine	antibacterial, anti-inflammatory, antiviral and antileishmanial, analgesic and antitumor, antioxidant activities (Venugopala *et al.*, 2017)
49.518	0.59	$C_{19}H_{12}F_2N_2$	306.3	Flavonoid	Acridin-9-yl-(2,4-difluoro-phenyl)-amine	antibacterial, anti-viral antiprotozoal, anti-viral, antitubercular, anti-fungal, anti-malarial and anti-cancer agents, larvicidal activities (Kalirajan, Jubie, & Gowramma, 2015)
49.924	0.35	$C_{15}H_{14}$	194.27	Alkene, resveratrol analogue phenol	.alpha.-Methylstilbene	antioxidant, antibacterial, and anticancer activity (Kasiotis, Pratsinis, Kletsas, & Haroutounian, 2013)
50.164	28.73	$C_{11}H_{14}O_2$	178.23	Phenol present	Methyleugenol	Mutagenic, anesthetic, antifungal,

				in essential oil, phenylpropene		antimicrobial, nematicide, antifeedant (Tan & Nishida, 2012) anti-allergic, anti-anaphylaxis, and anti-nociceptive
50.391	11.28	$C_{15}H_{12}$	192.26	Alkene	8,9,-Dihydrocyclopenta(def)phenathrene	cytotoxicity, antimicrobial, spasmolytic, anti-inflammatory, antiplatelet aggregation, anti-allergic, and phytotoxicity (Kovács, Vasas, & Hohmann, 2008)
50.511	2.25	$C_{14}H_{12}N_2$	208.26	Alkaloid pyridine	Indole,6-methyl-2-(4-pyridyl)	Not indicated
51.252	0.35	$C_{10}H_8OS$	176.24	Carboxylic coumarin	Benzo(b)thiophene-2-carboxaldehyde,7-methyl	anticoagulant, antibacterial, anthelmintic, hypothermal properties, and vasodilatory action (Nofal, El-Zahar, & El-Karim, 2000)
51.252	0.35	$C_9H_8N_2O_2$	176.18	alkaloid	1,3-Dihydroxy-6,7-dihydro-5H cyclopenta (c)pyridine-4-carbonitrile	Not indicated
51.551	3.46	$C_{19}H_{12}O_6$	336.3	Coumarin	Coumarine,6-(7-hydroxycoumarin-8-yl)-7-methoxy	anticoagulant, antibacterial, anthelmintic, hypothermal properties, and vasodilatory action (Nofal *et al.*,

						2000)
52.018	0.42	$C_{18}H_{18}O$	250.3	Lipid phenol	9,10,-Methanoanthracen-11-ol,9,10-dihydro-9,10,11-trimethyl	Not indicated
52.389	0.18	$C_{18}H_{45}AsO_3Si_3$	468.7	Organosilicon essential oil	Tris (tert-buthyldimethylsilyloxy) arsane	Not indicated
52.532	7.72	$C_{21}H_{20}O_4$	336.14	Carboxylic acid, essential oil	11H-Cyclopenta(a)phenanthrene-15-carboxylic acid,12,13,16,17-tetrahydro-3-methoxy-13-methyl-17-oxo-methyl ester (S)	Not indicated
52.951	0.38	$C_{12}H_{17}NO_2$	207.12	Aromatic piperidine hydrocarbon	Hexahydropyridine,1-methyl-4-(4,5-dihydroxyphenyl)	Not indicated
53.406	6.85	$C_{14}H_{12}N_2$	208.26	Member of benzimidazole	Indole,5-methyl-2-(4-pyridyl)	antitumor (Stojkovic *et al*., 2006), antimicrobial (Basser *et al*., 2001), antibacterial, antifungal (Hothi *et al*., 2008), anti-inflammatory (Pontiki *et al*., 2007), anticonvulsive, analgesic

						(Mruthyunjayaswamy *et al.*, 2000)
53.753	3.16	$C_{23}H_{22}O_6$	396.41	Flavonoid	Rotenone	antineoplastic agent, insecticide, anti-cancer, and fish poison (Beaulieu *et al.*, 2021; Xiao *et al.*, 2020)
53.992	0.86	$C_{18}H_{18}O$	250.30		9,10,-Methanoanthracen-11-ol,9,10-dihydro-9,10,11-trimethyl	Not indicated
54.458	1.19	$C_8H_5N_3O_4$	207.43	Ketone	2-p-Nitrophenyl-oxadiazol-1,3,4-one-5	anti-cancer, anti-microbial, anti-tuberculosis, antioxidant, and anti-inflammatory (Kavitha, Gnanavel, & Kannan, 2014)
54.650	1.97	$C_{23}H_{22}O_6$	396.41	Flavonoid	Rotenone	antineoplastic agent, insecticide, anti-cancer, and fish poison (Beaulieu *et al.*, 2021; Xiao *et al.*, 2020)

3.4 Conclusion

It can be concluded that the most effective way to harness the greatest number of different classes of phytochemicals from *N. brachypus* tubers is to use white tubers, air dry them at room temperature and then use the Soxhlet extraction method with methanol as the solvent. However, to get a high extract yield Chloroform 50%: Ethyl acetate 50% can be used in extraction. *Neorautanenia brachypus* tubers contained essential oils, terpenoids, quinones, saponins, coumarins, phenols, flavonoids, alkaloids, and tannins, according to phytochemical screening results. Methanol extracts had higher total phenolic and tannin content compared to the aqueous extracts. GC-MS analysis showed that the chemical constituents in methanol and Chloroform 50%: Ethyl acetate 50% were classified as esters, alkanes, alkenes, fatty acids, phenol, ketones, silicon-containing, Sulphur containing, coumarin, alkaloids, fluorine-containing, benzothiazoles, flavonoids, terpenoids, carbohydrates, benzimidazoles, cyclic carbonate, and aromatic piperidine hydrocarbons. The compounds in *N. brachypus* tuber extracts have antifungal, antibacterial, anthelmintic, antiviral, anti-tumor, antioxidant, anti-inflammatory, anticancer, insecticidal, pesticide, anti-tuberculosis, analgesic, antipyretic, anti-androgenic, allelopathic, anti-asthmatic, hemolytic, larvicidal, hepatoprotective, anti-arthritic, anti-malarial, anti-obesity, hypercholesterolemic, anticonvulsive, antidepressant, antiparasitic, antidiabetic, antiaging, antileukemia, immunomodulatory, cardioprotective, carcinogenic, mutagenic, anesthetic, anti-allergic, and anticoagulant applications. This affirms the therapeutic applications of *N. brachypus* tubers.

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Conflict of interest

The authors declare no conflict of interest.

CHAPTER 4 Effect of Phytochemical Extracts from *Neorautanenia brachypus* on Helminths Under Laboratory Conditions (*in vitro* experiments)

Mpofu, M.C., Gomo, C., Mugumbate, G., Mashingaidze, A.B., Chikwambi, Z., and Murungweni, C. 2022. Effect of Phytochemical Extracts from *Neorautanenia brachypus* on Helminths Under Laboratory Conditions. Journal of Transboundary and Emerging Diseases.

Abstract

Helminths pose a huge threat to the health and welfare of livestock. Helminths cause significant economic losses in ruminant systems due to death, weight loss, decreased milk and meat output, and lower reproductive efficiency. The objective of this research was to determine the effectiveness of different extracts on the death of helminths at egg and larval stages in the laboratory. The toxicity of *N. brachypus* extracts was determined on blood samples compared to positive control hydrogen peroxide using a spectrophotometer. Egg hatch inhibition test, larval mortality test, and adult worm mortality test were carried out to determine the efficacy of tuber extracts on the different stages of the development of helminths. *Eisenia foetida* earthworms were used as an assay for the Adult worm mortality test. The egg hatch inhibition percentage increased as the dilution factor of extracts increased. The undiluted (100%) fresh blended *N. brachypus* tuber extract and undiluted (100%) methanol *N. brachypus* tuber extracts had a statistically significant anthelminthic activity comparable to 75% Albendazole conventional drug (inhibition % 70.17-75.67%). The results showed that treatments *N. brachypus* Methanol 100%, *N. brachypus* Soaked 100% in distilled water, *N. brachypus* Distilled water 100%, *N. brachypus* Blended 100%, *N. brachypus* Blended 75%, *N. brachypus* Soaked 75% in distilled water, *N. brachypus* Distilled water 75%, and *N. brachypus* Methanol 75% had an anthelmintic activity comparable to Albendazole 75% and Albendazole 50% for egg hatch inhibition (inhibition % 60.33-70.17%). The larval mortality activity (%) was concentration-dependent. There was no statistical difference between Albendazole 75%, Albendazole 50%, *N. brachypus* Methanol 100%, *N. brachypus* Soaked 100% in distilled water, *N. brachypus* Distilled water 100%, *N. brachypus* Blended 100%, *N. brachypus* Blended 75%, *N. brachypus* Soaked 75% in distilled water, *N. brachypus* Distilled water 75%, and *N. brachypus* Methanol 75% (larval mortality % 51.67%-73.33%). *Neorautanenia tuber* extracts extracted using the soaking method in distilled water had the highest IC_{50} dilution factor for both egg hatch inhibition and larval

mortality activities 71.10% and 78.88% respectively. The mortality of *Eisenia foetida* worms was dependent on the concentration of *N. brachypus* plant extract concentration and on time of exposure. The highest mortality was recorded when extracts were undiluted and after 24 hours of exposure. Treatments Albendazole 100%, Albendazole 75%, *N. brachypus* Blended 100%, *N. brachypus* Soaked 100%, *N. brachypus* Methanol 100%, *N. brachypus* Methanol 75%, and *N. brachypus* Distilled Water 100% showed some anthelmintic activity after 1 hour of exposure. All treatments except for the negative control showed anthelmintic activity ±80% after 24 hours of exposure. The *in vitro* anthelmintic activity of tested plant preparations was characterized by a decrease in egg hatching ability, an increase in larvae larvae, and adult *Eisenia foetida* worms' mortality. These treatments may reduce the hatching of the helminths eggs excreted in the feces, resulting in both a reduced risk of reinfection and lightened worm loads by decreasing pasture contamination. Accordingly, they have the potential to contribute to controlling gastrointestinal parasites of ruminants. Methanol and Distilled water extracts of *N. brachypus* can be used as alternatives in drug discovery because of their low toxicity to erythrocytes. The use of fresh *N. brachypus* tuber extracts was recommended as the most effective way of controlling helminths eggs and larvae in cattle as they had the most significant IC_{50} dilutions in both assays.

Keywords: *Neorautanenia brachypus*, anthelmintic, extracts, *in vitro*, anthelmintic resistance, livestock

Abbreviations: Blen- *N. brachypus* blended juice extract, **Meth-** *N. brachypus* methanol extract, **DW**- *N. brachypus* distilled water extract, Soak- *N. brachypus* soaked extract in distilled water **Albe**-albendazole,

4.1 Background

Neorautanenia brachypus (Zhombwe in Shona) is a leguminous tuberous plant that is found in Zimbabwe's southeast Lowveld. The plant has a variety of purposes, including serving as a feed bridge between seasons to help cattle survive drought. It has therapeutic qualities and is an efficient anthelmintic in small ruminants and cattle *in vivo* (Murungweni *et al*., 2012). Its effectiveness on helminth eggs and larvae in vitro, however, has not been reported. This work fills in the research void in this area.

Helminths pose a huge threat to ruminant livestock's health and welfare. The helminths cause significant economic losses in ruminant systems due to death, weight loss, decreased milk and meat output, and lower reproductive efficiency (Ahmed *et al*., 2020). To control helminths, synthetic anthelmintics are routinely employed (Molento *et a*l., 2011). However, due to the inappropriate and exclusive use of synthetic medications, anthelmintic resistance has been a major downside to their usage (Dey, Begum, Alim, & Alam, 2020; Santiago-Figueroa *et al*., 2019). As a result, there are more residues in ruminant cattle meat and milk, as well as in the environment. The problem of anthelmintic resistance has harmed the livestock business by lowering flock and herd output due to mortalities (Torres-Acosta, Mendoza-de-Gives, Aguilar-Caballero, & Cuéllar-Ordaz, 2012). As a result, the demand for alternate control strategies is growing. Anthelmintic medicines made from medicinal plants are both inexpensive and effective (Boonmasawai, Sungpradit, Jirapattharasate, Nakthong, & Piasai, 2013; Chitura *et al.*, 2019).

In vitro investigations can be performed to assess the potential anthelmintic activities of plant extracts as a first step (Costa, Morais, Bevilaqua, Souza, & Leite, 2002). *In vitro* assays are advantageous for testing plant extracts for antiparasitic effects since they are inexpensive and have a quick turnaround time, allowing for large-scale screening of plants (Githiori, Athanasiadou, & Thamsborg, 2006; Tariq, 2018). To evaluate a similar number of extracts *in vivo*, a huge number of animals would be necessary, requiring a significant financial and time investment. *In vitro* assays also allow for fractionation (activity-guided fractionation) and testing of pure substances

4.2 Materials and methods

4.2.1 Study site

The research was carried out at Chinhoyi University of Technology (CUT) Laboratories. It is located along Harare to Chirundu road and also the Chinhoyi to Harare railway. It lies on the West side of the Hunyani River. CUT is in Agro-natural region 2b which receives rainfall ranging from 750-1000mm per year. It's a warm and temperate climate with an average annual temperature of around 20-24 degrees Celsius. It is in Mashonaland West province, Makonde district of Zimbabwe.

4.2.2 Extracts preparation

White *N. brachypus* tubers were collected from Zanamwi farm in Chikombezi GPS coordinates 21°45'0" S and 31°19'0" E. Zimbabwe, south of Gonarezhou National Park. The tubers were cleaned to remove soil and other adherent debris, the skins were peeled away, and prepared as displayed in Figure 4.1 below. The tubers were then cut into smaller pieces with a maximum volume of 1 cubic centimeter. The 1st batch of cut pieces was air-dried at room temperature and ground into powder. A quantity of 10 grams of the dried powder was extracted using each of the solvents Methanol and distilled water in a Soxhlet apparatus for 6 hours. The 2nd batch of fresh-cut pieces was soaked in distilled water for 12 hours at room temperature. After 12 hours of soaking, the sample was filtered using the Whatman filter paper No.1 and collected the filtrate for later use. The last batch of cut tuber pieces was put in a blender for 20 seconds to extract the juice component. The blended material was sieved using a mesh cloth to collect the juice. Each of the prepared *N. brachypus* extracts was divided into undiluted sample (100%) and diluted with PBS buffer to make 75% sample, 50% sample and 25% sample.

Figure 4.1: *Neorautanenia brachypus* extracts prepared for use during in vitro experiments. 1st bottle from the left side is a bottle of methanol extract followed by distilled water extract, then soaked extract, and lastly the blended extract.

4.2.3 Ethical clearance

This study was approved by the Academic Board of the Department of Animal Production and Technology, the Chinhoyi University of Technology, and the Department of Veterinary Services and Diagnostics under the Research and Specialist Services (DR&SS), Zimbabwe. Sample collection was carried out under the supervision of a qualified veterinarian and according to the Chinhoyi University of Technology 'Guidelines for Animal Handling and Sample Collection', which conforms to European Union Directive 2010/63 regarding the protection of animals used in scientific experiments.

4.2.4 Toxicity test

A hemolytic assay using goat serum was used to test for toxicity of each extract by using methods described by Reddy, Subramanyam, Vani, and Devi (2007) and Zohra and Fawzia (2014) with minor modifications. Blood was collected from the jugular vein of goats into vacutainers and stored in a cooler box with ice packs at 4^oC. Erythrocytes were then collected by centrifugation at 1500 rpm for 16 minutes. The supernatant was removed and the pellet was washed thrice with PBS buffer (Phosphate Buffered Saline) and centrifuged for 10 minutes at 300 rpm. 2% erythrocyte suspension was prepared in PBS at pH 7.4. 1 mL of each plant extract dilutions (undiluted-100%, 50%, 75%, and 25% (v/v)) were added to 1 mL of 0.9% NaCl (0.85 g NaCl + 100 mL distilled water) solution and received a 2% suspension of erythrocytes to make a final volume of 4 mL. The experiment was replicated thrice for each *N. brachypus* extract concentration, PBS negative control and hydrogen peroxide positive contro. The negative control was prepared without extract using sterile 2 mL PBS, 1 mL of 0.9% NaCl, and added 2% suspension erythrocytes to make a final volume of 4 mL. The positive control was prepared without extract using 2 mL of hydrogen peroxide, 1 mL of 0.9% NaCl, and added 2% suspension erythrocytes to make a final volume of 4 mL. All the prepared treatments were incubated for 30 mins at 37^oC. After incubation the cells were centrifuged cells at 5000 rpm for 5 mins to allow broken membranes and unbroken cells to settle at the bottom. The supernatant was removed and the liberated hemoglobin in the supernatant was measured spectrophotometrically as absorbance at 540 nm. A blank was prepared by adding PBS buffer to 0.9% NaCl, and hydrogen peroxide (Karim *et al*., 2020).

Percentage hemolysis was calculated as follows:

$$\% \text{ hemolysis} = A_t - A_c / A_n \times 100$$

Where: A_t is the absorbance of the test sample

A_n is the absorbance of the positive control

A_c is the absorbance of the negative control

The absorbance readings were recorded on an excel sheet.

4.2.5 Fecal sample preparation

Fecal collection

Fecal samples were collected directly from the rectum of the infected animals, obtaining about 20 grams of feces. Helminths eggs to use for the Egg Hatch Test were collected from the fecal samples.

In order, to test for the ability of extracts to inhibit egg hatching, the Egg Hatch Test (EHT) was conducted according to Coles *et al*. (1992) with some minor modifications. Fecal samples for analysis were prepared using the floatation and sedimentation methods.

Floatation procedure

Three grams of fecal sample were mixed with 42 mL of distilled water. The fecal mixtures were poured through a series of overlapping sieves (100, 150, 90, and 20 µm) and collected the filtrate when contained the helminths eggs. The filtrate was centrifuged for 4 minutes at 1500 rpm. After centrifugation the supernatant was decanted. The remaining sediment was agitated to loosen it. Saturated sodium chloride was added to the tube with sediment until a meniscus formed. The top of each tube was covered with a microscope coverslip and was left for 15 minutes. The contents on the coverslip were washed into a beaker using distilled water. The concentration of eggs in the sample was estimated by counting the number of eggs in aliquots of 50 µL at x 4/0.10 magnification using an Amscope MU1000 microscope. The concentration of 100 eggs in 100 µL was used for the experiment. The worm egg chart was used for the identification of species.

Sedimentation procedure

The collected fecal samples measuring 5 g were mixed with 200 mL of distilled in a beaker. The mixed fecal sample was poured through overlapping sieves (100, 150, 90, and 20 µm) and collected the filtrate. The filterate was left still for 10 minutes to allow for sedimentation.

After 10 minutes, approximately 70% of the supernatant was decanted and refilled in the beaker with fresh distilled water. The process from mixing with distilled water to sieving to filtration and to sedimentation was repeated 4 times until the supernatant was clear. Then poured off 90% of the supernatant and collected the sediment. The concentration of liver fluke eggs in the sample was estimated by counting the number of eggs in aliquots of 50 µL at x 4/0.10 magnification using an Amscope MU1000 microscope. The concentration of 50 eggs in 100 µL was used for the experiment.

4.2.6 Egg Hatch Test (EHT)

The first step was to measure 1600 µL of *N. brachypus* tuber extracts prepared by blending, soaking in distilled water, and Soxhlet extracted using methanol and distilled water into Petri dishes at final dilutions of undiluted-100%, 75%, 50%, and 25%, (v/v) in PBS. Albendazole (10% w/v) and distilled water were used as positive and negative controls respectively. There were 3 replications for each treatment. The entire experiment was carried out for 24 hr at $27^{o}{}_{c.}$ A drop of Lugol's iodine solution was added to each plate to stop further hatching. The number of eggs and first-stage (L_1) larvae were counted under an Amscope MU1000 microscope x 10/0.25 magnification as proposed by the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P) and described in detail by Powers, Wood, Eckert, Gibson, and Smith (1982).

Results were expressed as % inhibition of eggs hatch. Percent inhibition of egg hatching was calculated as follows:

Percent inhibition = $100(1- P_{test} / P_{negative}$ control), where P= number of eggs hatched in EHT

4.2.7 Fecal culturing

The fecal culture was done to cultivate larvae from eggs that hatch and develop into the infective 3rd stage (L_3) to carry out the larval mortality test. A mass of 100 g of the fecal sample was put in a 500ml glass container and mixed with 20 g of vermiculite to provide aeration and absorb excess moisture. A plastic lid with a 2 cm diameter hole covered with mesh was screwed to the glass container. The container with culture was incubated at 24 ^{o}C for 9 days. Everyday water was added to the culture and then mixed. The larvae were harvested using a modified Baermann technique by filling the container with water at a temperature of 37 ^{o}C. The container filled with water was placed down on a petri dish and allowed a little water to cover the bottom of the dish. The container was left in good light for

8 hours. The L3 larvae were recovered from the shallow layer of water in the petri dish by a pipette and concentrated the L3s by sedimentation in a small test tube. 5 mL of fluid was centrifuged at 1000 rpm for 2 minutes. The supernatant was removed and sediment was used for the larval mortality test. The concentration of 20 larvae in 100 µL was used for the experiment.

4.2.8 Larval Mortality test

Three replications per treatment were used and for each replicate 10 µL PBS and 100 µL of live L3s were randomly pipetted into Petri dishes. The L3s (Figure 4.2) were exposed to tuber crude extracts at final dilutions of undiluted-100%, 75%, 50%, and 25% (v/v) in PBS. Albendazole and distilled water were used as positive and negative controls respectively. Petri dishes were humidly incubated at 27^{o}C for 3 hours. After 3 hours added a drop of 1% staining dye and re-incubated the set up to 24 hr. The L3s were re-examined under an Amscope MU1000 microscope x 10/0.25 magnification for the uptake of the stain as displayed in Figure 4.3. Identified the larvae with cuticle damage based on the uptake of the blue stain and those with deformed body shapes and categorized them as dead. The larvicidal activity was expressed as the percentage of dead L3s after exposure.

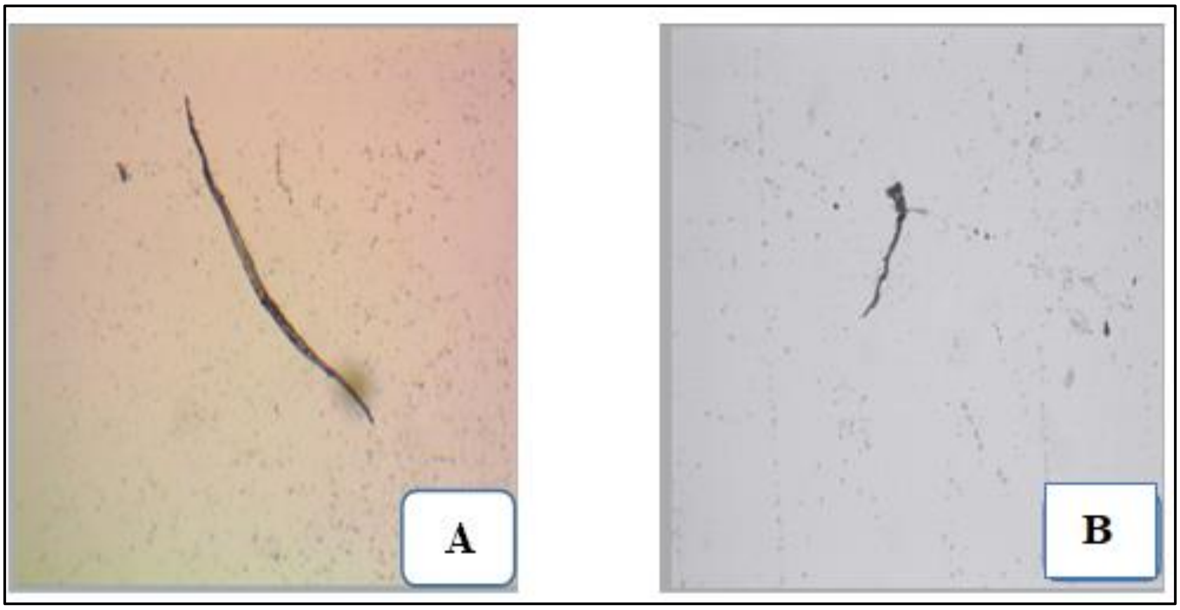

Figure 4.2: Picture of some of the larvae species before treatment with *N. brachypus* tuber preparations. A - nematode and B-fluke

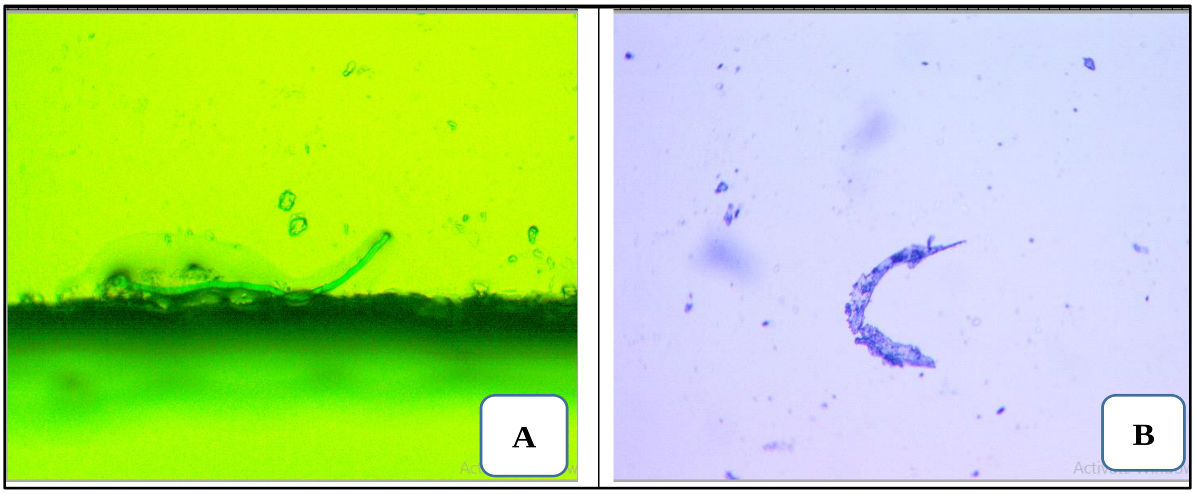

Figure 4.3: Pictures of A- larvae that had been stained showing death and B- larvae with a deformed body after exposure to *N. brachypus* treatments.

4.2.9 Adult worm mortality test

The anthelminthic experiment was carried out as per the method described by James and God (2014) and Hounzangbe-Adote, Paolini, Fouraste, Moutairou, and Hoste (2005). *Eisenia fetida* (red earthworms) were collected from local suppliers and washed in PBS. Five actively moving worms of the same length were selected and placed in the Petri dishes and filled with different dilutions (undiluted-100%, 75%, 50%, and 25%) of *N. brachypus* plant extracts in Dimethyl sulfoxide (DMSO) at 37°C as shown in Figure 4.4. Three replications were used for each treatment. Albendazole was used as a positive control and DMSO as the negative control. The number of dead worms in each petri dish was counted at different time intervals (0.5 hrs, 1 hr, 2 hrs, 4 hrs, 6 hrs, and 24 hrs). Death was confirmed when worms neither move when shaken nor when an external stimulus was given by putting the motionless worms in 50 degrees Celsius water. The mortality rate of each concentration of the extract was determined using the following formula:

Mortality rate = (number of dead worms in each petri dish/number of living worms in a petri dish)

× 100

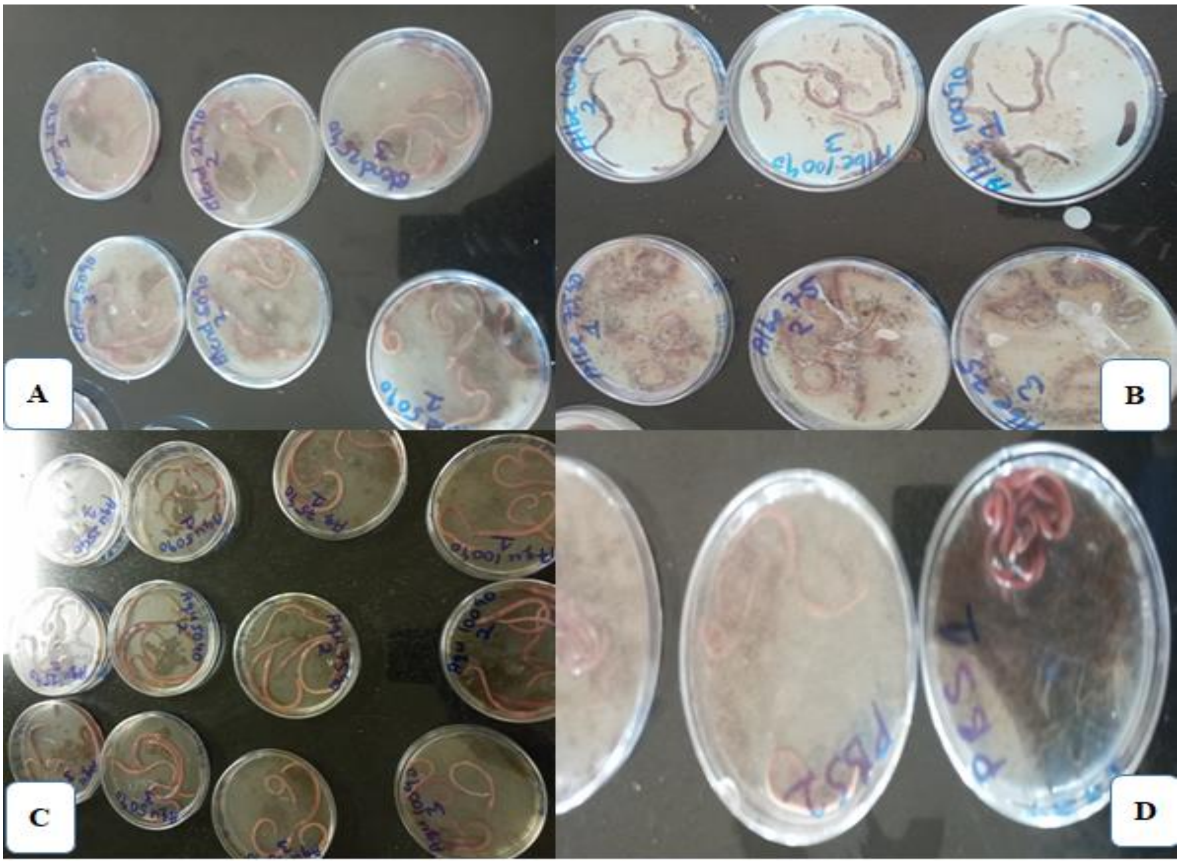

Figure 4.4: Experimental setup for testing the efficacy of *N. brachypus* tuber preparations against *Eisenia foetida*. A- worms exposed to the whole juice extract, B- worms exposed to Albendazole, C- worms exposed to the distilled water extract, and D- worms exposed to the negative treatment. DMSO was used a negative treatment.

4.2.10 Statistical treatment of data

Data was organized, edited, and analyzed using SAS version 9.4. Results generated from both assays were analyzed with ANOVA followed by Tukey's HSD multiple comparisons. A ***P***-value of less than 0.05 was considered statistically significant.

4.3 Results and discussion

4.3.1 The toxicity level of *N. brachypus* extracts

Data was tested for normality in SPSS version 20 and was found to be normally distributed (P>0.05) as determined by the Shapiro-Wilk statistic. Analysis of variance was done and results showed there was a significant difference between treatments on hemolysis of Red Blood Cells (***P***<0.0001) displayed in Table 4.1 below.

Table 4.1: ANOVA table for the toxicity level of *Neorautanenia brachypus* extracts on red blood cells (hemolysis).

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Treatment	9	15033.63	1670.40	501.12	<.0001

Table 4.2 shows that the effect of *N. brachypus* extracts on Red Blood Cells was dose-dependent. Percentage hemolysis increased as the concentration of extracts increased. The highest % hemolysis was recorded for 100% methanol extracts (Meth) 40% followed by 100% distilled water (DW) 35.67%, 75% Meth (32.33%), 50% Meth (23.33%), 75% DW (21%), 50% DW (17.67%), 25% Meth (12%), 25% DW (10%).

Table 4.2: Effect of N. brachypus extracts on hemolysis of Red Blood Cells compared to a positive control hydrogen peroxide and negative control PBS. Standard Error of Mean 1.05. LSMeans with the same superscript are significantly different (***P***<0.05)

Treatment	**LSMean -% Hemolysis**
Hydrop	85.00^{a}
Methanol 100%	40.00^{b}
Distilled Water 100%	35.67^{bc}
Methanol 75%	32.33^{c}
Methanol 50%	23.33^{d}
Distilled Water 75%	21.00^{d}
Distilled water 50%	17.67^{e}
Methanol 25%	12.00^{f}
Distilled water 25%	10.00^{f}
PBS	0.00^{g}

The quantity, quality, extraction velocity, inhibitory substances, toxicity, other biological activities, and biosafety of extracts are all affected by the solvent used for extraction (Do *et* *al*., 2014; Rafińska *et al*., 2019; Zhang *et al*., 2019). The differences between these solvents on hemolysis might be because of differences in the active compounds in the extracts that might be toxic to red blood cells. Methanol extracts have been shown to have higher phenolic content compared to aqueous extracts. Due to the presence of a hydroxyl group, phenolic compounds are more soluble in polar organic solvents (Aryal *et al*., 2019; Karim *et al*., 2020; Mahomoodally *et al*., 2021).

The hemolytic properties exhibited by *N. brachypus* tuber extracts mighty have been because of the presence of compounds that have been cited in the literature to have hemolytic or cytotoxic effects (Kovács *et al*., 2008; Shoaib *et al*., 2021; Zayed *et al*., 2019). GCMS analysis of methanol extracts of *N. brachypus* showed the presence of 1-Methylamino-1-propylclohexane (0.44%), Hexadecanoic acid-1-(hydroxymethyl-1,2-ethanediyl ester) (0.37%), 7-Isoquinolinol,1,2,3,4-tetrahydro-6-methoxy-1-salicyl (0.56%), and 8,9-dihydrocyclopenta(def)phenanthrene (11.28%). These compounds have been indicated to have hemolytic effects.

4.3.2 The effect of *N. brachypus* tuber extracts on helminths egg hatching ability

Data was tested for normality in SPSS version 20 and was found to be normally distributed (***P***>0.05) as determined by the Shapiro-Wilk statistic. The ANOVA Table 4.2 below shows that there was a significant difference between the treatments tested and blocking (***P***<0.05) on egg hatch inhibition percentage.

Table 4.3: ANOVA table for the effect of different *N. brachypus* preparations used against the hatching of helminth eggs from cattle and on types of helminths prepared by two different methods (Floatation and sedimentation).

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	20	43518.83	2175.94	94.40	<.0001
Block	1	104.96	104.96	4.55	0.04

The results displayed in Table 4.4 show that there was a concentration dependency on the effects of the different treatments used on egg hatch inhibition. The egg hatch inhibition percentage increased as the dilution factor of extracts decreased. The undiluted-100% fresh blended tuber extract and undiluted-100% methanol tuber extracts had a statistically significant anthelminthic activity comparable to 75% Albendazole conventional drug. The results showed that treatments with Methanol 100%, Soaked 100%, Distilled water 100%, Blended 100%, Blended 75%, Soaked 75%, Distilled water 75%, and Methanol 75% had an anthelmintic activity comparable to Albendazole 75% and Albendazole 50%. The lowest activity was recorded for Soak 25%, distilled water 25%, and methanol 25% inhibition % 28.33-31%) as they shared the same superscript J.

Table 4.4: Effect of different *N. brachypus* preparations used against the hatching of helminth eggs from cattle compared to a conventional drug Albendazole 10% (Standard Error of Mean 1.96). Means with the same superscript are statistically different (***P***<0.05).

Treatment	**LSMean Egg Hatch Inhibition %**
Albendaole 100%	84.50^{a}
Albendazole 75%	75.67^{ab}
Blended 100%	72.67^{b}
Methanol 100%	70.18^{bc}
Distilled Water 100%	62.50^{cd}
Blended 75%	62.17^{cd}
Albendazole 50%	61.33^{cde}
Soaked 100%	60.33^{cdef}
Methanol 75%	58.67^{def}
Distilled Water 75%	52.00^{efg}
Soaked 75%	51.67^{efg}
Blended 50%	51.17^{fg}
Albendazole 25%	50.83^{fgh}
Methanol 50%	47.17^{gh}
Soaked 50%	46.17^{gh}
Distilled Water 50%	43.50^{gh}
Blended 25%	41.00^{hi}
Methanol 25%	31.00^{ij}
Distilled Water 25%	29.33^{j}
Soaked 25%	28.33^{j}
PBS	0.00^{k}

The results showed that there was a difference between *N. brachypus* tuber preparations in their ability to inhibit egg hatching. This was probably because of the difference in the type of phytochemicals present in the extracts due to preparation differences. The blended sample had the highest inhibition activity probably because the sample was a whole juice extract of *N. brachypus* compared to methanol and aqueous sample where the sample was air-dried first and further subjected to continuous heating during soxhlet extraction. The high inhibition activity observed in blended samples suggests a possible synergistic relationship of compounds that can interact with multiple molecular targets in the developmental stages of the parasite (Oliveira *et al*., 2017). The degradation of phytochemicals upon thermal treatment of broccoli florets had been reported (Zhang & Hamauzu, 2004). In addition, thermal processing in the oven and sun-drying techniques rupture the cell structure of *T. arjuna* bark which may lead to the to the loss of thermolabile compounds (Bernard *et al.*, 2014). Differences in plant material, solvent, and method of extraction can lead to differences in secondary metabolites present in the plant extract (Zangueu *et al*., 2018). The ability of acetone and methanol to extract compounds of a wide polarity range at a high yield is greater than that of ethanol or water (Zangueu *et al*., 2018). The soaked sample probably had the lowest inhibition activity because of the limited time of soaking. As it is known, the solvents and protocols used for extraction promote variation in concentrations and the classes of secondary metabolites present in extracts (Marie-Magdeleine, Hoste, Mahieu, Varo, & Archimède, 2009), which could have large effects on the activities of botanical compounds (Eloff, 1998). The results in Figure 4.5 show that Floatation samples had a higher egg hatch inhibition percentage of 52.34% compared to sedimentation of 50.52%.

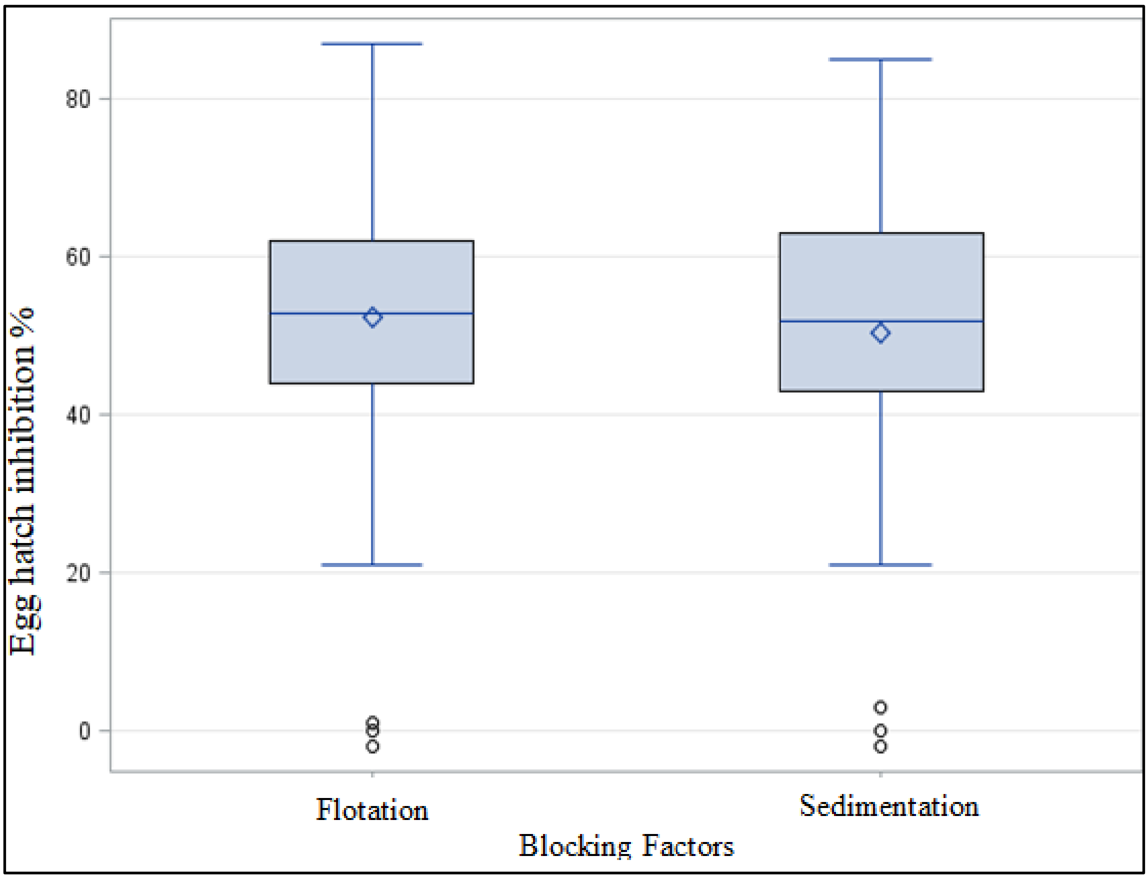

Figure 4.5: Effect of different *N. brachypus* preparations used on types of helminths prepared by two different methods (Floatation and sedimentation).

The ANOVA Table 4.5 below shows that there was a significant difference between IC_{50} values of the treatments tested (***P***<0.05) on egg hatch inhibition. There was no significant difference between IC_{50} values of *N. brachypus* preparations (***P***>0.05) on types of helminths prepared by different methods (Floatation and sedimentation).

Table 4.5: ANOVA table for the IC_{50} concentrations for the effect of different *N. brachypus* preparations used against the hatching of helminth eggs from cattle and on types of helminths prepared by two different methods (Floatation and sedimentation).

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
TRT	4	9759.50	2439.87	22.69	<.0001
Block	1	24.66	24.66	0.23	0.64

The results displayed in Figure 4.6 show that the fresh and soaked *N. brachypus* extracts had the highest IC_{50} concentration of 71.10% followed by dried and Soxhlet extracted with distilled water sample (70.33%), dried and Soxhlet extracted with methanol (59.11%), fresh and blended tuber sample (46.80%), and the positive control had the lowest IC_{50} concentration (22.54%). The Post Hoc analysis showed that there was no significant difference between the IC_{50} concentration of dried and Soxhlet extracted with methanol (59.11%) and fresh and blended tuber sample (46.80%).

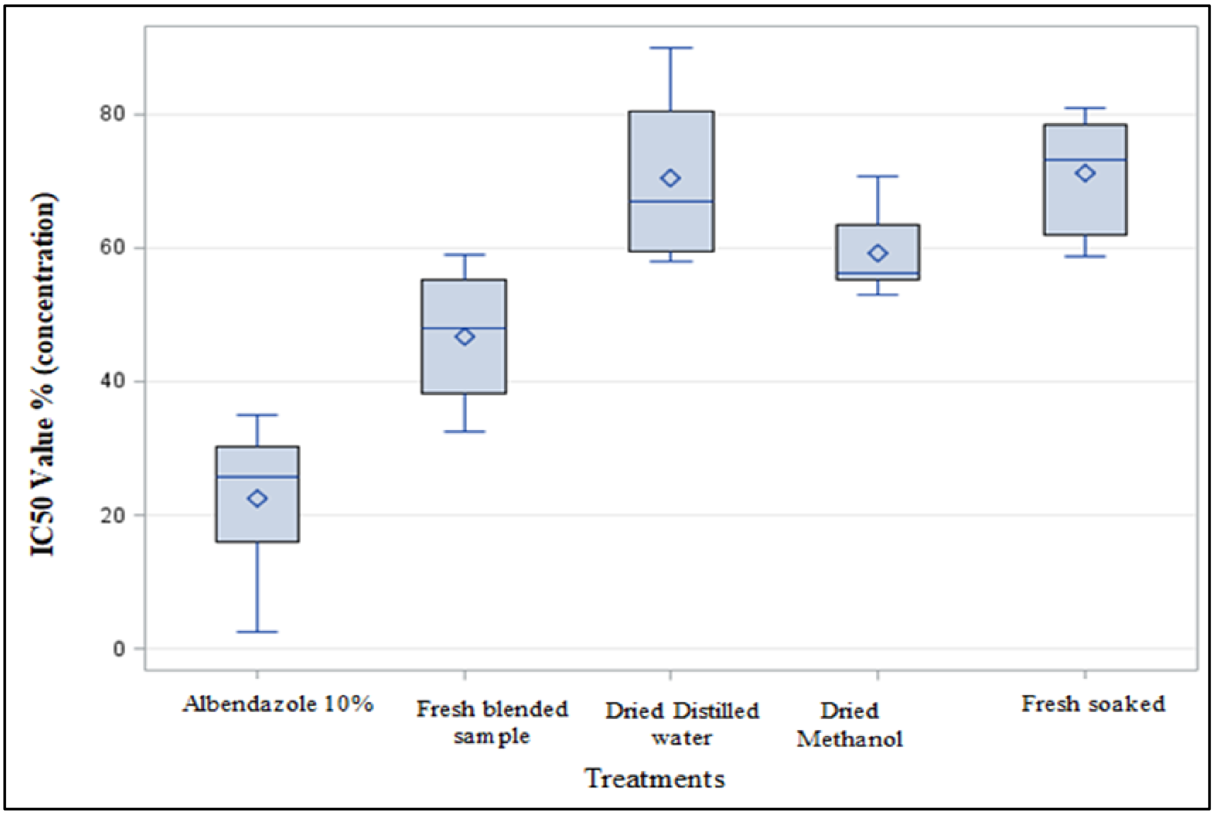

Figure 4.6: IC_{50} concentrations for the effect of different *N. brachypus* preparations that were tested against the hatching of helminth eggs from cattle.

4.3.3 The effect of *N. brachypus* tuber extracts on helminths larvae mortality

Data was tested for normality in SPSS version 20 and was found to be normally distributed ($\boldsymbol{P}>0.05$) as determined by the Shapiro-Wilk statistic. The ANOVA Table 4.6 below shows that there was a significant difference between the treatments tested ($\boldsymbol{P}<0.05$) on larval mortality.

Table 4.6: ANOVA table for the effect of different *N. brachypus* preparations on larval mortality of helminths associated with cattle.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	20	23372.22	1168.61	22.14	<.0001

The larval mortality activity (%) was dilution fcator-dependent. As the dilution factor of plant extracts decreased the larval mortality percentage also increased (Table 4.7). The Post Hoc analysis showed that there was no statistical difference between Albendazole 75%, Albendazole 50%, Methanol 100%, Soaked 100%, Distilled water 100%, Blended 100%, Blended 75%, Soaked 75%, Distilled water 75%, and Methanol 75% as they shared the same superscript B (larval mortality % 51.67%-73.33%). The highest larval mortality activity was recorded for Albendazole 100% (86.67%) and the lowest for Soaked 25%. The Post Hoc analysis showed that the activity of Soaked 25% was statistically similar to that of Blended 25%, Methanol 25%, Distilled water 25%, Methanol 50%, Soaked 50%, Blended 50%, and Distilled Water 50% as they shared the same superscript G.

Table 4.7: Effect of different *N. brachypus* preparations were used on larval mortality of helminths from cattle compared to a conventional drug Albendazole 10% (Standard Error was 4.19). Means with the same superscript are statistically different (P<0.05).

Treatment	**LSMean Larvae mortality %**
Albendazole 100%	86.67^{a}
Albendazole 75%	73.33^{ab}
Blended 100%	73.33^{ab}
Albendazole 50%	65.00^{abc}
Methanol 100%	61.67^{bcd}
Soaked 100%	58.33^{bcd}
Distilled Water 100%	56.67^{bcde}
Blended 75%	53.33^{bcde}
Soaked 75%	53.33^{bcde}
Distilled Water 75%	51.67^{bcde}
Methanol 75%	51.67^{bcde}
Albendazole 25%	43.33^{cdef}
Distilled Water 50%	43.33^{cdef}
Blended 50%	41.67^{defg}
Soaked 50%	40.00^{defg}
Methanol 50%	35.00^{efg}
Ditstilled Water 25%	28.33^{fgh}
Methanol 25%	25.00^{fgh}
Blended 25%	21.67^{fgh}
Soaked 25%	20.00^{gh}
PBS	8.33^{h}

The ANOVA Table 4.8 below shows that there was a significant difference between IC50 values of the treatments tested (***P***<0.05) on larval mortality.

Table 4.8: ANOVA table for the IC_{50} concentrations for the effect of different *N. brachypus* preparations used on larval mortality of helminths associated with cattle.

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Treatment	4	4392.74	1098.19	6.58	0.0073

The highest IC_{50} concentration was recorded for soaked tuber samples (78.88%), followed by Methanol (76.54%), Blended (66.49%), and Distilled water (65.75%), and the lowest for Albendazole (31.19%) (Table 4.9). There was no statistical difference between the IC_{50} concentration of Albendazole and Distilled water for larval mortality. There was also no statistical difference between the IC_{50} concentration of Soaked, Methanol, Blended, and Distilled water samples. These results are shown in Appendix 3 below.

It was noted that treatments with the highest egg hatch inhibition activity had the lowest IC_{50} concentration. On the other hand the lower the anthelmintic activity the higher the IC_{50} concentration. Soaked tuber samples had the highest IC_{50} concentrations for both egg hatch inhibition and larval mortality activities. The highest IC_{50} concentration was recorded for larval mortality compared to egg hatch inhibition. This probably meant that higher concentrations of *N. brachypus* plant extracts are needed to kill 50% of larvae compared to inhibiting 50% of eggs from hatching. This might also mean that eggs were more susceptible to the plant extracts compared to larvae. These differences in values have been noted to be attributed to the sensitivity of each developmental stage. L1 was reported to be the most sensitive stage because the larva’s pharynx is more sensitive to the paralysis caused by drugs, eggs are more resistant than L1 due to their hard and resistant shell, and L3 larvae are more resilient due to their double sheath (Molan, Waghorn, & McNabb, 2002). These facts lead to the requirements for high or low contents of active compounds to achieve IC_{50} values for egg hatch inhibition and larval mortality.

Table 4.9: IC50 dilution factor for the effect of different *N. brachypus* preparations used on larval mortality of helminths associated with cattle. (Standard Error of Mean was 7.46). Means with the same superscript are statistically different (P<0.05).

Treatment	**LSMean IC50 Larvae mortality**
Soaked	78.88[a]
Methanol	76.54[a]
Blended	66.49[a]
Distilled Water	65.75[ab]
Albendazole	31.19[b]

4.4 The effect of *N. brachypus* extracts on adult worms

Data was tested for normality in SPSS version 20 and was found to be normally distributed (***P***>0.05) as determined by the Shapiro-Wilk statistic. The ANOVA Table 4.10 below shows that there was a significant difference between the treatments tested (***P***<0.05) and the time of exposure to adult worm mortality. There was a significant one-way interaction between the treatments and time of exposure (***P***<0.05).

Table 4.10: ANOVA table for the effect of different *N. brachypus* preparations and time of exposure on the mortality of *Eisenia foetida* worms.

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Treatment	20	267.62	13.38	80.29	<0.0001
Time (hour)	5	949.13	189.83	1138.96	<0.0001
Treatment*Time (hour)	100	192.76	1.93	11.57	<0.0001

The mortality of worms was plant extract dilution factor-dependent and time-dependent. The mortality of worms increased as the dilution factor decreased of tuber preparations increased. Also, mortality increased as the time of exposure increased. The highest mortality was recorded at undiluted-100% and after 24 hours of exposure. The lowest mortality was recorded at 25% dilution and after 30 minutes of exposure. All treatments showed no worm mortality after 30 minutes of exposure. Treatments Albe100, Albe75, Blend100, Soak100, Meth100, Meth75, and DW100 showed some anthelmintic activity after 1 hour of exposure. The treatment Albe100 showed 100% worm mortality after 2 hours of exposure. Meth100, Soak100, and Blend100 showed 100% worm mortality after 6 hours of exposure. Albe75, Albe50, Albe25, Blend75, Blend50, Soak75, Soak50, Meth75, Meth50, and Meth25 exhibited 100% worm mortality after 24 hours of exposure. All treatments except for the negative control showed anthelmintic activity ± 80% after 24 hours of exposure.

A number of tuberous plants have been cited to exhibit anthelmintic action against *Eisenia foetida*. These are *Azadirachta indica* (Priya et al., 2015), *Curcuma amada* (Gill *et al*., 2011), *Curcuma caesia* (Randeep *et al*., 2011), *Costus speciosus*, *Dioscorea bulbifera* (Kosalge *et al.*, 2009b), and *Gloriosa superba* (Suryavanshi *et al*., 2012).

Table 4.11: Effect of different *N. brachypus* preparations and time of exposure on the mortality of *Eisenia foetida* worms.

TIME (hours)	**0.5**	**1**	**2**	**4**	**6**	**24**
TREATMENTS	**ADULT WORM MORTALITY % (LSMEANS)**					
Albe100	0	73.33	100	100	100	100
Albe75	0	33.33	46.67	73.33	86.67	100
Albe50	0	0	26.67	60	80	100
Albe25	0	0	0	20	26.67	100
Blen100	0	26.67	26.67	60	100	100
Blen75	0	0	26.67	53.33	73.33	100
Blend50	0	0	0	26.67	53.33	100
Blend25	0	0	0	0	20	86.67
Soak100	0	20	26.67	66.67	100	100
Soak75	0	0	13.33	40	53.33	100
Soak50	0	0	0	26.67	46.67	100
Soak25	0	0	0	0	26.67	93.33

DMSO	0	0	0	0	0	6.67
Meth100	0	6.67	33.33	66.67	100	100
Meth75	0	6.67	20	53.33	73.33	100
Meth50	0	0	6.67	20	26.67	100
Meth25	0	0	0	0	33.33	100
DW100	0	6.67	26.67	40	66.67	100
DW75	0	0	0	40	66.67	86.67
DW50	0	0	0	26.67	53.33	80
DW25	0	0	0	0	26.67	80

The exhibited anthelmintic effect of the *N. brachypus* extracts might be attributed to the existing secondary metabolites. Preliminary phytochemical research of *N. brachypus* tubers indicated the presence of phytochemicals such as saponins, flavonoids, essential oils, alkaloids, tannins, phenols, and coumarins. It has been reported that these compounds may work either singly or in combination to control helminths. Kaufman, Cseke, Warber, Duke, and Brielmann (2003), described the synergistic interactions to cause the effectiveness of phytomedicines that lead to better activity of some individual constituents. In addition, phytochemicals action may be additive or antagonistic in manner acting at single or at multiple target sites (Wynn & Fougere, 2007).

GCMS analysis of *N. brachypus* showed the presence of anthelmintic compounds such as Coumarine,6-(7-hydroxycoumarin-8-yl)-7-methoxy, Benzo(b)thiophene-2-carboxaldehyde,7-methyl, Methyleugenol, n- Hexadecanoic acid, Tetradecane, and 4-hydroxy-3-methoxycinnamic acid. Joshi, Kommuru, *et al*. (2011), assimilated that tannins may exert anthelmintic activity by reducing hatching, blocking its development to the infective larval stage, and decrease in adults' motility. The observed ovicidal activity of plant extracts could be because the active compounds penetrate the eggshell and stop the segmentation of the blastomeres or paralyze the larvae inside embryonated eggs (Jeannette, Olivia, Komtangi, CF, & Mbida, 2011). Saponins have been reported to inhibit egg hatching (Camurça-Vasconcelos et al., 2007; Eguale, Tilahun, Debella, Feleke, & Makonnen, 2007). Polyphenols and tannins increase the supply of digestible proteins by animals via forming protein complexes in the rumen and interfere with energy generation in the helminths parasites by uncoupling the oxidative phosphorylation, causing a decrease in gastrointestinal metabolism which leads to paralysis and death of helminths (Tiwari, Kumar, Kaur, Kaur, & Kaur, 2011).

Anthelmintic activity increased as the concentration of plant *N. brachypus* plant extracts increased. This was probably because of an increase in the saturation of target receptors. This was reported by Lullman, Morh, and Bieger (2017) who said that receptors get saturated with increasing doses of the active ingredients. At higher concentrations, all binding receptors on the worms were likely occupied, thus, leading to hyperpolarization of membranes thereby limiting excitation and impulse transmission which leads to flaccid paralysis of worm muscles and their death (Wasswa & Olila, 2006).

4.4 Conclusion and recommendations

Ethno-medicinal plants are potential sources of active compounds to develop sustainable commercial anthelmintics. The *in vitro* anthelmintic activity of tested plant preparations was characterized by a decrease in egg hatching, larvae, and adult worm mortality. These treatments may reduce the hatchability of the eggs excreted in the feces, resulting in both a reduced risk of reinfection and lightened worm loads by decreasing pasture contamination. Accordingly, they have the potential to contribute to controlling gastrointestinal parasites of ruminants. Methanol and Distilled water extracts of *N. brachypus* can be used as alternatives in drug discovery because of their low toxicity to erythrocytes. The use of fesh blended N. brachypus extracts was recommendend for effective control of helminths eggs and larvae control in cattle as they had lower IC_{50} concentrations in both assays. Also, *N. brachypus* plant extracts had significant adult worm control.

Once a plant has proven its efficiency in vitro, further in vivo testing will be necessary to confirm the obtained results and evaluate risks, side effects, and future applicability. Therefore, *in vivo* anthelmintic evaluation of these plants is imperative before their clinical use.

CHAPTER 5: Efficacy of Phytochemical Extracts from *Neorautanenia brachypus* on the control of Strongyloides and Coccidia in ruminant livestock

Mpofu, M.C., Gomo, C., Mugumbate, G., Mashingaidze, A.B., Chikwambi, Z., and Murungweni, C. 2022. Efficacy of Phytochemical Extracts from *Neorautanenia brachypus* on Helminths control in ruminant livestock. Journal of Verterinary Medicine and Science

Abstract

In the face of anthelmintic resistance to traditional medications, plant extracts have gained appeal as a viable way for controlling helminths. One of the plants that have shown efficiency against helminths is *Neorautanenia brachypus*. In past studies, its tubers were cut and fed directly to ruminant livestock as samples in *in vivo* testing. *In vivo* efficacy of *N. brachypus* extracts against helminths in ruminants has not been reported. This work fills that void in the literature. Seventeen goats blocked by sex into 8 does and 9 bucks and 12 steers blocked by breed into 6 Brahman and 6 Mashona were selected and were randomly divided into three groups. Animals in each group were randomly allocated to 1 negative control, 2 positive control-Albendazole drug, and 3 test treatments *N. brachypus* herbal formulation. Fecal samples were collected on days 0, 7, 14, 28, and 35 after treatment. The efficacy of the treatments against gastrointestinal worms was determined by counting eggs per gram. The results showed that there was no significant difference (***P***>0.05) in eggs per gram reduction of Strongyloides between untreated group, group treated with herbal-based drug, and group treated with conventional drug in week 1 and week 2 for both cattle and goats. Significant differences in eggs per gram reduction of Strongyloides (P<0.05) were noticed from week 3 to week 5. The results showed that there was no significant difference (***P***>0.05) in eggs per gram reduction of coccidia between untreated group, group treated with herbal-based formulation, and group treated with conventional drug in week 1 and week 3 for both cattle and goats. Significant differences in eggs per gram reduction of Strongyloides (***P***<0.05) were noticed from week 4 to week 5. The ranks for reduction in eggs per gram for both coccidia and Strongyloides across species increased from week 1 to week 5 for the untreated group. However, the ranks for reduction in eggs per gram for both coccidia and Strongyloides in goats and cattle decreased from week 1 to week 5. There was no significant change in the weight of goats and cattle between the start and end of the experimental period (***P***>0.05). The results of this study are suggestive of promising anthelmintic activity of the herbal-based drug for both Strongyloides and coccidia in cattle and goats.

Keywords: Anthelmintic, *in vivo*, *Neorautanenia brachypus*, herbal-based drug, anthelmintic resistance, tubers, Strongyloides, coccidia

5.1 Background

Anthelmintic resistance is on the rise throughout the world. The short time it takes for livestock parasites to develop resistance to standard anthelmintics (between 2 and 10 years) is a major source of concern (French, 2018). In many parts of the United States, South America, and South Africa, anthelmintics like benzimidazoles and ivermectins are no longer effective (Kaplan & Vidyashankar, 2012; Shalaby, 2013). Overuse of commercial medications and changes in cattle management are thought to be the causes of anthelmintic resistance. Other disadvantages of using synthetic medications for helminths control include environmental, ecological, and economic consequences. Due to medication residues in feces, synthetic anthelmintics have been shown to diminish soil invertebrate diversity (Cooke, Morgan, & Dungait, 2017; Numa, Verdú, Rueda, & Galante, 2012).

Gastrointestinal nematodes such as Strongyloides are responsible for substantial loss in the production of goats and cattle. They are a major barrier to efficient and profitable livestock production by causing economic losses due to reduced weight gains, poor growth rates, and visceral organ condemnation at slaughter and mortality. Coccidia are among the factors that interfere in livestock development because of economic losses that they cause concerning low herd productivity, delayed animal development, death, and significant expenses on management and medication (Alam *et al*., 2014; Dkhil, 2013). Anticoccidial drugs (e.g. dindamycin, narasin, and decoquinate) against coccidia are harmful to the host tissues due to several side effects (Wunderlich, Al-Quraishy, Steinbrenner, Sies, & Dkhil, 2014).

Plant-derived anthelmintics are promising alternatives in the face of anthelmintic resistance. Plants naturally manufacture about 60,000 chemical compounds to repel herbivores, eliminate diseases, and interact with other species such as pollinators, according to Wink (2010). *Cyperus compressus* methanol root extract was found to be efficient in the control of *H. diminuta* and *S. obvelata* in *in vitro* investigations (Soren & Yadav, 2020). The anthelmintic efficacy of *Praecitrullus fistulosus* against *Pheretima posthuma* was evaluated in a study, and results revealed that 5% of methanol extracts of the plant had anthelmintic activity (Ishnava & Patel, 2020). Plant extracts from *Justicia adhatora*, *Vernonia amygdalina*, *Mikania micrantha*, and *Momordica charantia* demonstrated considerable anthelmintic action causing *Pheretima posthuma* mortality (Ikbal, Rajkhowa, Singh, Choudhury, & Sahu, 2020). However, farmers and shepherds who hold this ethnobotanical knowledge are rapidly disappearing, with them perhaps a potential long-term solution to anthelmintic resistance (French, 2018). Thus there is a need to investigate these medicinal plants and document their use.

Plant-based anthelmintic research uses ethnobotanical information as a guide, but not as a foundation. These results are "hearsay" in the absence of chemical analyses and *in vitro* and *in vivo* studies (French, 2018). As a result, there was a need to investigate the in vivo efficacy of *Neorautanenia brachypus* herbal formulation against ruminant helminths. *Neorautanenia brachypus* (Zhombwe in Shona) is a leguminous tuberous plant that is found in Zimbabwe's southeast Lowveld. The plant has a variety of purposes, including serving as a feed bridge between seasons to help cattle survive drought. It also has therapeutic qualities and has proven to be an effective anthelmintic properties (Murungweni *et al*., 2012).

5.2 Materials and methods

5.2.1 Study site

The research was carried out at Chinhoyi University of Technology (CUT) farm, GPS coordinates -17.34943, 30.21029. It is located along Harare to Chirundu road and also the Chinhoyi to Harare railway. It lies on the West side of the Hunyani River. Chinhoyi University of Technology farm is in Agro-natural region 2b which receives rainfall ranging from 750-1000mm per year. It’s a warm and temperate climate with an average annual temperature of around 20-24 degrees Celsius. It is in Mashonaland West province, Makonde district of Zimbabwe.

5.2.2 Sample preparation

Five white tubers of *N. brachypus* were collected from Zanamwi farm in Chikombezi, Zimbabwe, south of Gonarezhou National Park, GPS coordinates 21°45'0" S and 31°19'0" E. The tubers were cleaned to remove soil and other adherent debris, and then the skins were peeled off. The tubers were then cut into smaller pieces with a maximum volume of 1 cubic centimeter. The juice containing phytoconstituents was extracted from the tubers by blending for 20 seconds and straining off the fibrous material leaving behind the white liquid extracts.

5.2.3 Ethical clearance

This study was approved by the Academic Board of the Department of Animal Production and Technology, Chinhoyi University of Technology, and the Department of Research and

Specialist Services (DR&SS), Zimbabwe. Sample collection was carried out under the supervision of a qualified veterinarian and according to the Chinhoyi University of Technology 'Guidelines for Animal Handling and Sample Collection', which conforms to European Union Directive 2010/63 regarding the protection of animals used in scientific experiments.

5.2.4 Feacal Egg Count Reduction Test

Firstly, on Day 0 fecal samples were collected, and carried out fecal counts to determine the worm burden of each experimental animal. Seventeen goats blocked by sex into 8 does and 9 bucks and 12 steers blocked by breed into 6 Brahman and 6 Mashona were selected as experimental units for this experiment. Each block of animals was randomly allocated to pens for housing. The experimental units were divided into 3 clusters (high, medium, and low) according to the worm load of coccidia and Strongyloides. Animals in each cluster were randomly allocated to the three treatments, 1 untreated group- negative control, 2 group treated with conventional drug- positive control, and 3 the group treated with herbal drug- test treatment.

Experiment 1 (Goats)

Group 1 not treated- 3 females, 3 males

Group 2 conventional drug- 2 females, 3 males

Group 3 herbal drug- 3 females, 3 males

Experiment 2 (Cattle)

Group 1 not treated- 2 Mashona, 2 Brahman

Group 2 conventional drug- 2 Mashona, 2 Brahman

Group 3 herbal drug- 2 Mashona, 2 Brahman

Animals were dosed on day 1 using Albendazole (positive control) at the rate of 10 mg/kg thus 40 mL per animal for cattle and 7.5 mg/kg thus 1.5 mL per animal for goats. All animals were weighed on day 1 and day 35. Cattle and goats exposed to the herbal mixture were dosed at the rate of 40 mL per animal for cattle and 1.5 mL per animal for goats. Cattle were fed with 25 kg of beef maintenance meal and 22 kg of Katambora hay per pen in the morning and 22 kg of Katambora hay per pen in the afternoon. Does were fed with 5 kg goat maintenance pellets and 6 kg of Katambora hay in the morning and 6 kg hay in the afternoon. Bucks were given 6 kg goat pellets and 7 kg of Katambora hay in the morning and 7 kg hay in the afternoon. Water was provided all the time. Fecal samples were collected on days 7, 14, 28, and 35 after treatment. A minimum of 5 g of feces was collected from each animal directly from the rectum. Samples were placed in individually sealed containers and returned rapidly to the laboratory for egg counts. The floatation and sedimentation procedures were carried out to determine egg counts per gram. Eggs per gram (EPG) were counted using the McMaster Method.

5.2.5 Statistical analysis

Data was tested for normality in SPSS version 20 and was found to be not normally distributed (***P***<0.05) as determined by the Shapiro-Wilk statistic. The results were analyzed using the Wilcoxon Non- Parametric test using SAS version 9.4. Kruskal-Wallis Chi-square was used to determine significant differences between treatments.

5.3 Results and discussion

The results displayed in Figure 5.1 show that there was no significant difference between treatments for Strongyloides EPG reduction in cattle at week 1 and week 2 (***P***>0.05). Significant differences between treatments for Strongyloides load are recorded from week 3 to week 5 (***P***<0.05). The rank of Strongyloides EPG reduction increased from week 1 (6.9) up to week 5 (10.5) for the untreated group (control). The EPG reduction ranks were highest at week 1 and started to decrease from week 2 up to week 5 for the groups treated with conventional drug (Albendazole) and herbal-based drug. The rank for the group treated with the conventional drug was higher at week 3 (5.1) compared to the group treated with herbal-based drug (4.5). However, the trend was vice versa in week 4. The ranks for the group treated with the conventional drug and the group treated with the herbal drug were the same in week 2 (5) and week 5 (4.2).

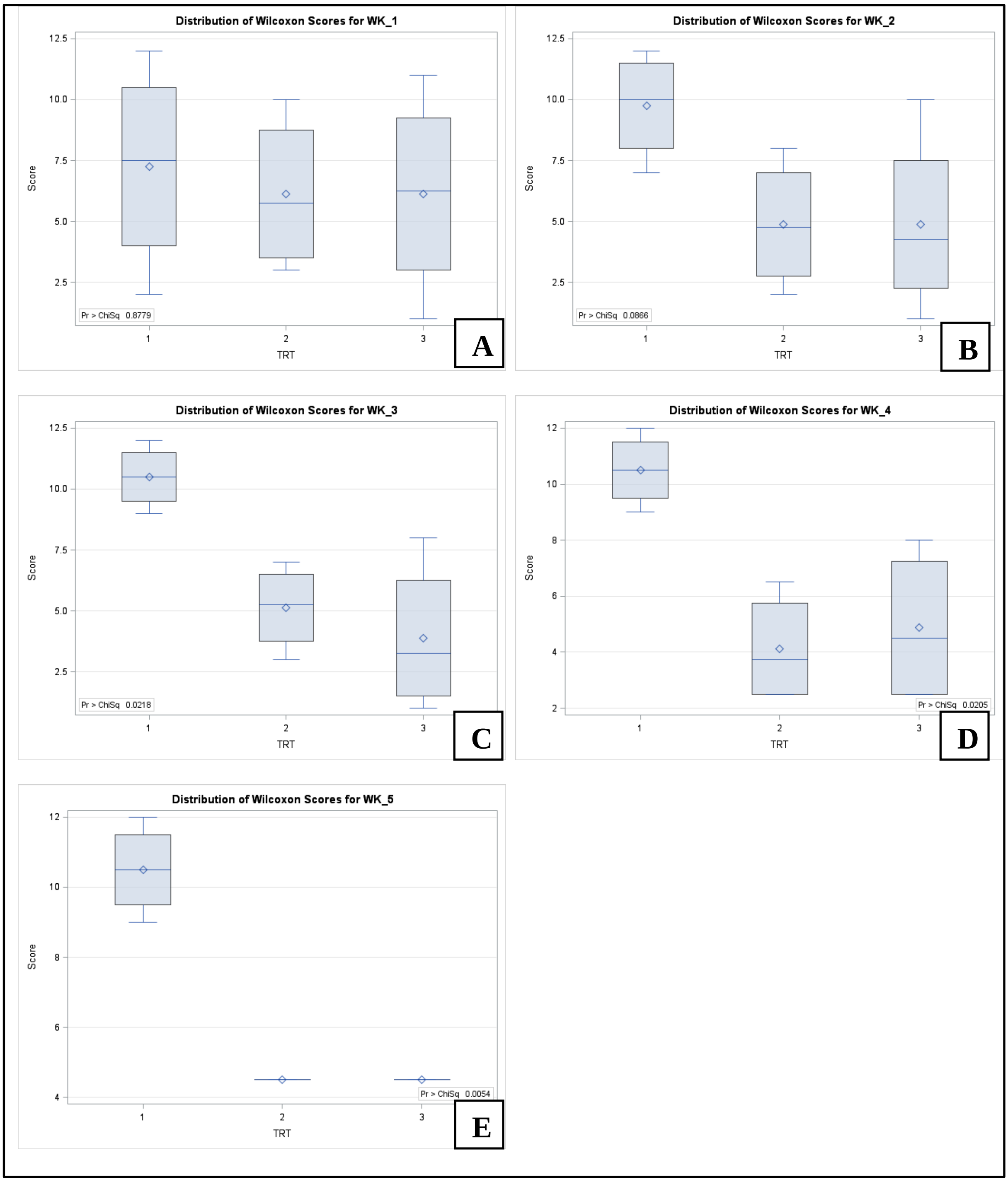

Figure 5.1: Load of intestinal Strongyloides ranks in cattle from Week 1 to Week 5 (A-E) of research testing the effectiveness of the formulated herbal-based anthelmintic drug. TRT- Treatment, 1- no treatment group (negative control), 2- group treated with conventional dosing drug (positive control), and 3- group treated with herbal dosing drug (test treatment).

The results displayed in Figure 5.2 below show that there was no significant difference between Strongyloides EPG reduction in goats at week 1 and week 2 (***P***>0.05). Significant differences between treatments for Strongyloides EPG reduction load are recorded from week 3 to week 5 (***P***<0.05). The drop in EPG may be due to the direct effect of *N. brachypus* and Albendazole through paralysis and expulsion of worms (Cormanes, Portugaliza, & Quilicot, 2016; Jabbar, Zaman, Iqbal, Yaseen, & Shamim, 2007). The rank of Strongyloides EPG reduction increased from week 1 (7) up to week 5 (14) for the untreated group (control). The ranks were highest at week 1 and started to decrease from week 2 up to week 5 for the groups treated with conventional drug (Albendazole) and herbal-based drug. The ranks for the group treated with the conventional drug were higher from week 2 to week 5 compared to the group treated with herbal-based drug.

The results for the effect of *N. brachypus* on Strongyloides were similar to those reported by Murungweni *et al*. (2012), where the conventional drug and *N. brachypus* reduced the load of Strongyloides in goats and cattle (***P***<0.05) after a month. A tuberous plant in the same genera as *N. brachypus*, *N. mitis* was reported to have anthelmintic activity against helminths (Adebayo, Olubisi, Adebisi, & Idowu, 2018). The reduction in EPG in this study could have been due to the conventional drug and the herbal-based drugs having affected the egg-laying ability of the female adult worms (Jabbar *et al*., 2007; Maphosa *et al*., 2012b). Other tuberous plants that have been reported to have anthelmintic effects against Strongyloides are *Zingiber officinale* (Kiambom *et al*., 2021), *Oroxylum indicum* (Downing, 2000), *Allium sativum* (Tavassoli *et al*., 2018), and *Azadirachta indica* (Suhaimi *et al*., 2016).

The results displayed in Figure 5.3 show that there were no significant differences between treatments for coccidia load in cattle from week 1 to week 3 (***P***>0.05). Significant reductions in EPG for coccidia between treatments were noticed from week 4 to week 5 (***P***<0.05). The rank of coccidia EPG increased from week 1 (9) up to week 5 for the untreated group (control). The EPG reduction ranks were lower for the group treated with the herbal-based drug compared to the group treated with the conventional drug from week 2 to week 4. The rank for EPG reduction was the same for the group treated with the herbal-based drug compared to the group treated with the conventional drug was the same at week 5. The EPG reduction was quicker for the group treated with the herbal-based drug compared to the group treated with the conventional drug. The synergistic activity of constituents of the herbal-based drug may have caused a quicker reduction in EPG for coccidia in cattle compared to the conventional drug (Abbas *et al*., 2020).

The results displayed in Figure 5.4 show that there were no significant differences between coccidia EPG reduction in goats from week 1 to week 3 ($\boldsymbol{P}$>0.05). Significant differences between treatments for coccidia load were recorded from week 4 to week 5 ($\boldsymbol{P}$<0.05). The rank of coccidia EPG reduction in goats increased from week 1 (8) to week 5 (14) for the untreated group. The rank started to decrease from week 2 (7) for the group treated with the herbal-based drug and from week 3 (8) for the group treated with the conventional drug. The ranks were lower for the group treated with herbal drug at week 2 (7) and week 3 (6) compared to the group treated with conventional drug (9). However, the trend was vice versa in week 4 and week 5.

Tuberous plants that have been reported to have anthelmintic effects against coccidia are *Zingiber officinale*, *Curcuma longa* (Ashraf *et al*., 2020), and *Allium Sativum* (Worku *et al*., 2009). The anticoccidial activity of *N. brachypus* might have been attributed to the antioxidant properties of its phytochemicals. Plants that are rich in antioxidant compounds such as V*itis vinifera*, *Humulus lupulus*, *Camellia sinensis*, *Ageratum conyzoides*, *Sideritis scardica*, *Pinus radiate*, and *Artemisia vestita* have been reported to have excellent anticoccidial properties (Abbas *et al.*, 2017). The groups treated with the herbal-based drug showed lower coccidia EPG as compared to the untreated group. This was probably because the phytoconstituents in the herbal based mixture acted directly against the parasites' developmental stages or indirectly by interaction with intestinal microflora (Abbas *et al*., 2017). Plant extracts also exact anticoccidial activity through elevation of parasite-specific IgA that can bind and damage sporozoites, impairing their extracellular differentiation and thereby preventing parasite invasion and intracellular development (Guo *et al*., 2004; Yang *et al*., 2019). They also inhibit oocytes sporulation (Fatemi, Razavi, Asasi, & Torabi Goudarzi, 2015; Yang *et al*., 2019).

The anthelmintic effects exhibited by the herbal-based preparation might have been due to the presence of phytochemicals with anthelmintic properties (Dkhil *et al*., 2019; Wamburu *et al*., 2013). Phytochemical screening of *N. brachypus* extracts showed the presence of saponins, phenols, alkaloids, coumarins, tannins, terpenoids, essential oils, quinones, and flavonoids. Some phytochemicals such as flavonoids, essential oils, alkaloids, saponins, triterpenes, and tannins have been reported to control gastrointestinal nematodes (Barrau, Fabre, Fouraste, & Hoste, 2005; Wamburu *et al*., 2013). Tannins act by impairing the feeding and reproduction activities of the parasites and also cause parasite cuticle damage. Saponins cause cytolytic action by affecting the cell membranes and increasing the permeability of cells (Geidam, Ambali, & Onyeyili, 2007). Alkaloids disturb the nervous activities of nematodes and affect their gastric motility also (Lateef, Iqbal, Rauf, & Jabbar, 2006).

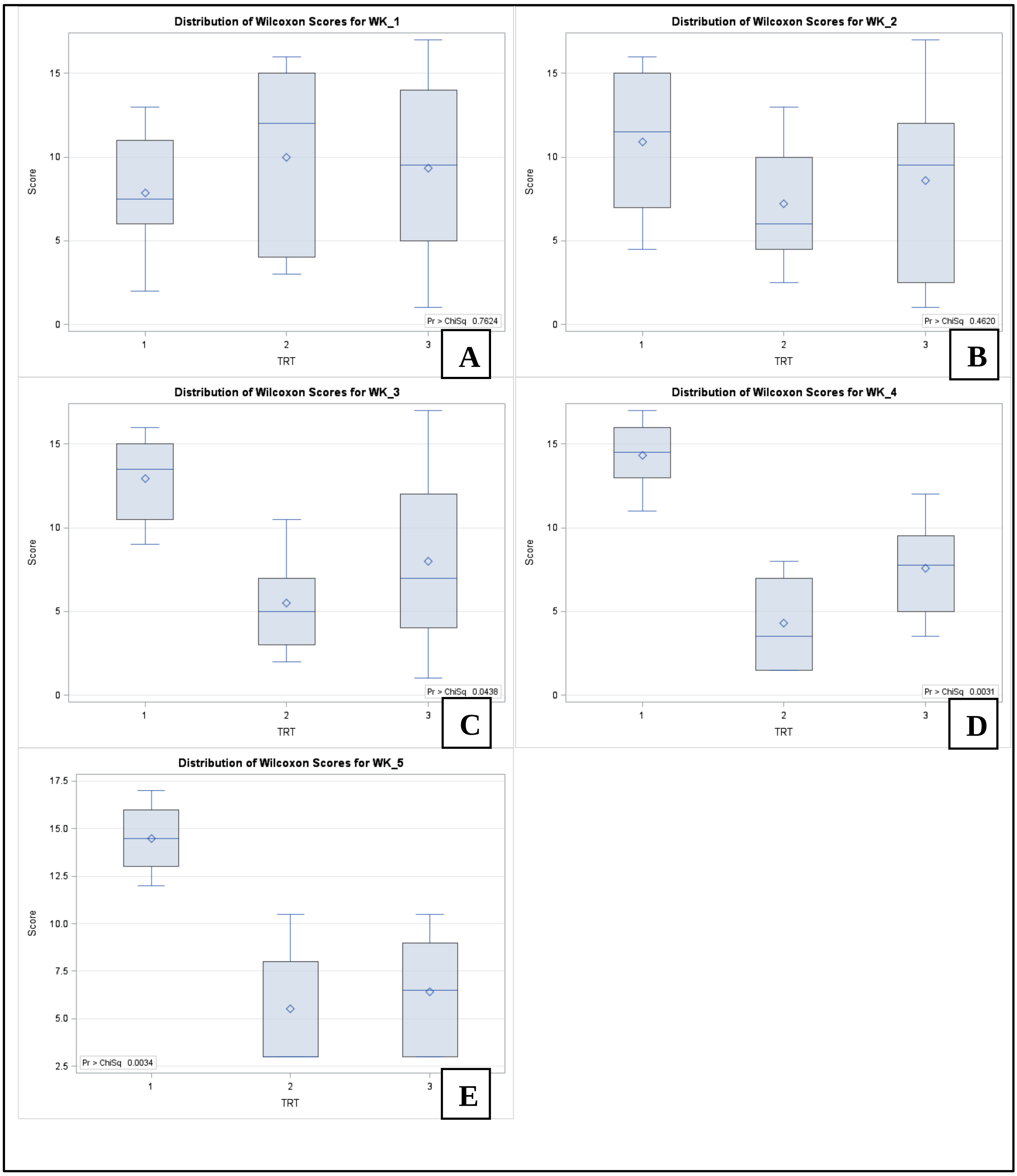

Figure 5.2: Load of intestinal Strongyloides in goats from Week 1 to Week 5 (A-E) of research testing effectiveness of the formulated herbal-based anthelmintic drug. TRT-Treatment, 1- no treatment group (negative control), 2- group treated with conventional dosing drug (positive control), and 3- group treated with herbal dosing drug (test treatment).

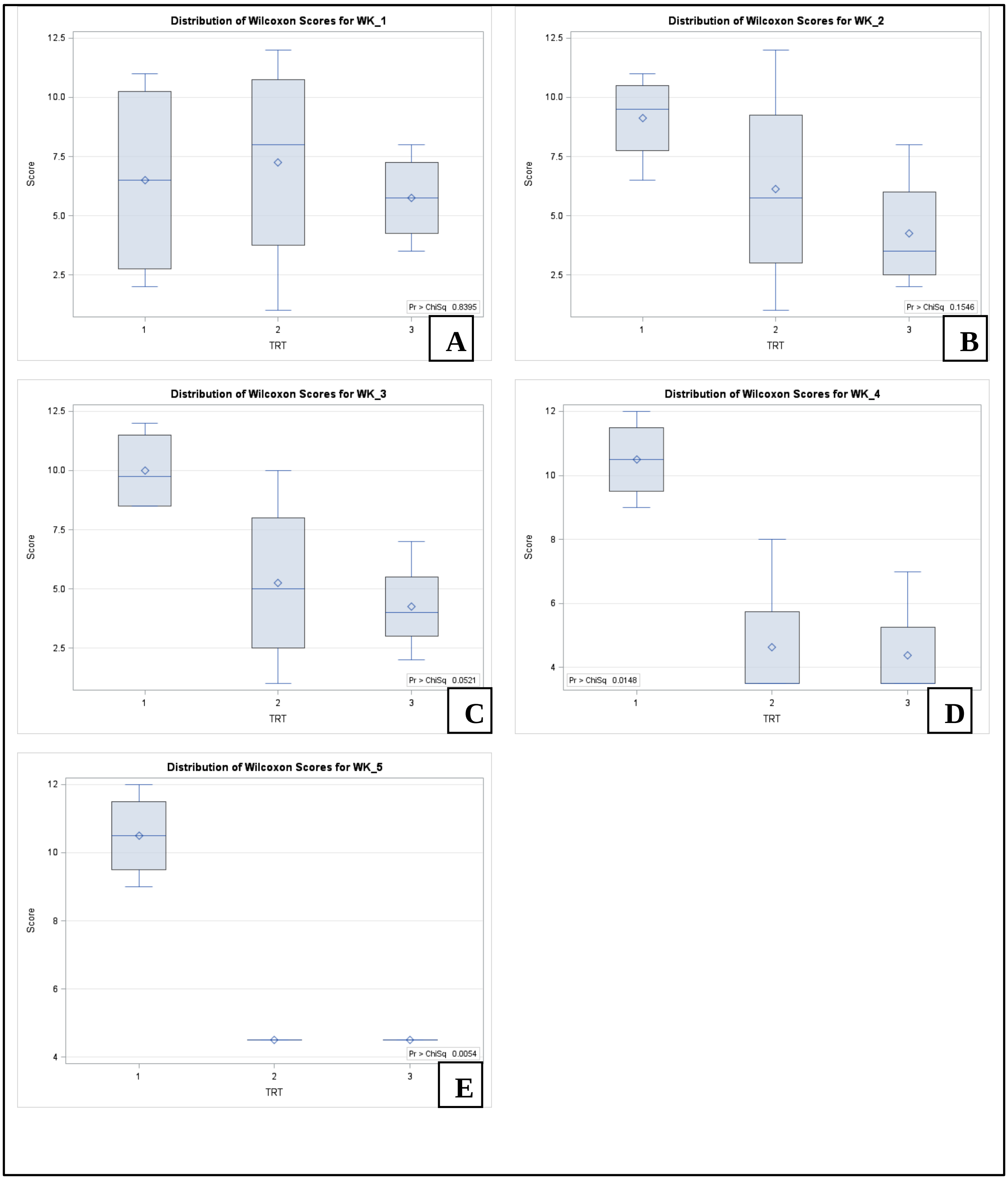

Figure 5.3: Load of intestinal coccidia in cattle from Week 1 to Week 5 (A-E) of research testing effectiveness of the formulated herbal-based anthelmintic drug. TRT- Treatment, 1- no treatment group (negative control), 2- group treated with conventional dosing drug (positive control), and 3- group treated with herbal-based dosing drug (test treatment).

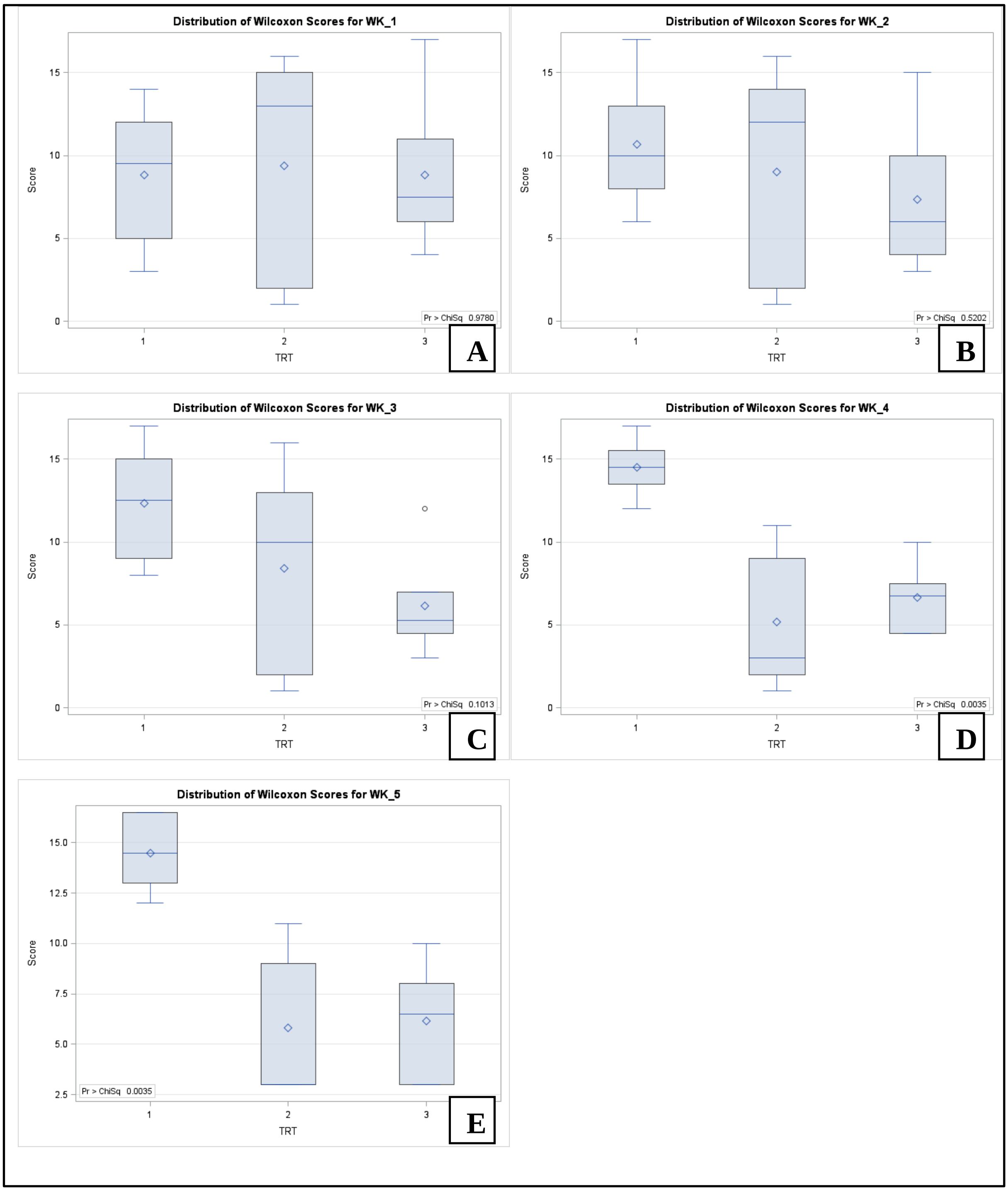

Figure 5.4: Load of intestinal coccidia in goats from Week 1 to Week 5 (A-E) of research testing effectiveness of the formulated herbal-based anthelmintic drug. TRT- Treatment, 1- no treatment group (negative control), 2- group treated with conventional dosing drug (positive control), and 3- group treated with herbal-based dosing drug (test treatment).

Table 5.1 shows there was no significant change in the weight of steers between the start and end of the experimental period *(**P**>0.05)*. The body weights of goats from days 1 to 9 were not significantly different from the control after drenching with *Aloe ferox* (Mill)., *Elephantorrhiza elephantina* Bruch. Skeels. and *Leonotis leonurus* (L) R. BR. (Maphosa *et al*., 2012b). However, the live weight seemed to have increased for the groups treated with the conventional drug and herbal-based drug and decreased for the untreated group.

Table 5. 1: Average weights and associated standard errors of steers at the start (Day 1) and end (Day 35) of research testing the effectiveness of the formulated herbal-based anthelmintic drug.

Period	**Control Group**	**Group Treated with Conventional dosing drug**	**Group Treated with formulated herbal dosing drug**
Initial	438 (19.1)	442 (8.73)	373 (23.9)
Final	431 (19.1)	456 (8.73)	402 (23.9)
P-value	>0.05	>0.05	>0.05

Table 5.2 shows there was no significant change in the weight of goats between the start and end of the experimental period (***P***>0.05). The results were similar to those by Costa *et al*. (2006) where there were no significant differences in live weight gain (***P***>0.05). However, the live weight seemed to have increased for the groups treated with the conventional drug and herbal-based formulation and decreased for the untreated group.

Table 5. 2: Average weights and associated standard errors of goats at the start (Day 1) and end (Day 35) of research testing the effectiveness of the formulated herbal-based anthelmintic drug.

Period	**Control Group**	**Group Treated with Conventional dosing drug**	**Group Treated with formulated herbal dosing drug**
Initial	15.6 (0.73)	16.6(2.37)	18.6(1.23)
Final	13.6 (0.73)	18.8(2.37)	21.5(1.23)
P-value	>0.05	>0.05	>0.05

An improvement in the weights of test animals has been reported in literature after treatment with herbal formulations. This might have been because of the elimination of competition for nutrients between the animals and helminths thus reducing weight losses (Al-Rofaai, Rahman, & Abdulghani, 2013).

5.4 Conclusion and recommendations

This in vivo anthelmintic activity is in full agreement with the in vitro potential reported for *N. brachypus.* The results of this research are suggestive of promising anthelmintic activity of the herbal-based preparation for both Strongyloides and coccidia in cattle and goats. This meant that the herbal-based drug had broad spectrum action. However, a large-scale evaluation of the anthelmintic efficacy of this herbal-based preparation against other parasites is needed.

CHAPTER 6: Synthesis

6.1 General Discussion

6.1.1 Tuberous plants as alternatives for helminths control

Helminths impair weight increase, cause anemia, diarrhea, decreased reproductive performance, low live mass, dull rough coat, organ condemnation, and mortality (Johansson, 2017; León et al., 2019; Morgan et al., 2013). Because of their accessibility, affordability, and availability, ethnoveterinary plants have become more relevant in managing helminths. Plants could thus provide a long-term replacement for synthetic anthelmintics. This study showed that at least 43 tuberous plants found worldwide have the potential to successfully control helminths in livestock. Some of the culturally important tuber-producing plants in the control of helminths are *Dioscorea deltoidea*, *Dioscorea bulbifera*, *Dioscorea alata*, *Gloriosa superba*, *Curcuma longa*, *Dioscorea pentaphylla*, *Cyperus rotundus*, and *N. brachypus.* The classes of phytochemicals that are common in all these plants are phytosterols, tannins, alkaloids, saponins, essential oils, flavonoids, and terpenoids. This study has paved the way for further research on the other less culturally important plants to prevent the extinction of the culturally important plants. *Neorautanenia brachypus* was one of the less culturally important plants that were further researched to identify its phytochemicals that are active against helminths and its tubers was used to formulate a herbal based preparation against helminths. Also, systematic review results indicated to researchers plants that need conservation and propagation strategies to prevent their extinction. There is potential to identify plants that might be introduced to pastures and used as feed supplements to naturally prevent and treat the parasitic infestation. This would also help resource-limited farmers and those in drought-prone areas by providing perpetual feed for livestock. *Neorautanenia brachypus* has been reported to clear gastrointestinal worms when fed to cattle and small ruminants (Murungweni *et al*., 2012). The introduction of these plants into modern agricultural systems has the potential to help slow down the emergence of anthelmintic resistance in animals (French, 2018).

6.1.2 Phytochemicals in *N. brachypus* and their effect on helminths

The anthelmintic effect of *N. brachypus* was attributed to the presence of phytochemicals in its tubers. Phytochemical analysis showed the presence of saponins, phenols, alkaloids, coumarins, tannins, terpenoids, essential oils, quinones, and flavonoids in *N. brachypus* tubers. Tannins, alkaloids, flavonoids, terpenoids, phenols, saponins, and essential oils have all been shown to have anthelmintic properties (Ajah *et al*., 2010; Athanasiadou *et al*., 2001; Wang, Zhou, *et al*., 2010). Phenols affect the decoupling of the oxidative phosphorylation responsible for ATP production interfering with energy production and leading to the death of parasites (Salhan *et al*., 2011). Tannins have ovicidal action related to their interaction with enzymes responsible for the hatching of eggs (Molan *et al*., 2010). In addition, they can interact with metabolites, increasing cell permeability, which leads to their interaction with free proteins or cuticle glycoproteins of parasites hindering nutrient absorption, mobility, reproduction, and consequently causing their death (Botura *et al*., 2013). Quinones inhibit cell development by different mechanisms, such as apoptosis induction, intercalation and binding with DNA, and inhibition of the enzyme topoisomerase (Pe´rez-Pertejo *et al*., 2019). Terpenes in essential oils exhibit anthelmintic effects by enhancing the suppression effects of many biochemical targets such as tyramine receptors, chloride channels, and acetylcholinesterase (Lynagh *et al*., 2014; Miyazawa *et al*., 2016; Trailović *et al*., 2015). Alkaloids and coumarins effect result from both competitive and non-competitive inhibition of parasitic acetylcholine receptors (Basumatary *et al*., 2020; Dubois *et al*., 2019). Flavonoids cause oxidative stress by increasing the production of the reactive oxygen species (ROS), thus affecting the normal physiology of parasites (Wang, Tidrick, *et al*., 2013). Terpenoids inhibit the motility and egg-hatching ability of worms (Ferreira *et al*., 2016; Katiki *et al*., 2017). Anthelmintic effects of saponins are due to their interaction with cell membranes causing changes in cell permeability (Doligalska *et al*., 2011; Tava *et al*., 2006; Vo *et al.*, 2017).

6.1.3 *Neorautanenia brachypus* herbal formulation as an alternative anthelmintic

The herbal formulation of *N. brachypus* can be used as an alternative anthelmintic drug because of several reasons. Phytochemical analysis showed the presence of saponins, phenols, alkaloids, coumarins, tannins, terpenoids, essential oils, quinones, and flavonoids in *N. brachypus* tubers. Tannins, alkaloids, flavonoids, terpenoids, phenols, saponins, and essential oils have all been shown to have anthelmintic properties (Ajah *et al.*, 2010; Athanasiadou *et al.*, 2001; Wang, Zhou, *et al.*, 2010). The methanol and distilled water *N. brachypus* extracts exhibited low toxicity levels to erythrocytes. Plant extracts that are not toxic are a viable alternative for further *in vitro* and *in vivo* studies. The use of plant extracts that have reduced toxicity contributes to environmental conservation by toxic residues present in the excrement of treated animals and decreases the use of chemical anthelmintics and parasitic resistance (da Silva Felix *et al*., 2022). The *in vitro* anthelmintic activity of *N. brachypus* preparations was characterized by a decrease in egg hatching, larvae mortality, and adult worm mortality. This means that *N. brachypus* has the potential to mediate both rapid and long-term effects. The treatments may reduce the hatchability of the eggs excreted in the feces, resulting in both a reduced risk of reinfection and lightened worm loads by decreasing pasture contamination. An increased IC_{50} concentration of *N. brachypus* plant extracts used for larval mortality compared to egg hatch inhibition probably meant that higher concentrations of *N. brachypus* plant extracts are needed to kill 50% of larvae compared to inhibiting 50% of eggs from hatching. In addition, probably beacuse eggs were more susceptible to the plant extracts compared to larvae. These differences in values have been noted to be attributed to the sensitivity of each developmental stage. The efficacy of *N. brachypus* extracts against adult worms (*Eisenia foetida*) ranged from 80-100% across all test treatments after 24 hours of exposure. This was higher compared to its efficacy *in vivo* where the efficacy of the herbal-based prepation reached 0 egg counts in coccidia and Strongyloides in week 4 and week 5. Since most conventional anthelmintic drugs are not effective against coccidia, it is, therefore, advantageous to drench with the *N. brachypus* herbal-based preparation as it had some significant activity against coccidia. Thus it may be used to control coccidiosis, which is problematic in young animals. Though *in vitro* investigations might help find potential anthelmintic mechanisms in plants, such as parasite motility inhibition or worm structural activity, a variation of activity may occur when tested in an in vivo experiment (Nalule, Mbaria, Kimenju, & Olila, 2012a). Because of pharmacological considerations such as ruminal pH, destruction of active constituents and biodegradation by rumen flora, bioavailability, absorption, metabolism, and excretion, some researchers have argued that the adulticidal, larvicidal, and ovicidal potency of a plant extract in vitro is not a reflection of its efficacy *in vivo* (Katiki et al., 2012; Pervez, Ashraf, & Hanjra, 1994; Taíse et al., 2009). However, the results for this research showed that there was no variation in the efficacy *of N. brachypus* tuber extracts between *in vitro* and *in vivo* investigations.

The herbal-based formulation reduced Strongyloides and coccidia egg counts in ruminants. The conventional drug caused a decrease in Strongyloides in cattle at a faster rate compared to the herbal-based drug as shown by its lower rank in week 2. The herbal-based preparation caused a decrease in coccidia in goats at a faster rate compared to the conventional drug as shown by its lower ranks in week 2 and week 3. Tuberous plants have the potential to contribute to controlling gastrointestinal parasites of ruminants. Several medications from the

Orchidaceae family have been produced for the treatment of helminthiases, including Agrimophol from *Agrimonia supatoria*, Arecoline from *Areca catechu*, and qulsqualic acid from *Quisqualis indica* (Kong *et al*., 2003). Several potent anthelmintic compounds have also been isolated from various plant sources, including Atanine, Santonin, Phenanthrenes, Eugenol, Palasonin, Santovin, Alantalactone, Benzoquinone, Tetre-hydroharmine, Kestoxin, Ascaridole, azadirachtin, Bromclain, Allicin, Kaurenoic acid, and Genistein (Kar *et al*., 2002; Tariq, 2018). The live weight of cattle and goats increased from the start to the end of the experimental period after dosing with *N. brachypus* herbal formulation and the conventional drug. The live weights decreased for the untreated group. This showed the advantages of deworming in reducing the competition between the animal and helminths for nutrients and reducing economic losses due to weight loss.

6.1.4 Implications to further study

1. Standardization of herbal formulation

This research ended with the testing of the herbal formulation in vivo. However, there is a need to standardize this formulation and also carry out stability tests. Standardization of herbal formulations involves confirmation of their identity, quality, and purity (Kulkarni, Jagtap, & Magdum, 2019). Usually, the quality of herbal products is assessed through stability testing studies. Standardization tests include testing for (i) Organoleptic Properties, (ii) pH, (iii) Viscosity, (iv) Determination of Crystal Growth, (v) Thin Layer Chromatography, and Accelerated stability studies.

2. Testing for the presence of herbal formulation meat residues

Due to helminths' infections-induced economic losses, conventional drugs are repeatedly used in livestock, and thus have been associated with the appearance of residues in edible animal products. The main roots of drug residue accumulation in food-producing animals include improper observation of withdrawal periods, and failure to maintain animal treatment (Beyene, 2016). Methods for meat drug residue testing are the use of biosensors, chromatographic techniques, immunological techniques, and inhibition assays (Falowo & Akimoladun, 2019).

3. In silico evaluation (Computer-Aided drug discovery)

There is a need to evaluate the mode of action of the phytochemicals isolated from *N. brachypus* on the essential proteins (enzymes) involved in sustaining the lives of helminths using Computer-aided drug discovery (CAAD) tools. Some of the essential proteins in helminths tested against are Acetylcholinesterase, β-tubulin (Basumatary et al., 2020), NOS (nitric oxide synthase) (Chetia *et al*., 2018), and L-AChRs and UNC-49 (GABA) receptors (Hernando, Turani, & Bouzat, 2019). Computer-aided drug design (CADD) techniques use computational approaches to discover, develop, and analyze drugs and similar biologically active molecules (Baig, Ahmad, Rabbani, Danishuddin, & Choi, 2018). The CADD approach involves the analysis of ligands known to interact with a target of interest. These methods use a set of reference structures collected from compounds known to interact with the target of interest and analyze their 2D or 3D structures. The basic objective of these methods is to predict the nature and strength of binding of a given molecule to a target. The density functional theory is frequently used to deliver optimized parameters for the molecular mechanics calculations to predict the conformation of the small molecule and to model conformational changes in the biological target that may occur when the small molecule binds to it. The data also provide an estimate of the electronic properties (electrostatic potential, polarizability, etc.) of the drug candidate that will influence binding affinity. In silico drug design plays an important role in all stages of drug development including preclinical discovery to clinical development (Rathi, Harwalkar, Jayashree, Sharma, & Rao, 2017). In silico screening and validation produce the best results in a short space of time thus saving money and time (Bharath, Manjula, & Vijaychand, 2011).

4. Efficient *N. brachypus* herbal based phytochemical liquid extraction method

There is a need to identify a more efficient herbal based phytochemical liquid extraction method when formulating the herbal based preparation on a large scale. The process of extracting the active compounds can affect yield, quality, and productivity. One method that can be used is the use of centrifugal force.

5. Use of residues after *N. brachypus* herbal based phytochemical liquid extraction

The residues can be used in animal feed formulations as a source of fiber. Additionally, they can be used in the industry to manufacture biodegradable packaging material.

6. Conservation and propagation strategies

Since many of the plants used in ethnoveterinary systems are native to an area they may be endangered. Conservation efforts should encourage the active usage of these plants to help local populations survive (French, 2018). There is a need to think of future conservation and propagation strategies for the *N. brachypus* plant as it might face extinction in the future because of its pharmaceutical importance and since its tubers are the ones harvested for use as cattle feed in drought-prone areas. In this context, animal feed that provides necessary dietary requirements while ensuring parasite control could contribute to increasing farming sustainability in developed and low resource settings. Propagation of *N. brachypus* in the wild is by sexual means involving the use of seeds. There is room to carry out the asexual propagation of *N. brachypus.* Asexual propagation involves the use of vegetative parts of the plant for propagation (Kesari, Krishnamachari, & Rangan, 2008). Common methods of asexual propagation include cuttings, grafting, layering, and micropropagation using tissue culture techniques. Micropropagation through tissue culture techniques is mostly practiced in the propagation of important plants with pharmaceutical properties (Espinosa-Leal, Puente-Garza, & García-Lara, 2018). The technique is advantageous in that it produces healthy and vigorous plants within a short time and can be used to produce secondary natural metabolites of commercial value.

6.2 Conclusion

Phytochemical analysis of *N. brachypus* revealed the presence of several phytochemicals. The therapeutic properties of *N. brachypus* were affirmed experimentally, GCMS analysis and literature search. The plant had an anthelmintic effect against the helminths eggs, larvae, and the adult stage. The *N. brachypus* herbal-based preparation had broad spectrum activity against coccidia and Strongyloides in goats and cattle. This feeds into the knowledge of plant biology and pharmacology. This study showed that *N. brachypus* herbal formulation can potentionally be successfully used as an alternative to conventional drugs.

CHAPTER 7

References

Abbas, A., Iqbal, Z., Abbas, R. Z., Khan, M. K., Khan, J. A., Mahmood, M. S., & Saleemi, M. K. (2017). In vivo anticoccidial effects of Beta vulgaris (sugar beet) in broiler chickens. *Microbial pathogenesis, 111*, 139-144.

Abbas, R. Z., Zaman, M. A., Sindhu, D., Sharif, M., Rafique, A., Saeed, Z., . . . Akram, M. S. (2020). Anthelmintic Effects and Toxicity Analysis of Herbal Dewormer against the Infection of Haemonchus contortus and Fasciola hepatica in Goat. *Pakistan Veterinary Journal, 40*(4).

Abdullah, A.-S. H., Mirghani, M. E. S., & Jamal, P. (2011). Antibacterial activity of Malaysian mango kernel. *African Journal of Biotechnology, 10*(81), 18739-18748.

Abdullah, B. M., Mehdi, M. A. H., Khan, A. R., & Pathan, J. M. (2020). Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Ajwain (Trachyspermum ammi) Seed Extract. *International Journal of Pharmaceutical Quality Assurance, 11*(02), 228-231.

Abidi, A., Sebai, E., Dhibi, M., Alimi, D., Rekik, M., B'chir, F., . . . Akkari, H. (2018). Chemical analyses and anthelmintic effects of Artemisia campestris essential oil. *Veterinary parasitology, 263*, 59-65.

Abidi, A., Sebai, E., Dhibi, M., Darghouth, M. A., & Akkari, H. (2020). Chemical analyses and evaluation of the anthelmintic effects of Origanum majorana essential oil, in vitro and in vivo studies. *Veterinární medicína, 65*(11), 495-505.

Abubacker, M. N., & Devi, P. K. (2014). In vitro antifungal potentials of bioactive compound oleic acid, 3-(octadecyloxy) propyl ester isolated from Lepidagathis cristata Willd.(Acanthaceae) inflorescence. *Asian Pacific journal of tropical medicine, 7*, S190-S193.

Abubakar, M. N., & Majinda, R. R. (2016). GC-MS analysis and preliminary antimicrobial activity of Albizia adianthifolia (Schumach) and Pterocarpus angolensis (DC). *Medicines, 3*(1), 3.

Abuseir, S., Epe, C., Schnieder, T., Klein, G., & Kühne, M. (2006). Visual diagnosis of Taenia saginata cysticercosis during meat inspection: is it unequivocal? *Parasitology research, 99*(4), 405-409.

Adebayo, L. A., Olubisi, A. E., Adebisi, S., & Idowu, M. (2018). Anthelmintic principles from the tuberous roots of Neorautanenia mitis (A. Rich) Verdcourt–Papilonaceae. *Journal of Chemical Society of Nigeria, 43*(4).

Adebayo, O. L., Olasehinde, E. F., Lajide, L., & Oloruntoba, D. (2019). Effect of Drying Method on Phytochemical Compositions and Inhibition Efficiency of Alchornea Laxiflora and Mucuna Flagellepes Leaves Extracts in Corrosion Prevention.

Adedapo, A. A., & Mubo, A. S. (2013). In vitro potential anthelmintic activity of bulbils of *Dioscorea bulbifera* L. on earthworms and liverflukes. *Journal of Pharmacognosy and Phytotherapy, 5*(12), 196-203.

Adeniji, S. A., Adediran, O. A., Ososanya, T. O., & Uwalaka, E. C. (2017). Antihelmintic and anticoccidial effects of *Zingiber officinale* Roscoe fortified diets fed yankasa rams.

Adeniran, S., Olowokudejo, J., & Kadiri, A. (2021). Comparative analysis of GC-MS of Isolona Engl.(Annonaceae) in Nigeria and the Cameroons. *Anatolian Journal of Botany, 5*(2), 102-111.

Adewusi, E., & Afolayan, A. (2009). Safety evaluation of the extract from the roots of Pelargonium reniforme Curtis in male wistar rats. *African Journal of Pharmacy and Pharmacology, 3*(8), 368-373.

Aggarwal, R., & Bagai, U. (2014). Evaluation of anthelmintic activity of ethanolic and aqueous leaf extracts of Azadirachta indica on phosphatases in Gastrothylax indicus. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS), 9*, 98-104.

Aggarwal, R., Kaur, K., Suri, M., & Bagai, U. (2016). Anthelmintic potential of Calotropis procera, Azadirachta indica and Punica granatum against Gastrothylax indicus. *Journal of Parasitic Diseases, 40*(4), 1230-1238.

Ahmad, M., Mahayrookh, M., Rehman, A. B., & Jahan, N. (2012). Analgesic, antimicrobial and cytotoxic effect of Cyperus rotundus ethanol extract. *Pakistan Journal of Pharmacology, 29*(2), 7-13.

Ahmad, S. (2001). Traditional uses of economically important plants of Chitral District, Malakand Division, NWFP, Pakistan. *Pak. J. Bot.*, 587-598.

Ahmed, A. H., Ejo, M., Feyera, T., Regassa, D., Mummed, B., & Huluka, S. A. (2020). In Vitro Anthelmintic Activity of Crude Extracts of Artemisia herba-alba and Punica granatum against Haemonchus contortus. *Journal of Parasitology Research, 2020*.

Ahmed, M., Laing, M., & Nsahlai, I. (2013). In vitro anthelmintic activity of crude extracts of selected medicinal plants against Haemonchus contortus from sheep. *Journal of Helminthology, 87*(2), 174-179.

Ahmed, M., Laing, M. D., & Nsahlai, I. V. (2014). In vivo effect of selected medicinal plants against gastrointestinal nematodes of sheep. *Tropical Animal Health and Production, 46*(2), 411-417.

Ajabe, J., Borikar, S., Digraskar, S., Siddiqui, M., Narladkar, B., & Kumbhar, N. (2018). Therapeutic efficacy of Azadirachta indica (Neem) leaves powder against helminthosis in donkeys. *Group, 640*(27.38efg), 22.89.

Ajah, P., & Eteng, M. (2010). Phytochemical screening and histopathological effects of single acute dose administration of Artemisia annua L. on testes and ovaries of Wistar rats. *African Journal of Biochemistry Research, 4*(7), 179-185.

Ajaib, M., Farooq, S., Khan, K. M., Perveen, S., & Shah, S. (2019). Phytochemical Analysis and Anthelmintic Activity of Salsola imbricata. *Journal of the Chemical Society of Pakistan, 41*(1), 198-198.

Ajayi, G., Olagunju, J., Ademuyiwa, O., & Martins, O. (2011). Gas chromatography-mass spectrometry analysis and phytochemical screening of ethanolic root extract of Plumbago zeylanica, Linn. *Journal of Medicinal Plants Research, 5*(9), 1756-1761.

Akalya, S., & Subasri, G. (2016). Phytochemical screening and pharmacognostical study of Dioscorea deltoidea Wall. Ex Griseb. *World Journal of Science and Research, 1*(2), 4-8.

Akhter, S., Dey, A., Hossain, S., Dey, T., & Begum, N. (2015). In vitro anthelmintic effect of some medicinal plants against Haemonchus contortus. *J. Anim. Sci. Adv, 5*, 1162-1170.

Al-Rofaai, A., Rahman, W., & Abdulghani, M. (2013). Sensitivity of two in vitro assays for evaluating plant activity against the infective stage of Haemonchus contortus strains. *Parasitology Research, 112*(2), 893-898.

Al-Rofaai, A., Rahman, W., Sulaiman, S., & Yahaya, Z. (2012). In vitro activity of neem (Azadirachta indica) and cassava (Manihot esculenta) on three pre-parasitic stages of susceptible and resistant strains of Teladorsagia (Ostertagia) circumcincta. *Veterinary parasitology, 188*(1-2), 85-92.

Al-Tameme, H. J., Hadi, M. Y., & Hameed, I. H. (2015). Phytochemical analysis of Urtica dioica leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy, 7*(10), 238-252.

Alafiatayo, A. A., Lai, K.-S., Syahida, A., Mahmood, M., & Shaharuddin, N. A. (2019). Phytochemical evaluation, embryotoxicity, and teratogenic effects of Curcuma longa extract on zebrafish (Danio rerio). *Evidence-Based Complementary and Alternative Medicine, 2019*.

Alajmi, M. F., Mothana, R. A., Al-Rehaily, A. J., & Khaled, J. M. (2018). Antimycobacterial activity and safety profile assessment of Alpinia galanga and Tinospora cordifolia. *Evidence-Based Complementary and Alternative Medicine, 2018*.

Alam, M., Alam, K., Begum, N., & Amin, M. (2014). Comparative efficacy of different herbal and modern anthelmintics against gastrointestinal nematodiasis in fowl. *International Journal of Biological Research, 2*(2), 145-148.

Alawa, C., Adamu, A., Gefu, J., Ajanusi, O., Abdu, P., & Chiezey, N. (2010). In vivo efficacy of Vernonia amygdalina (compositae) against natural helminth infection in Bunaji (Bos indicus) calves. *Pakistan Veterinary Journal, 30*(4), 215-218.

Aleem, H. (1992). Gloriosa superba poisoning. *The Journal of the Association of Physicians of India, 40*(8), 541-542.

Ali, H., & Yaqoob, U. (2021). Traditional uses, phytochemistry, pharmacology and toxicity of Arisaema (Areaceae): a review. *Bulletin of the National Research Centre, 45*(1), 1-19.

Ali, M., Aldosari, A., Tng, D. Y., Ullah, M., Hussain, W., Ahmad, M., . . . Sher, H. (2019). Traditional uses of plants by indigenous communities for veterinary practices at Kurram District, Pakistan. *Ethnobotany Research and Applications, 18*, 1-19.

Ali, M., Beguh, N., Rahman, A., & Shanta, I. (2006). In vitro anthelmintic effects of some medicinal plants against Ascaridia galli of indigenous chickens. *Progress Agric, 17*(2), 59-66.

Ali, N., Nabi, M., Subhan, Z., Ullah, S., Sultana, U., & Shams, B. (2020). Acute toxicity and antinociceptive activity of saponins rich fraction of Dioscorea deltoidea (wall). *Khyber Medical University Journal, 12*(2), 107-112.

Almalki, E., Al-Shaebi, E. M., Al-Quarishy, S., El-Matbouli, M., & Abdel-Baki, A. S. (2017). *In vitro* effectiveness of *Curcuma longa* and *Zingiber officinale* extracts on *Echinococcus protoscoleces*. *Saudi journal of biological sciences, 24*(1), 90-94.

Alnajar, Z. A. A., Abdulla, M. A., Ali, H. M., Alshawsh, M. A., & Hadi, A. H. A. (2012). Acute toxicity evaluation, antibacterial, antioxidant and immunomodulatory effects of Melastoma malabathricum. *Molecules, 17*(3), 3547-3559.

Alowanou, G., Olounladé, P., Akouèdegni, G., Faihun, A., Koudandé, D., & Hounzangbé-Adoté, S. (2019). In vitro anthelmintic effects of Bridelia ferruginea, Combretum glutinosum, and Mitragyna inermis leaf extracts on Haemonchus contortus, an abomasal nematode of small ruminants. *Parasitology research, 118*(4), 1215-1223.

AlSalhi, M. S., Elumalai, K., Devanesan, S., Govindarajan, M., Krishnappa, K., & Maggi, F. (2020). The aromatic ginger Kaempferia galanga L.(Zingiberaceae) essential oil and its main compounds are effective larvicidal agents against Aedes vittatus and Anopheles maculatus without toxicity on the non-target aquatic fauna. *Industrial Crops and Products, 158*, 113012.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D., & Lightfoot, D. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants, 6*(4), 42.

Ambriani, D. (2012). Daya Antihelmintik Ekstrak Air Bawang Putih (Allium sativum) Terhadap Cacing Kait Anjing In Vitro.

Amin, M., Mostofa, M., Awal, M., & Hossain, M. (2008). Effects of neem (Azadirachta indica) leaves against gastrointestinal nematodes in cattle. *Journal of the Bangladesh Agricultural University, 6*(452-2018-4018), 87-92.

Amin, M., Mostofa, M., Awal, M., & Sultana, M. (2008). Effects of garlic, turmeric and betel leaf against gastrointestinal nematodes in cattle. *Bangladesh Journal of Veterinary Medicine, 6*(1), 115-119.

Amjad, M. S., Zahoor, U., Bussmann, R. W., Altaf, M., Gardazi, S. M. H., & Abbasi, A. M. (2020). Ethnobotanical survey of the medicinal flora of Harighal, Azad Jammu & Kashmir, Pakistan. *Journal of ethnobiology and ethnomedicine, 16*(1), 1-28.

Amuamuta, A., Plengsuriyakarn, T., & Na-Bangchang, K. (2017). Anticholangiocarcinoma activity and toxicity of the Kaempferia galanga Linn. Rhizome ethanolic extract. *BMC Complementary and Alternative Medicine, 17*(1), 1-11.

Andrean, D., Prasetyo, S., Kristijarti, A. P., & Hudaya, T. (2014). The extraction and activity test of bioactive compounds in Phaleria macrocarpa as antioxidants. *Procedia Chemistry, 9*, 94-101.

Angunawela, R., & Fernando, H. (1971). Acute ascending polyneuropathy and dermatitis following poisoning by tubers of Gloriosa superba. *The Ceylon medical journal, 16*(4), 233-235.

Anjukrishna, S., Hafza, S., Poorna, C., Lekhya, P., & Bhaskara, R. (2015). Pharmacological properties, phytochemical and GC-MS analysis of Bauhinia acuminata Linn. *J Chem Pharm Res, 7*, 372-380.

Annadurai, T., Cyril, J. S., & Narayanan, V. (2020). GC-MS ANALYSIS OF VARIOUS EXTRACTS FROM SEED KERNELS OF CAESALPINIA BONDUCELLA LINN. *WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES, 9*(12), 1321-1331

Aparna, V., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C., & Haridas, M. (2012). Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. *Chemical biology & drug design, 80*(3), 434-439.

Aratanechemuge, Y., Komiya, T., Moteki, H., Katsuzaki, H., Imai, K., & Hibasami, H. (2002). Selective induction of apoptosis by ar-turmerone isolated from turmeric (Curcuma longa L) in two human leukemia cell lines, but not in human stomach cancer cell line. *International journal of molecular medicine, 9*(5), 481-484.

Arcadi, F., Costa, C., Imperatore, C., Marchese, A., Rapisarda, A., Salemi, M., . . . Costa, G. (1998). Oral toxicity of bis (2-ethylhexyl) phthalate during pregnancy and suckling in the Long–Evans rat. *Food and Chemical Toxicology, 36*(11), 963-970.

Arora, S., & Kumar, G. (2018). Phytochemical screening of root, stem and leaves of Cenchrus biflorus Roxb. *Journal of Pharmacognosy and Phytochemistry, 7*(1), 1445-1450.

Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants, 8*(4), 96.

Arzamastsev, E., Mironova, M., Krepkova, L., Bortnikova, V., & IuV, K. (1983). Preclinical study of the safety of the new Soviet tranquilizer gindarin. *Farmakologiia i Toksikologiia, 46*(4), 107-112.

Ashour, M. L., El-Readi, M., Youns, M., Mulyaningsih, S., Sporer, F., Efferth, T., & Wink, M. (2009). Chemical composition and biological activity of the essential oil obtained from Bupleurum marginatum (Apiaceae). *Journal of Pharmacy and Pharmacology, 61*(8), 1079-1087.

Ashraf, A., Shahardar, R., Bulbul, K., Wani, Z., Allaie, I., Makhdoomi, D., . . . Rather, M. (2020). Anticoccidial efficacy of Curcuma longa (turmeric) and Zingiber officinale (ginger) in goats in central Kashmir. *J. Pharmacogn. Phytochem., 9*(4S), 354-360.

Ataba, E., Katawa, G., Ritter, M., Ameyapoh, A. H., Anani, K., Amessoudji, O. M., . . . Ameyapoh, Y. (2020). Ethnobotanical survey, anthelmintic effects and cytotoxicity of plants used for treatment of helminthiasis in the Central and Kara regions of Togo. *BMC Complementary Medicine and Therapies, 20*(1), 1-13.

Athanasiadou, S., Githiori, J., & Kyriazakis, I. (2007). Medicinal plants for helminth parasite control: facts and fiction. *Animal, 1*(9), 1392-1400.

Athanasiadou, S., Kyriazakis, I., Jackson, F., & Coop, R. (2001). Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: in vitro and in vivo studies. *Veterinary parasitology, 99*(3), 205-219.

Awa, E., Ibrahim, S., & Ameh, D. (2012). GC/MS analysis and antimicrobial activity of diethyl ether fraction of methanolic extract from the stem bark of Annona senegalensis Pers. *International Journal of Pharmaceutical Sciences and Research, 3*(11), 4213.

Ayaz, E., Turel, I., Gul, A., & Yilmaz, O. (2008). Evaluation of the anthelmentic activity of garlic (Allium sativum) in mice naturally infected with Aspiculuris tetraptera. *Recent patents on anti-infective drug discovery, 3*(2), 149-152.

Ayonga, D. N. (2014). *Efficacy of Schitozim: A Herbal Medicine in the Treatment of Schistosoma Mansoni Infections in Experimentally Infected Balb/C Mice.* University of Nairobi.

Azra, A., Kaleemullah, M., Khattak, B., Asma, N., Safi, A., Qaiser, J., . . . Farhan, Y. (2019). Comparative efficacy of domestic garlic (allium sativum) and neem (azadirachta indica) against haemonchus contortus in small ruminants. *Applied Ecology and Environmental Research, 17*, 10389-10397.

ba Ndob, I. B., Mengome, L. E., Bourobou, H.-P. B., Banfora, Y. L., & Bivigou, F. (2016). Ethnobotanical survey of medicinal plants used as anthelmintic remedies in Gabon. *Journal of Ethnopharmacology, 191*, 360-371.

Babaiwa, U. F., Erharuyi, O., Falodun, A., & Akerele, J. O. (2017). Antimicrobial activity of ethyl acetate extract of Citrullus lanatus seeds. *Tropical Journal of Pharmaceutical Research, 16*(7), 1631-1636.

Babalola, O., & Areola, J. (2010). Interactive roles of terpenoid extract from the leaves of neem plant (Azadirachta indica, A. Juss) on lead induced toxicity in pregnant rabbits. *Journal of Medicinal Plants Research, 4*(12), 1102-1107.

Babu, N. D., Moulika, V., Krishna, B. M., Anitha, S., Mounika, B., & Satyanarayana, T. (2017). Phytochemical and anthelmintic activity of Alpinia galanga Linn. *Journal of Pharmacognosy and Phytochemistry, 6*(4), 2049-2051.

Badwaik, H., Giri, T. K., Tripathi, D., Singh, M., & Khan, A. H. (2011). A Review on Pharmacological Profile for Phytomedicine Known as Gloriosa superba Linn. *Research Journal of Pharmacognosy and Phytochemistry, 3*(3), 103-107.

Baig, M. H., Ahmad, K., Rabbani, G., Danishuddin, M., & Choi, I. (2018). Computer aided drug design and its application to the development of potential drugs for neurodegenerative disorders. *Current neuropharmacology, 16*(6), 740-748.

Balaji, S., & Chempakam, B. (2010). Toxicity prediction of compounds from turmeric (Curcuma longa L). *Food and Chemical Toxicology, 48*(10), 2951-2959.

Bannerjee, A., Nigam, S. S., & (1978). *In vitro* anthelmintic activity of the essential oils derived from the various species of the genus Curcuma Linn. *Sci Cult, 44*, 503,504.

Barrau, E., Fabre, N., Fouraste, I., & Hoste, H. (2005). Effect of bioactive compounds from Sainfoin (Onobrychis viciifolia Scop.) on the in vitro larval migration of Haemonchus contortus: role of tannins and flavonol glycosides. *Parasitology, 131*(4), 531-538.

Basser, M., & Mote, N. (2001). Synthesis and antimicrobial activity of some Schiff bases from benzothiazoles. *Asian Journal of Chemistry, 13*(2), 496.

Basumatary, G., Dhar, E. D., Das, D., Deka, R. C., Yadav, A. K., & Bez, G. (2020). Coumarin-based trisubstituted methanes as potent anthelmintic: synthesis, molecular docking and in vitro efficacy. *Journal of Chemical Sciences, 132*(1), 1-12.

Bate-Smith, E. (1962). The phenolic constituents of plants and their taxonomic significance. I. Dicotyledons. *Botanical Journal of the Linnean Society, 58*(371), 95-173.

Bazh, E. K., & El-Bahy, N. M. (2013). In vitro and in vivo screening of anthelmintic activity of ginger and curcumin on Ascaridia galli. *Parasitology Research, 112*(11), 3679-3686.

Beaulieu, J., Trépanier-Leroux, D., Fischer, J., Olson, M., Thibodeau, S., Humphries, S., . . . Derry, A. (2021). Rotenone for exotic trout eradication: nontarget impacts on aquatic communities in a mountain lake. *Lake and Reservoir Management*, 1-16.

Begum, I. F., Mohankumar, R., Jeevan, M., & Ramani, K. (2016). GC–MS analysis of bio-active molecules derived from Paracoccus pantotrophus FMR19 and the antimicrobial activity against bacterial pathogens and MDROs. *Indian journal of microbiology, 56*(4), 426-432.

Beigh, S. Y., Nawchoo, I. A., & Iqbal, M. (2006). Cultivation and conservation of Aconitum heterophyllum: A critically endangered medicinal herb of the northwest Himalayas. *Journal of herbs, spices & medicinal plants, 11*(4), 47-56.

Berihulay, H., Abied, A., He, X., Jiang, L., & Ma, Y. (2019). Adaptation mechanisms of small ruminants to environmental heat stress. *Animals, 9*(3), 75.

Bernard, D., Kwabena, A. I., Osei, O. D., Daniel, G. A., Elom, S. A., & Sandra, A. (2014). The effect of different drying methods on the phytochemicals and radical scavenging activity of Ceylon cinnamon (Cinnamomum zeylanicum) plant parts. *European Journal of Medicinal Plants*, 1324-1335.

Besier, B. (2007). New anthelmintics for livestock: the time is right. *Trends in parasitology, 23*(1), 21-24.

Beyene, T. (2016). Veterinary drug residues in food-animal products: its risk factors and potential effects on public health. *J Vet Sci Technol, 7*(1), 1-7.

Bhandari, M. R., & Kawabata, J. (2005). Bitterness and toxicity in wild yam (Dioscorea spp.) tubers of Nepal. *Plant Foods for Human Nutrition, 60*(3), 129-135.

Bhandary, B. S. K., Sharmila, K., Kumari, N. S., Bhat, V. S., & Fernandes, R. (2017). Acute and subacute toxicity profile of Asparagus racemosus root extract, isoprinosine and shatvari syrup in Swiss albino mice. *Journal of Applied Pharmaceutical Science, 7*(5), 129-135.

Bharath, E., Manjula, S., & Vijaychand, A. (2011). In silico drug design tool for overcoming the innovation deficit in the drug discovery process. *Int J Pharm Pharm Sci, 3*(2), 8-12.

Bhardwaj, M., Bharadwaj, L., Trigunayat, K., & Trigunayat, M. M. (2011). Insecticidal and wormicidal plants from Aravalli hill range of India. *Journal of ethnopharmacology, 136*(1), 103-110.

Bhat, M. N., Singh, B., Surmal, O., Singh, B., Shivgotra, V., & Musarella, C. M. (2021). Ethnobotany of the Himalayas: safeguarding medical practices and traditional uses of Kashmir regions. *Biology, 10*(9), 851.

Bhattacharjee, K., Islam, S., Roy, K., Bora, S., Deka, D., & Sarmah, P. (2021). Efficacy of plant extract based feed-block as anthelmintics against gastro-intestinal nematodes of goats in Assam. *Medicinal Plants-International Journal of Phytomedicines and Related Industries, 13*(1), 164-168.

Bhokare, P., Khadke, A., Kuchekar, G., & Kulkarni, S. (2018). Comparative study of different extraction technique and phytochemical screening of Delonix regia. *J Pharmacognosy Phytochem, 7*(4), 133-138.

Bloedon, L. T., Jeffcoat, A. R., Lopaczynski, W., Schell, M. J., Black, T. M., Dix, K. J., . . . Crowell, J. A. (2002). Safety and pharmacokinetics of purified soy isoflavones: single-dose administration to postmenopausal women. *The American journal of clinical nutrition, 76*(5), 1126-1137.

Boonmasawai, S., Sungpradit, S., Jirapattharasate, C., Nakthong, C., & Piasai, L. (2013). Effects of alcoholic extract from pomegranate (Punica granatum L.) peels on gastrointestinal nematode egg counts in doe.

Bopana, N., & Saxena, S. (2008). In vitro propagation of a high value medicinal plant: Asparagus racemosus Willd. *In Vitro Cellular & Developmental Biology-Plant, 44*(6), 525-532.

Borah, A., Kumar, D., Paw, M., Begum, T., & Lal, M. (2020). A review on ethnobotany and promising pharmacological aspects of an endangered medicinal plant, Curcuma caesia Roxb. *Turkish Journal of Botany, 44*(3), 205-213.

Borch, J., Axelstad, M., Vinggaard, A. M., & Dalgaard, M. (2006). Diisobutyl phthalate has comparable anti-androgenic effects to di-n-butyl phthalate in fetal rat testis. *Toxicology letters, 163*(3), 183-190.

Borhan, M., Ahmad, R., Rusop, M., & Abdullah, S. (2013). *Impact of nanopowders on exraction yield of Centella asiatica.* Paper presented at the Advanced Materials Research.

Botelho, R. G., Inafuku, M. M., Maranho, L. A., Neto, L. M., de Olinda, R. A., Dias, C. T., & Tornisielo, V. L. (2010). Toxicidade aguda e crônica do extrato de nim (Azadirachta indica) para Ceriodaphnia dubia. *Pesticidas: revista de ecotoxicologia e meio ambiente, 20*.

Botura, M. B., dos Santos, J. D. G., da Silva, G. D., de Lima, H. G., de Oliveira, J. V. A., de Almeida, M. A. O., . . . Branco, A. (2013). In vitro ovicidal and larvicidal activity of Agave sisalana Perr.(sisal) on gastrointestinal nematodes of goats. *Veterinary Parasitology, 192*(1-3), 211-217.

Boussaada, O., Ammar, S., Saidana, D., Chriaa, J., Chraif, I., Daami, M., . . . Mighri, Z. (2008). Chemical composition and antimicrobial activity of volatile components from capitula and aerial parts of Rhaponticum acaule DC growing wild in Tunisia. *Microbiological research, 163*(1), 87-95.

Buranrat, B., Noiwetch, S., Suksar, T., Ta-ut, A., & Boontha, S. (2018). Cytotoxic and antimigration effects of different parts of Oroxylum Indicum extract on human breast cancer MCF-7 cells. *Science & Technology Asia*, 42-52.

Burnett, B. P., Pillai, L., Bitto, A., Squadrito, F., & Levy, R. M. (2011). Evaluation of CYP450 inhibitory effects and steady-state pharmacokinetics of genistein in combination with cholecalciferol and citrated zinc bisglycinate in postmenopausal women. *International Journal of Women's Health, 3*, 139.

Burungale, S. D., & Bhitre, M. (2013). Synthesis of 2, 4, 5-triphenyl imidazole derivatives and biological evaluation for their antibacterial and anti-inflammatory activity. *International Journal of Pharmaceutical Sciences and Research, 4*(10), 4051.

Busari, I., Soetan, K., Aiyelaagbe, O., & Babayemi, O. (2021). Phytochemical screening and in vitro anthelmintic activity of methanolic extract of Terminalia glaucescens leaf on Haemonchus contortus eggs. *Acta tropica, 223*, 106091.

Butler, R. A. (2020). Total number of plant species by country.

Cadmus, S., & Adesokan, H. (2009). Causes and implications of bovine organs/offal condemnations in some abattoirs in Western Nigeria. *Tropical animal health and production, 41*(7), 1455.

Camurça-Vasconcelos, A., Bevilaqua, C., Morais, S., Maciel, M., Costa, C., Macedo, I., . . . Vieira, L. (2007). Anthelmintic activity of Croton zehntneri and Lippia sidoides essential oils. *Veterinary parasitology, 148*(3-4), 288-294.

Cannel, R. J. (1998). Natural products isolation. *Methods in Biotechnology, 4*, 220-222.

Capraro, H. (1984). In the alkaloids.(Ed.): A. Brossi. *Academic Press, 23*(1984), 1-70.

Cervantes-Valencia, M. E., Alcala-Canto, Y., Salem, A. Z., Kholif, A. E., Ducoing-Watty, A. M., Bernad-Bernad, M. J., & Gutiérrez-Olvera, C. (2015). Influence of curcumin (Curcuma longa) as a natural anticoccidial alternative in adult rabbits: first results. *Italian Journal of Animal Science, 14*(3), 3838.

Cezar, A. S., Toscan, G., Camillo, G., Sangioni, L. A., Ribas, H. O., & Vogel, F. S. F. (2010). Multiple resistance of gastrointestinal nematodes to nine different drugs in a sheep flock in southern Brazil. *Veterinary parasitology, 173*(1-2), 157-160.

Chadalavada, V., & Budala, S. (2017). Study on anthelmintic activity of curcuma caesia. *Journal of Pharmaceutical Research, 7*(07).

Chagas, A. C. S. (2015). Medicinal plant extracts and nematode control. *CAB International Review, 10*, 1-8.

Chamuah, J., Mech, A., Perumal, P., & Dutta, P. (2014). Efficacy of chemical and herbal anthelmintic drug against naturally infested gastrointestinal helminthiasis in mithun calves (Bos frontalis). *Indian J. Anim. Res.*

Chanda, S., & Ramachandra, T. (2019). Phytochemical and pharmacological importance of turmeric (Curcuma longa): A review. *Research & Reviews: A Journal of Pharmacology, 9*(1), 16-23.

Chandra, P., & Khuda-Bukhsh, A. (2004). Genotoxic effects of cadmium chloride and azadirachtin treated singly and in combination in fish. *Ecotoxicology and environmental safety, 58*(2), 194-201.

Chandra, S., Saklani, S., & Dimari, M. (2012). Evaluation of Garhwal Himalaya wild edible tuber Dioscorea deltoidea *International Research Journal of Pharmacy, 3* . (3), 152–156.

Chandrawathani, P., Adnan, M., & Zaini, C. (2000). Preliminary study on Neem (Azadirachta indica) as an alternative anthelmintic for sheep. *Proc. 12th Vet. Assoc. Malaysia Scientific Cong., Malaysia, Kuanyan*.

Chang, C. J., Tzeng, T.-F., Liou, S.-S., Chang, Y.-S., & Liu, I.-M. (2012a). Absence of genotoxic and mutagenic effects of Zingiber zerumbet (L.) Smith (Zingiberaceae) extract. *Evidence-Based Complementary and Alternative Medicine, 2012*.

Chang, C. J., Tzeng, T.-F., Liou, S.-S., Chang, Y.-S., & Liu, I.-M. (2012b). Acute and 28-day subchronic oral toxicity of an ethanol extract of Zingiber zerumbet (L.) Smith in rodents. *Evidence-Based Complementary and Alternative Medicine, 2012*.

Charlier, J., Thamsborg, S., Bartley, D., Skuce, P., Kenyon, F., Geurden, T., . . . Höglund, J. (2018). Mind the gaps in research on the control of gastrointestinal nematodes of farmed ruminants and pigs. *Transboundary and emerging diseases, 65*, 217-234.

Chathuranga, K., Weerawardhana, A., Dodantenna, N., Ranathunga, L., Cho, W.-K., Ma, J. Y., & Lee, J.-S. (2021). Inhibitory Effect of Sargassum fusiforme and Its Components on Replication of Respiratory Syncytial Virus In Vitro and In Vivo. *Viruses, 13*(4), 548.

Chauhan, K. (2020). Role of Ethnobotany on Indian Society: A Review. *Journal of Arts, Culture, Philosophy, Religion, Language and Literature, 4*(2), 109-111.

Chavunduka, G. (1999). Christianity, African religion and African medicine. *World Council of Churches*.

Chelvan, Y., Chelvan, T., Pushpam, A. C., Karthik, R., Ramalingam, K., & Vanitha, M. (2016). Extraction and Purification of Antimicrobial Compounds from Marine Actinobacteria. *Research Journal of Pharmacy and Technology, 9*(4), 381-385.

Chen, Y., Zhao, Y.-Y., Wang, X.-Y., Liu, J.-T., Huang, L.-Q., & Peng, C.-S. (2011). GC-MS analysis and analgesic activity of essential oil from fresh rhizoma of Cyperus rotundus. *Zhong yao cai= Zhongyaocai= Journal of Chinese Medicinal Materials, 34*(8), 1225-1229.

Cheng, Z., Jiang, J., Yang, X., Chu, H., Jin, M., Li, Y., . . . Shang, L. (2017). The research of genetic toxicity of β-phellandrene. *Environmental toxicology and pharmacology, 54*, 28-33.

Chetia, M., & Das, R. (2018). Effect of (-)-epicatechin, a flavonoid on the NO and NOS activity of Raillietina echinobothrida. *Acta tropica, 178*, 311-317.

Chhetri, D., Parajuli, P., & Subba, G. (2005). Antidiabetic plants used by Sikkim and Darjeeling Himalayan tribes, India. *Journal of Ethnopharmacology, 99*(2), 199-202.

Chitura, T., Shiba, M. R., Afful, D. B., Shai, K., Muvhali, P. T., & Tsotetsi-Khambule, A. M. (2019). In vitro anthelmintic activity of seven medicinal plants used to control livestock internal parasites in chief Albert Luthuli municipality, South Africa. *Livestock Research for Rural Development, 31*(2).

Choi, D., Kang, W., & Park, T. (2020). Anti-allergic and anti-inflammatory effects of Undecane on mast cells and keratinocytes. *Molecules, 25*(7), 1554.

Choudhary, N., Khatik, G. L., Choudhary, S., Singh, G., & Suttee, A. (2021). In vitro anthelmintic activity of Chenopodium album and in-silico prediction of mechanistic role on Eisenia foetida. *Heliyon, 7*(1), e05917.

Choudhary, S., Kaurav, H., & Chaudhary, G. (2021). Vaibidang (Embelia ribes): A Potential Herbal Drug in Ayurveda with Anthelmintic Property. *International Journal for Research in Applied Sciences and Biotechnology, 8*(2), 237-243.

Chowdhury, N. S., Karim, M. R., & Rana, M. S. (2005). In vitro studies on toxicological property of the root and stem bark extracts of Oroxylum indicum. *Dhaka University Journal of Pharmaceutical Sciences, 4*(1).

Chybowski, J. (1997). Study of the anthelminthic activity of garlic extracts. *Herba Polonica, 43*, 383-387.

Clayton, M. J., Davis, T. Z., Knoppel, E. L., & Stegelmeier, B. L. (2020). Hepatotoxic plants that poison livestock. *Veterinary Clinics of North America: Food Animal Practice, 36*(3), 715-723.

Clerici, F., Pocar, D., Guido, M., Loche, A., Perlini, V., & Brufani, M. (2001). Synthesis of 2-amino-5-sulfanyl-1, 3, 4-thiadiazole derivatives and evaluation of their antidepressant and anxiolytic activity. *Journal of medicinal chemistry, 44*(6), 931-936.

Coles, G. (2005). Anthelmintic resistance–looking to the future: a UK perspective. *Research in veterinary Science, 78*(2), 99-108.

Coles, G., Bauer, C., Borgsteede, F., Geerts, S., Klei, T., Taylor, M., & Waller, P. (1992). World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary parasitology, 44*(1-2), 35-44.

Condy, J. (1962). Fascioliasis-a disease of major economic importance. *Rhodesia Agricultural Journal, 59*(5), 259-269.

Cooke, A. S., Morgan, E. R., & Dungait, J. A. (2017). Modelling the impact of targeted anthelmintic treatment of cattle on dung fauna. *Environmental Toxicology and Pharmacology, 55*, 94-98.

Cormanes, J. M. Y., Portugaliza, H. P., & Quilicot, A. M. M. (2016). In vivo anthelmintic activity of pineapple (Ananas comosus Merr.) fruit peeling juice in semi-scavenging Philippine native chicken naturally co-infected with Ascaridia galli and Heterakis gallinarum. *Livest. Res. Rural Dev, 28*(5), 82.

Costa, C., Bevilaqua, C., Camurça-Vasconcelos, A., Maciel, M., Morais, S., Castro, C., . . . Oliveira, L. (2008). In vitro ovicidal and larvicidal activity of Azadirachta indica extracts on Haemonchus contortus. *Small Ruminant Research, 74*(1-3), 284-287.

Costa, C., Bevilaqua, C., Maciel, M., Camurça-Vasconcelos, A., Morais, S., Monteiro, M., . . . Souza, M. (2006). Anthelmintic activity of Azadirachta indica A. Juss against sheep gastrointestinal nematodes. *Veterinary parasitology, 137*(3-4), 306-310.

Costa, C., Morais, S. d., Bevilaqua, C., Souza, M. d., & Leite, F. (2002). Efeito ovicida de extratos de sementes de Mangifera indica L. sobre Haemonchus contortus. *Rev Bras Parasitol Vet, 11*, 57-60.

Cragg, G. M., & Newman, D. J. (2013). Natural products: a continuing source of novel drug leads. *Biochimica et Biophysica Acta (BBA)-General Subjects, 1830*(6), 3670-3695.

da Silva Felix, R. C., Barbosa, T. N., Marques, H. P., de Oliveira Rebouças, C. K., da Silveira Pereira, J. C., Batista, J. I. L., . . . Bezerra, A. C. D. S. (2022). In vitro nematocidal activity of Punica granatum L. against gastrointestinal helminths in goats. *Journal of Parasitic Diseases, 46*(1), 236-242.

Da Silva, V. C. L., de França Silva, F. M., Meunier, I. M. J., Da Silva, T. M. S., da Silva Júnior, V. A., & Maia, F. C. L. (2015). Post-natal development of ratsoffspring treated with the ethanol extract of Neem leaves (Azadirachta indica A. Juss) during pregnancy and lactation. *Acta Scientiarum. Biological Sciences, 37*(2), 219-224.

Daniel, U., Ohalete, C., Ibiam, U., & Okechukwu, R. (2015). Medicinal plants effectiveness against helminths of cattle. *Journal of Applied Biosciences, 86*(1), 7900–7917.

Das, B., Tandon, V., Lyndem, L. M., Gray, A. I., & Ferro, V. A. (2009). Phytochemicals from Flemingia vestita (Fabaceae) and Stephania glabra (Menispermeaceae) alter cGMP concentration in the cestode Raillietina echinobothrida. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 149*(3), 397-403.

Das, B., Tandon, V., & Saha, N. (2004a). Anthelmintic efficacy of Flemingia vestita (Fabaceae): alteration in the activities of some glycolytic enzymes in the cestode, Raillietina echinobothrida. *Parasitology research, 93*(4), 253-261.

Das, B., Tandon, V., & Saha, N. (2004b). Anthelmintic efficacy of Flemingia vestita (Fabaceae): alterations in glucose metabolism of the cestode, Raillietina echinobothrida. *Parasitology International, 53*(4), 345-350.

Das, B., Tandon, V., & Saha, N. (2006). Effect of isoflavone from Flemingia vestita (Fabaceae) on the Ca2+ homeostasis in Raillietina echinobothrida, the cestode of domestic fowl. *Parasitology International, 55*(1), 17-21.

Das, B., Tandon, V., & Saha, N. (2007). Genistein from Flemingia vestita (Fabaceae) enhances NO and its mediator (cGMP) production in a cestode parasite, Raillietina echinobothrida. *Parasitology, 134*(10), 1457-1463.

Das, B., Tandon, V., Saxena, J., Joshi, S., & Singh, A. (2013). Purification and characterization of phosphoenolpyruvate carboxykinase from Raillietina echinobothrida, a cestode parasite of the domestic fowl. *Parasitology, 140*(1), 136-146.

Das, G., Dongre, S., Nath, S., Dixit, A., & Agrawal, V. (2015). Anthelmintic effeicacy of Azadirachta indica (neem) against strongyles in goats. *The Indian Journal of Veterinary Sciences and Biotechnology, 10*(03), 18-21.

Das, U. N. (2006). Essential fatty acids: biochemistry, physiology and pathology. *Biotechnology Journal: Healthcare Nutrition Technology, 1*(4), 420-439.

Dash, P. R., Mou, K. M., Erina, I. N., Ripa, F. A., Masud, K. N., & Ali, M. S. (2017). Study of anthelmintic and insecticidal activities of different extracts of Kaempferia galanga. *Int J Pharm Sci Res. , 8*(2), :29-33.

Dash, P. R., Nasrin, M., & Ali, M. S. (2014). In vivo cytotoxic and In vitro antibacterial activities of Kaempferia galanga. *Journal of pharmacognosy and Phytochemistry, 3*(1).

Dasheiff, R. M., & Ramirez, L. F. (1985). The effects of colchicine in mammalian brain from rodents to rhesus monkeys. *Brain Research Reviews, 10*(1), 47-67.

Datta, A., & Sukul, N. C. (1987). Antifilarial effect of Zingiber officinale on Dirofilaria immitis. *Journal of Helminthology, 61*(3), 268-270.

Davuluri, T., Chennuru, S., Pathipati, M., Krovvidi, S., & Rao, G. (2020). In vitro anthelmintic activity of three tropical plant extracts on Haemonchus contortus. *Acta parasitologica, 65*(1), 11-18.

De Graef, J., Claerebout, E., & Geldhof, P. (2013). Anthelmintic resistance of gastrointestinal cattle nematodes. *Vlaams Diergeneeskundig Tijdschrift, 82*(3), 113-123.

de Matos, A. F. I. M., Nobre, C. O. R., Monteiro, J. P., Bevilaqua, C. M. L., Smith, W. D., & Teixeira, M. (2017). Attempt to control Haemonchus contortus in dairy goats with Barbervax®, a vaccine derived from the nematode gut membrane glycoproteins. *Small Ruminant Research, 151*, 1-4.

de Oliveira, B., Brandão, R., da Silva, R., Rosa, C., Farias, S., & dos Santos, S. (2021). In vitro anthelmintic efficacy of essential oils in the control of Neoechinorhynchus buttnerae, an endoparasite of Colossoma macropomum. *Journal of Essential Oil Research, 33*(5), 509-522.

De, S., Dey, Y., & Ghosh, A. (2010). Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of Amorphaphallus paeoniifolius (Araceae). *Int J Pharm Biol Res, 1*(5), 150-157.

De Silva, G. O., Abeysundara, A. T., & Aponso, M. M. W. (2017). Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *American Journal of Essential Oils and Natural Products, 5*(2), 29-32.

de Sousa, C. P., de Amorim, W. R., do Nascimento Martins, G., Santos, A. R. S. S., de Sousa Sá, I., Dada, J. M. V., . . . Dantas, J. B. G. (2019). Plantas tóxicas de interesse pecuário em municípios da microrregião do Alto Médio Gurguéia–Piauí. *Pubvet, 13*, 162.

Deepak, J. K. (2019). Anthelmintic Activity of Aqueous Leaf Extract of Clerodendrum Serratum. *International Journal of Science and Research (IJSR), 8*(5), 998-1001.

Dehpour, A., Babakhani, B., Khazaei, S., & Asadi, M. (2011). Chemical composition of essential oil and antibacterial activity of extracts from flower of Allium atroviolaceum. *Journal of Medicinal Plants Research, 5*(16), 3667-3672.

Deori, K., & Yadav, A. K. (2016). Anthelmintic effects of Oroxylum indicum stem bark extract on juvenile and adult stages of Hymenolepis diminuta (Cestoda), an in vitro and in vivo study. *Parasitology Research, 115*(3), 1275-1285.

Deshpande, S., Lalitha, V., Ingle, A., Raste, A., Gadre, S., & Maru, G. (1998). Subchronic oral toxicity of turmeric and ethanolic turmeric extract in female mice and rats. *Toxicology letters, 95*(3), 183-193.

Dewanto, V., Wu, X., & Liu, R. H. (2002). Processed sweet corn has higher antioxidant activity. *Journal of agricultural and Food Chemistry, 50*(17), 4959-4964.

Dey, A. R., Begum, N., Alim, M. A., & Alam, M. Z. (2020). Multiple anthelmintic resistance in gastrointestinal nematodes of small ruminants in Bangladesh. *Parasitology International*, 102105.

Dey, P., Bardalai, D., Kumar, N. R., Subramani, C., Mukherjee, M., & Bhakta, T. (2012). Ethnomedicinal knowledge about various medicinal plants used by the tribes of Tripura. *Research Journal of Pharmacognosy and Phytochemistry, 4*(6), 297.

Dey, P., Roy Chowdhuri, S., Sarkar, M. P., & Chaudhuri, T. K. (2016). Evaluation of anti-inflammatory activity and standardisation of hydro-methanol extract of underground tuber of Dioscorea alata. *Pharmaceutical biology, 54*(8), 1474-1482.

Dhawan, D., & Gupta, J. (2017). Research Article Comparison of Different Solvents for Phytochemical Extraction Potential from Datura metel Plant Leaves. *Int. J. Biol. Chem, 11*, 17-22.

Dhiman, A. (2017). Comparison of antimicrobial, larvicidal and anthelmintic activity of *Zingiber officinale* Rose. cow urine extract. *International Journal of Green Pharmacy (IJGP), 11*(02).

Dimitrijević, B., Borozan, S., Katić-Radivojević, S., & Stojanović, S. (2012). Effects of infection intensity with Strongyloides papillosus and albendazole treatment on development of oxidative/nitrosative stress in sheep. *Veterinary Parasitology, 186*(3-4), 364-375.

Dkhil, M. A. (2013). Anti-coccidial, anthelmintic and antioxidant activities of pomegranate (Punica granatum) peel extract. *Parasitology Research, 112*(7), 2639-2646.

Dkhil, M. A., Thagfan, F. A., Abdel-moniem, S. H., Al-Shaebi, E. M., Abdel-Gaber, R., & Al-Quraishy, S. (2019). Anthelmintic, anticoccidial and antioxidant activity of Salvadora persica root extracts. *Saudi journal of biological sciences, 26*(6), 1223-1226.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y.-H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *Journal of food and drug analysis, 22*(3), 296-302.

Dobson, R., Hosking, B., Jacobson, C., Cotter, J., Besier, R., Stein, P., & Reid, S. (2012). Preserving new anthelmintics: a simple method for estimating faecal egg count reduction test (FECRT) confidence limits when efficacy and/or nematode aggregation is high. *Veterinary parasitology, 186*(1-2), 79-92.

Dogo, A. G., Karaye, G. P., Patrobas, M., Galadima, M., & Gosomji, I. (2017). Prevalence of gastrointestinal parasites and their impact in domestic animals in Vom, Nigeria.

Doligalska, M., Jóźwicka, K., Kiersnowska, M., Mroczek, A., Pączkowski, C., & Janiszowska, W. (2011). Triterpenoid saponins affect the function of P-glycoprotein and reduce the survival of the free-living stages of Heligmosomoides bakeri. *Veterinary Parasitology, 179*(1-3), 144-151.

Dorababu, D., Joshi, M., Kumar, B., Chaturvedi, A., & Goel, R. (2006). Effect of aqueous extract of neem (Azadirachta indica) leaves on offensive and defensive gastric mucosal factors in rats. *Indian journal of physiology and pharmacology, 50*(3), 241.

Dorny, P., Phiri, I., Gabriël, S., Speybroeck, N., & Vercruysse, J. (2002). A sero-epidemiological study of bovine cysticercosis in Zambia. *Veterinary parasitology, 104*(3), 211-215.

Downing, J. (2000). Anthelmintic activity of Oroxylum indicum against equine strongyles in vitro compared to the anthelmintic activity of Ivermectin. *Journal of Biological Research, 1*.

Dubey, R., Paroha, S., Wani, V., Pandey, A., Sahu, P., Verma, S., & Daharwal, S. (2010). In Vitro Comparative Studies of Anthelmintic Activity of Allium sativum and Ficus religiosa. *Research Journal of Science and Technology, 2*(4), 84-86.

Dubey, R. D., Verma, S., Rane, D., Wani, V. K., Pandey, A. K., & Paroha, S. (2010). Comparative studies of anthelmintic activity of *Zingiber officinale* and *Cassia tora*. *International Journal of Chemical and Pharmaceutical Sciences, 1*(1), 1-4.

Dubois, O., Allanic, C., Charvet, C., Guégnard, F., Février, H., Théry-Koné, I., . . . Fassier, T. (2019). Lupin (Lupinus spp.) seeds exert anthelmintic activity associated with their alkaloid content. *Scientific reports, 9*(1), 1-12.

Duguma, G., Mirkena, T., Haile, A., Okeyo, A., Tibbo, M., Rischkowsky, B., . . . Wurzinger, M. (2011). Identification of smallholder farmers and pastoralists' preferences for sheep breeding traits: choice model approach. *Animal, 5*(12), 1984-1992.

Dutta, A., Dahiya, A., Prakash, A., & Agrawala, P. K. (2021). Acute toxicity of diallyl sulfide derived from Allium sativum (garlic) in mice and its possible mechanisms. *Phytomedicine Plus, 1*(3), 100084.

Dutta, B. (2015). Food and medicinal values of certain species of Dioscorea with special reference to Assam. *Journal of Pharmacognosy and Phytochemistry, 3*(5).

Eddleston, M. (2000). Patterns and problems of deliberate self-poisoning in the developing world. *Qjm, 93*(11), 715-731.

Edeoga, H., Okwu, D., & Mbaebie, B. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African journal of biotechnology, 4*(7), 685-688.

Eguale, T., Tilahun, G., Debella, A., Feleke, A., & Makonnen, E. (2007). Haemonchus contortus: in vitro and in vivo anthelmintic activity of aqueous and hydro-alcoholic extracts of Hedera helix. *Experimental parasitology, 116*(4), 340-345.

El-Bahy, N. M., & Bazh, E. K. (2015). Anthelmintic activity of ginger, curcumin, and praziquentel against *Raillietina cesticillus* (*in vitro* and *in vivo*). *Parasitology Research, 114*(7), 2427-2434.

El-Kamali, H., & El-Khalifa, K. (1999). Folk medicinal plants of riverside forests of the Southern Blue Nile district, Sudan. *Fitoterapia, 70*(5), 493-497.

El-Sakka, S., Soliman, A., & Imam, A. (2009). Synthesis, antimicrobial activity and Electron Impactof Mass Spectra of Phthalazine-1, 4-dione Derivatives. *Afinidad, 66*(540).

El-Sayed, N. M. (2017). Efficacy of *Zingiber officinale* ethanol extract on the viability, embryogenesis and infectivity of *Toxocara canis* eggs. *Journal of Parasitic Diseases, 41*(4), 1020-1027.

Eleazu, C. (2016). Characterization of the natural products in cocoyam (Colocasia esculenta) using GC–MS. *Pharmaceutical biology, 54*(12), 2880-2885.

Elkhishin, I. A., & Awwad, I. A. (2009). A study of the cardiovascular toxic effects of Zingiber officinale (ginger) in adult male albino rats and its possible mechanisms of action. *Mansoura Journal of Forensic Medicine and Clinical Toxicology, 17*(2), 109-127.

Eloff, J. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Journal of ethnopharmacology, 60*(1), 1-8.

Elsayed, T. R., Galil, D. F., Sedik, M. Z., Hassan, H. M., & Sadik, M. W. (2020). Antimicrobial and anticancer activities of actinomycetes isolated from Egyptian soils. *Journal homepage: http://www. ijcmas. com, 9*(9), 2020.

Espinosa-Leal, C. A., Puente-Garza, C. A., & García-Lara, S. (2018). In vitro plant tissue culture: means for production of biological active compounds. *Planta, 248*(1), 1-18.

Fakchich, J., & Elachouri, M. (2021). An overview on ethnobotanico-pharmacological studies carried out in Morocco, from 1991 to 2015: systematic review (part 1).

Falowo, A. B., & Akimoladun, O. F. (2019). Veterinary drug residues in meat and meat products: Occurrence, detection and implications. *Veterinary Medicine and Pharmaceuticals, 3*, 194.

Faruque, M. O., Feng, G., Khan, M. N. A., Barlow, J. W., Ankhi, U. R., Hu, S., . . . Hu, X. (2019). Qualitative and quantitative ethnobotanical study of the Pangkhua community in Bilaichari Upazilla, Rangamati District, Bangladesh. *Journal of ethnobiology and ethnomedicine, 15*(1), 1-29.

Fatemi, A., Razavi, S. M., Asasi, K., & Torabi Goudarzi, M. (2015). Effects of Artemisia annua extracts on sporulation of Eimeria oocysts. *Parasitology Research, 114*(3), 1207-1211.

Ferrandiz, M., & Alcaraz, M. (1991). Anti-inflammatory activity and inhibition of arachidonic acid metabolism by flavonoids. *Agents and actions, 32*(3-4), 283-288.

Ferreira, L. E., Benincasa, B. I., Fachin, A. L., Franca, S. C., Contini, S. S., Chagas, A. C., & Beleboni, R. O. (2016). Thymus vulgaris L. essential oil and its main component thymol: Anthelmintic effects against Haemonchus contortus from sheep. *Veterinary parasitology, 228*, 70-76.

Feussom, N. G., Jatsa, H. B., Kenfack, M. C., Nkondo, E. T., Femoe, U. M., Fassi, J. B. K., . . . Tchuente, L.-A. T. (2020). In vitro Activity of Ozoroa pulcherrima Schweinf. Extracts and Fractions on Schistosoma mansoni Cercariae and Adult Worms. *European Journal of Medicinal Plants*, 17-30.

Fomum, S. W., & Nsahlai, I. V. (2017). In vitro nematicidal activity of plant species possessing alkaloids and tannins. *Cogent Food & Agriculture, 3*(1), 1334295.

Foroumadi, A., Mirzaei, M., & Shafiee, A. (2001). Antituberculosis agents, I: Synthesis and antituberculosis activity of 2-aryl-1, 3, 4-thiadiazole derivatives. *Die Pharmazie, 56*(8), 610-612.

Fowotade, A., Fowotade, A., Enaibe, B., & Avwioro, G. (2017). Evaluating toxicity profile of garlic (Allium sativum) on the liver, kidney and heart using Wistar rat model. *International Journal of Tropical Disease & Health, 26*(2), 1-12.

French, K. E. (2018). Plant-based solutions to global livestock anthelmintic resistance. *Ethnobiology Letters, 9*(2), 110-123.

Gakuya, D. W. (2001). *Harmacological and clinical evaluation Anthelmintic activity of Albizia Anthelmintica Maerua Edulis de wolf and Maerua Subcordata plant extracts in sheep and mice.*

Gao, H., Hou, B., Kuroyanagi, M., & Wu, L. (2007). Constituents of anti-tumor promoting active part of Dioscorea bulbifera in JB6 mouse epidermal cells. *Asiaan Journal of Traditional Medicine, 2*, 104-109.

Gao, H., Kuroyanagi, M., Wu, L., Kawahara, N., Yasuno, T., & Nakamura, Y. (2002). Antitumor-promoting constituents from Dioscorea bulbifera L. in JB6 mouse epidermal cells. *Biological and Pharmaceutical Bulletin, 25*(9), 1241-1243.

Gatsing, D., Aliyu, R., Kuiate, J. R., Garba, I. H., Jaryum, K. H., Tedongmo, N., . . . Adoga, G. I. (2005). Toxicological evaluation of the aqueous extract of Allium sativum bulbs on laboratory mice and rats. *Cameroon Journal of Experimental Biology, 1*(1), 39-45.

Geary, T. G. (2005). Ivermectin 20 years on: maturation of a wonder drug. *Trends in parasitology, 21*(11), 530-532.

Geidam, Y., Ambali, A., & Onyeyili, P. (2007). Preliminary phytochemical and antibacterial evaluation of crude aqueous extract of Psidium guajava leaf. *Journal of Applied Sciences, 7*(4), 511-514.

Gelfland, M., Mavi, S., Drummond, R., & Ndemera, B. (1985). The traditional medical practitioner in Zimbabwe: his principles of practice and pharmacopoeia. *The traditional medical practitioner in Zimbabwe: his principles of practice and pharmacopoeia.*

Gemechu, E. C. (2021). Assessment of indigenous knowledge of medicinal plants used for livestock treatment in five selected Kebeles of Kersa District, Jimma Zone, South Western Ethiopia. *Journal of Scientific Agriculture, 5*, 49-54.

George, L. O., Radha, H., & Somasekariah, B. (2018). In vitro anti-diabetic activity and GC-MS analysis of bioactive compounds present in the methanol extract of Kalanchoe pinnata.

Ghafar, A., Arbabi, M., Mosayebi, M., Hooshyar, H., & Nickfarjam, A. M. (2021). Evaluation of anti-helmintic activity of *Zingiber officinale* roscoe extract on *Fasciola hepatica* miracidia *In vitro*. *International Archives of Health Sciences, 8*(1), 45.

Ghimire, S. K., Gimenez, O., Pradel, R., McKey, D., & Aumeeruddy-Thomas, Y. (2008). Demographic variation and population viability in a threatened Himalayan medicinal and aromatic herb Nardostachys

grandiflora: matrix modelling of harvesting effects in two contrasting habitats. *Journal of Applied Ecology, 45*(1), 41-51.
Ghosh, C. (2017). Ethnobotanical survey in the Bamangola Block of Malda District, West Bengal (India): II. Medicinal and Aromatic plants.
Ghosh, M., Ghosh, T., Sinha Babu, S., & Sukul, N. (1992). *Antifilarial effect of a plant, Zingiber officinale on Setaria cervi in rats.* Paper presented at the Proc Zool Soc.
Ghosh, S., Parihar, V., More, P., Dhavale, D., & Chopade, B. (2015). Phytochemistry and therapeutic potential of medicinal plant: Dioscorea bulbifera. *Med chem, 5*(4), 154-159.
Giday, M., Asfaw, Z., Elmqvist, T., & Woldu, Z. (2003). An ethnobotanical study of medicinal plants used by the Zay people in Ethiopia. *Journal of ethnopharmacology, 85*(1), 43-52.
Gideon, V. A. (2015). GC-MS analysis of phytochemical components of Pseudoglochidion
anamalayanum Gamble: An endangered medicinal tree *Asian Journal of Plant Science and Research, 5*(12), 36-41.
Gill, R., Kalsi, V., & Singh, A. (2011). Phytochemical investigation and evaluation of anthelmintic activity of Curcuma amada and Curcuma caesia-A comparative study. *Inventi Impact Ethnopharmacol, 2011*.
Girija, S., Duraipandiyan, V., Kuppusamy, P. S., Gajendran, H., & Rajagopal, R. (2014). Chromatographic characterization and GC-MS evaluation of the bioactive constituents with antimicrobial potential from the pigmented ink of Loligo duvauceli. *International Scholarly Research Notices, 2014*.
Githiori, J. B., Athanasiadou, S., & Thamsborg, S. M. (2006). Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. *Veterinary parasitology, 139*(4), 308-320.
Githiori, J. B., Höglund, J., Waller, P. J., & Baker, R. L. (2003). Evaluation of anthelmintic properties of extracts from some plants used as livestock dewormers by pastoralist and smallholder farmers in Kenya against Heligmosomoides polygyrus infections in mice. *Veterinary parasitology, 118*(3-4), 215-226.
Goel, R., Prabha, T., Kumar, M. M., Dorababu, M., & Singh, G. (2006). Teratogenicity of Asparagus racemosus Willd. root, a herbal medicine.
Gooneratne, B. (1966). Massive generalized alopecia after poisoning by Gloriosa superba. *British Medical Journal, 1*(5494), 1023.
Goswami, S., Pandey, A., Tripathi, P., Singh, A., & Rai, A. (2011). An *in vitro* evaluation of the anthelmintic activity of *Hedychium spichatum* rhizomes and *Zingiber zerumbet* rhizomes on the *Pheritima Posthuma* model: A comparative study. *Pharmacognosy research, 3*(2), 140.
Goto, C., Kasuya, S., Koga, K., Ohtomo, H., & Kagei, N. (1990). Lethal efficacy of extract from *Zingiber officinale* (traditional Chinese medicine) or [6]-shogaol and [6]-gingerol in Anisakis larvae *in vitro*. *Parasitology Research, 76*(8), 653-656.
Goyal, R., Singh, J., & Lal, H. (2003). Asparagus racemosus-an update. *Indian journal of medical sciences, 57*(9), 408-414.
Grasso, S., De Sarro, G., De Sarro, A., Micale, N., Zappalà, M., Puja, G., . . . De Micheli, C. (2000). Synthesis and anticonvulsant activity of novel and potent 6, 7-methylenedioxyphthalazin-1 (2 H)-ones. *Journal of medicinal chemistry, 43*(15), 2851-2859.
Guan, X.-R., Zhu, L., Xiao, Z.-G., Zhang, Y.-L., Chen, H.-B., & Yi, T. (2017). Bioactivity, toxicity and detoxification assessment of Dioscorea bulbifera L.: a comprehensive review. *Phytochemistry Reviews, 16*(3), 573-601.
Guest, D., Hamilton, M. L., Deisinger, P. J., & DiVincenzo, G. D. (1984). Pulmonary and percutaneous absorption of 2-propoxyethyl acetate and 2-ethoxyethyl acetate in beagle dogs. *Environmental health perspectives, 57*, 177-183.
Gunbatan, T., Gurbuz, I., & Ozkan, A. M. G. (2016). The current status of ethnopharmacobotanical knowledge in Çamlıdere (Ankara, Turkey). *Turkish Journal of Botany, 40*(3), 241-249.
Guo, F., Kwakkel, R., Williams, B., Parmentier, H., Li, W., Yang, Z., & Verstegen, M. (2004). Effects of mushroom and herb polysaccharides on cellular and humoral immune responses of Eimeria tenella-infected chickens. *Poultry Science, 83*(7), 1124-1132.
Gupta, D., & Singh, J. (1989). p-Hydroxy acetophenone derivatives from Dioscorea bulbifera. *Phytochemistry, 28*(3), 947-949.
Gupta, R. R., Kumar, M., & Gupta, V. (2013). *Heterocyclic Chemistry: Volume II: Five-Membered Heterocycles*: Springer Science & Business Media.
Gusha, B., Palmer, A., & Villano, R. (2018). Performance of livestock production in north eastern cape communal areas: a stochastic frontier analysis.
Gutiérrez, R. M. P. (2010). Orchids: A review of uses in traditional medicine, its phytochemistry and pharmacology. *Journal of Medicinal Plants Research, 4*(8), 592-638.

Gwalwanshi, D. R., Salunkhe, O., Shukla, A., Bishwas, A. J., & Vyas, D. (2014). Indigenous Knowledge and Documentation of Ethno-Medicinal Plants of Panna District, Central India: A Case Study. *the Journal of Ethnobiology and Traditional Medicine. Photon, 122*, 868-876.
Haile, M., & Kang, W. H. (2019). Antioxidant activity, total polyphenol, flavonoid and tannin contents of fermented green coffee beans with selected yeasts. *Fermentation, 5*(1), 29.
Hamayun, M. (2007). Traditional uses of some medicinal plants of Swat Valley, Pakistan.
Hatcher, H., Planalp, R., Cho, J., Torti, F., & Torti, S. (2008). Curcumin: from ancient medicine to current clinical trials. *Cellular and molecular life sciences, 65*(11), 1631-1652.
Hatta, G. M. (2020). Indigenous knowledge of Ethnic Bugis Pagatan in using of medicinal plants, South Kalimantan, Indonesia.
Hayajneh, F. M., Titi, H. H., Alnimer, M. A., & Irshaid, R. (2019). Evaluation of anthelmintics resistance against gastrointestinal parasites infection in Awassi sheep in Jordan and the use of alternative herbal anthelmentics. *Am. J. Anim. Vet. Sci, 14*(2), 122-126.
Hazrat, A., Nisar, M., Shah, J., & Ahmad, S. (2011). Ethnobotanical study of some elite plants belonging to Dir, Kohistan valley, Khyber Pukhtunkhwa, Pakistan. *Pak J Bot, 43*(2), 787-795.
Heckel, B., Phillips, E., Edwards, E., Sherwood Lollar, B., Elsner, M., Manefield, M. J., & Lee, M. (2019). Reductive dehalogenation of trichloromethane by two different Dehalobacter restrictus strains reveal opposing dual element isotope effects. *Environmental science & technology, 53*(5), 2332-2343.
Hellawi, H., & Ibrahim, O. M. (2021). Evaluation of Anthelmintic Activity of N-hexane Extract of Cucurbita Maxima and Azadirachta Indica Pulp Seed Against Ascaridia Galli in Vitro.
Hendrickx, E., Thomas, L. F., Dorny, P., Bobić, B., Braae, U. C., Devleesschauwer, B., . . . Torgerson, P. R. (2019). Epidemiology of Taenia saginata taeniosis/cysticercosis: a systematic review of the distribution in West and Central Africa. *Parasites & vectors, 12*(1), 1-10.
Hernando, G., Turani, O., & Bouzat, C. (2019). Caenorhabditis elegans muscle Cys-loop receptors as novel targets of terpenoids with potential anthelmintic activity. *PLoS neglected tropical diseases, 13*(11), e0007895.
Hidalgo, W., Duque, L., Saez, J., Arango, R., Gil, J., Rojano, B., . . . Otalvaro, F. (2009). Structure− activity relationship in the interaction of substituted perinaphthenones with Mycosphaerella fijiensis. *Journal of agricultural and food chemistry, 57*(16), 7417-7421.
Hodgson, B., & Mulvaney, C. (2017). Resistance to a triple-combination anthelmintic in Trichostrongylus spp. on a commercial sheep farm in New Zealand. *New Zealand Veterinary Journal, 65*(5), 277-281.
Hogade, M. G., Jalalpure, S., Bhinge, S. D., Kuthar, S., & Kosgi, S. (2013). Invitro Anthelmintic Activity of Bark of Azadirachta indica against Ascardi galli and Eudrilus eugeniae. *Journal of Natural Remedies, 14*(1), 48-51.
Hölscher, D., Dhakshinamoorthy, S., Alexandrov, T., Becker, M., Bretschneider, T., Buerkert, A., . . . Heckel, D. G. (2014). Phenalenone-type phytoalexins mediate resistance of banana plants (Musa spp.) to the burrowing nematode Radopholus similis. *Proceedings of the National Academy of Sciences, 111*(1), 105-110.
Hossain, M. M. (2011). Therapeutic orchids: traditional uses and recent advances—an overview. *Fitoterapia, 82*(2), 102-140.
Hossain, S., Khatun, A., & Miajee, U. (2010). Medicinal plants used by folk medicinal practitioners in three villages of Natore and Rajshahi districts, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture, 4*(2), 211-218.
Hoste, H., & Torres-Acosta, J. (2011). Non chemical control of helminths in ruminants: adapting solutions for changing worms in a changing world. *Veterinary parasitology, 180*(1-2), 144-154.
Hothi, H., Makkar, A., Sharma, J., & Manrao, M. (2008). Synthesis and antifungal 8 potential of Schiff bases of 2'-hydroxyacetophenone and their Cu (II) complexes. *Ind. J. Agri. Chemi, 41*, 53-58.
Hounzangbe-Adote, M., Paolini, V., Fouraste, I., Moutairou, K., & Hoste, H. (2005). In vitro effects of four tropical plants on three life-cycle stages of the parasitic nematode, Haemonchus contortus. *Research in veterinary Science, 78*(2), 155-160.
Huang, L., Zhu, X., Zhou, S., Cheng, Z., Shi, K., Zhang, C., & Shao, H. (2021). Phthalic Acid Esters: Natural Sources and Biological Activities. *Toxins, 13*(7), 495.
Hussein, H. J., Hameed, I. H., & Hadi, M. Y. (2017). Using gas chromatography-mass spectrometry (GC-MS) technique for analysis of bioactive compounds of methanolic leaves extract of Lepidium sativum. *Research Journal of Pharmacy and Technology, 10*(11), 3981-3989.
Hutchings, A. (1996). *Zulu medicinal plants: An inventory*: University of Natal press.
Hynniewta, S., & Kumar, Y. (2008). Herbal remedies among the Khasi traditional healers and village folks in Meghalaya.
IA Mgbeje, B., Abu, C., O Ugoanyanwu, F., & E Ebong, P. (2020). Towards Establishing Chemical Markers for Antidiabetic Plants: A Comparative Analysis of the Chemical Fingerprints of Three Validated

Antidiabetic Plants, Nauclea latifolia, Azadirachta indica and Moringa oleifera. *Merit Research Journal of Medicine and Medical Sciences, 8*(10), 537-547.

Ibekwe, H. (2019). In vitro anthelmintic activities of aqueous crude extract of Azadirachta indica on Paramphistomum cervi and Fasciola hepatica. *International Journal of Veterinary Sciences and Animal Husbandry, 4*(1), 14-18.

Ibukun, O., & Oluwadare, E. E. (2021). In vitro Antioxidant Property and Acute Toxicity Study of Methanol Extract of Leaves of Zingiber officinale and Curcuma longa. *Free Radicals and Antioxidants, 11*(2), 42-45.

Idan, S. A., Al-Marzoqi, A. H., & Hameed, I. H. (2015). Spectral analysis and anti-bacterial activity of methanolic fruit extract of Citrullus colocynthis using gas chromatography-mass spectrometry. *African Journal of Biotechnology, 14*(46), 3131-3158.

Idang, E. O., Yemitan, O. K., Mbagwu, H. O., Udom, G. J., Ogbuagu, E. O., & Udobang, J. A. (2019). Toxicological assessment of Zingiber officinale Roscoe (Ginger) root oil extracts in Albino rats. *Toxicology Digest, 4*(1), 108-119.

Ikbal, A. M. A., Rajkhowa, A., Singh, P. C., Choudhury, P. D., & Sahu, R. K. (2020). Assessment of Phytochemical and Anthelmintic Activity of Some Selected Ethnomedicinal Plants from Barak Valley Region of Assam. *Biomedical and Pharmacology Journal*, *13*(4), 1825-1831.

Ingle, K. P., Deshmukh, A. G., Padole, D. A., Dudhare, M. S., Moharil, M. P., & Khelurkar, V. C. (2017). Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. *Journal of Pharmacognosy and Phytochemistry, 6*(1), 32-36.

Innocent, T., & Deogracious, O. (2006). The anthelmintic activity of selected indigenous medicinal plants used by the anyankole of Western Uganda. *Journal of animal and veterinary advances*.

Iqbal, Z., Asim, M., Ahmad, A., Abbas, R. Z., & Aslam, B. (2014). In vitro ovicidal and wormicidal activity of six medicinal plants against Haemonchus contortus. *International Journal of Agriculture & Biology, 16*(6).

Iqbal, Z., Babar, W., Abbas, R. Z., & Sajid, M. S. (2012). Evaluation of Anthelmintic Activity of Different Fractions of Azadirachta indica A. Juss Seed Extract. *Pakistan Veterinary Journal, 32*(4).

Iqbal, Z., Lateef, M., Akhtar, M. S., Ghayur, M. N., & Gilani, A. H. (2006). *In vivo* anthelmintic activity of ginger against gastrointestinal nematodes of sheep. *Journal of ethnopharmacology, 106*(2), 285-287.

Iqbal, Z., Lateef, M., Jabbar, A., & Gilani, A. (2010). In vivo anthelmintic activity of Azadirachta indica A. Juss seeds against gastrointestinal nematodes of sheep. *Veterinary parasitology, 168*(3-4), 342-345.

Iqbal, Z., Nadeem, Q. K., Khan, M., Akhtar, M., & Waraich, F. N. (2001). In vitro anthelmintic activity of Allium sativum, Zingiber officinale, Curcurbita mexicana and Ficus religiosa. *International Journal of Agriculture and Biology, 3*(4), 454-457.

Ishnava, K. B., & Konar, P. S. (2020). In vitro anthelmintic activity and phytochemical characterization of Corallocarpus epigaeus (Rottler) Hook. f. tuber from ethyl acetate extracts. *Bulletin of the National Research Centre, 44*(1), 1-10.

Ishnava, K. B., & Patel, K. S. (2020). In vitro study of Praecitrulus fistulosus (Stocks) Pangalo (Cucurbitaceae) fruit–A potential candidate of Anthelmintic activity. *Bulletin of the National Research Centre, 44*(1), 1-10.

Ishtiyak, P., & Hussain, S. A. (2017). Traditional use of medicinal plants among tribal communities of Bangus valley, Kashmir Himalaya, India. *Studies on Ethno-Medicine, 11*(4), 318-331.

Islam, M., Rahman, M. M., Hasan, M., Rahaman, M., Khan, M., & Al-Faysal, A. (2016). Evaluation of anthelmintic activity of crude extracts and different fractions of stem bark and fruits of Oroxylum indicum. *World J. Pharm. Res, 5*, 287-294.

Ittiyavirah, S. P., Jumimol, T., Krishnapriya, T., Krishnaja, K., & Manjusha, V. (2012). Comparative evaluation of aqueous extracts of some selected medicinal plants for anthelmintic activity. *International Journal of Pharmacology & Biological Sciences, 6*(1).

Jabbar, A., Zaman, M. A., Iqbal, Z., Yaseen, M., & Shamim, A. (2007). Anthelmintic activity of Chenopodium album (L.) and Caesalpinia crista (L.) against trichostrongylid nematodes of sheep. *Journal of ethnopharmacology, 114*(1), 86-91.

Jafari, E., Khajouei, M. R., Hassanzadeh, F., Hakimelahi, G. H., & Khodarahmi, G. A. (2016). Quinazolinone and quinazoline derivatives: recent structures with potent antimicrobial and cytotoxic activities. *Research in pharmaceutical sciences, 11*(1), 1.

Jagtap, S., & Satpute, R. (2014). Phytochemical screening, antioxidant, antimicrobial and flavonoid analysis of Gloriosa superba Linn. Rhizome extracts. *Journal of academia and industrial research, 3*(6), 247-254.

Jaja, I. F., Mushonga, B., Green, E., & Muchenje, V. (2017). Financial loss estimation of bovine fasciolosis in slaughtered cattle in South Africa. *Parasite epidemiology and control, 2*(4), 27-34.

James, O., & God, U. O. T. (2014). In-vitro Anthelmintic Activity of Saba Florida (Benth) Extracts Against Nigerian Adult Earth Worm (Terrestris lumbricoides).

Jamnah, O., Khadijah, S., & Vincent, N. (2006). Daily feeding of fresh Neem leaves (Azadirachta indica) for worm control in sheep. *J. ournal of Tropical Biomedicine, 23*(1), 23-30.

Jamra, N., Das, G., Singh, P., & Haque, M. (2015). Anthelmintic efficacy of crude neem (Azadirachta indica) leaf powder against bovine strongylosis. *Journal of Parasitic Diseases, 39*(4), 786-788.

Jana, S., & Shekhawat, G. (2011). Critical review on medicinally potent plant species: Gloriosa superba. *Fitoterapia, 82*(3), 293-301.

Jansen, P., & Cardon, D. (2005). *Plant resources of tropical Africa 3. Dyes and tannins*: Programme PROTA.

Jeannette, Y., Olivia, F. T., Komtangi, M. C., CF, B. B., & Mbida, M. (2011). The in vitro effects of Chenopodium ambrosioides (Chenopodiaceae) extracts on the parasitic nematode Heligmosomoides bakeri (Nematoda, Heligmosomatidae). *Journal of Pharmacognosy and Phytotherapy, 3*(4), 56-62.

Jebamalar, U. J. J. A., Gajalakshmi, D., & Sivakumar, T. (2019). Phytochemical analysis, and evaluation of antimicrobial activity in the whole plant extracts of Gloriosa superb. *Asian journal of Pharmaceutical and clinical research, 12*, 245-249.

Jebasingh, D., Jackson, D., Venkataraman, S., & Emerald, B. (2012). Physiochemical and toxicological studies of the medicinal plant Cyperus rotundus L (Cyperaceae). *International Journal of applied Research in natural products, 5*(4), 1-8.

Jefferson, W. N., & Williams, C. J. (2011). Circulating levels of genistein in the neonate, apart from dose and route, predict future adverse female reproductive outcomes. *Reproductive toxicology, 31*(3), 272-279.

Jenkins, E. J., Schurer, J. M., & Gesy, K. M. (2011). Old problems on a new playing field: Helminth zoonoses transmitted among dogs, wildlife, and people in a changing northern climate. *Veterinary parasitology, 182*(1), 54-69.

Jeong, S.-M., Kim, S.-Y., Kim, D.-R., Jo, S.-C., Nam, K., Ahn, D., & Lee, S.-C. (2004). Effect of heat treatment on the antioxidant activity of extracts from citrus peels. *Journal of agricultural and Food Chemistry, 52*(11), 3389-3393.

Jeyasheela, R., Chairman, K., Padmalatha, C., & Ranjit Singh, J. (2014). Isolation and phytochemical characterization of bioactive compounds from the rhizomes of Cyperus rotundus. *Global Journal of Science Frontier Research; C Biological Science, 14*(2), 1-8.

Jeyathilakan, N., Murali, K., Anandaraj, A., & Abdul Basith, S. (2010). *In vitro* evaluation of anthelmintic property of herbal plants against *Fasciola gigantica*. *Indian Journal of Animal Sciences, 80*(11), 1070.

Jeyathilakan, N., Murali, K., Anandaraj, A., & Abdul Basith, S. (2012). In vitro evaluation of anthelmintic property of ethno-veterinary plant extracts against the liver fluke Fasciola gigantica. *Journal of Parasitic Diseases, 36*(1), 26-30.

Jha, M., Alam, M., Hossain, M., & Islam, A. (2010). In vitro antioxidant and cytotoxic potential of Costus speciosus (Koen.) Smith rhizome. *International Journal of Pharmaceutical Sciences and Research, 1*(10), 138.

Ji, M., Choi, J., Lee, J., & Lee, Y. (2004). Induction of apoptosis by ar-turmerone on various cell lines. *International journal of molecular medicine, 14*(2), 253-256.

Johansson, L. (2017). *The impact of gastrointestinal parasites on weight gain, activity patterns and behaviours in cattle on pasture.* Institutionen för husdjurens miljö och hälsa.

Joseph, C., Ndoile, M., Malima, R., & Nkunya, M. (2004). Larvicidal and mosquitocidal extracts, a coumarin, isoflavonoids and pterocarpans from Neorautanenia mitis. *Transactions of the Royal Society of Tropical Medicine and hygiene, 98*(8), 451-455.

Joshi, B., Kommuru, D., Terrill, T., Mosjidis, J., Burke, J., Shakya, K., & Miller, J. (2011). Effect of feeding sericea lespedeza leaf meal in goats experimentally infected with Haemonchus contortus. *Veterinary Parasitology, 178*(1-2), 192-197.

Joshi, P. (1993). Tribal remedies against snake bites and scorpion stings in Rajasthan. *Glimpses Plant Res, 10*, 23-25.

Joshi, S. V., Vyas, B. A., Shah, P. D., Shah, D. R., Shah, S. A., & Gandhi, T. R. (2011). Protective effect of aqueous extract of Oroxylum indicum Linn.(root bark) against DNBS-induced colitis in rats. *Indian Journal of Pharmacology, 43*(6), 656.

Jucá, M. M., Cysne Filho, F. M. S., de Almeida, J. C., Mesquita, D. d. S., Barriga, J. R. d. M., Dias, K. C. F., . . . Ribeiro, J. E. (2020). Flavonoids: biological activities and therapeutic potential. *Natural product research, 34*(5), 692-705.

Jung, S.-H., Kim, S. J., Jun, B.-G., Lee, K.-T., Hong, S.-P., Oh, M. S., . . . Choi, J.-H. (2013). α-Cyperone, isolated from the rhizomes of Cyperus rotundus, inhibits LPS-induced COX-2 expression and PGE2 production through the negative regulation of NFκB signalling in RAW 264.7 cells. *Journal of ethnopharmacology, 147*(1), 208-214.

Kadam, P. U., Kharde, M. R., Lamage, S. T., Borse, S., Borse, L., & Pawar, S. (2015). Anthelmintic activity of Allium sativum. *Pharma Science Monitor, 6*(2).

Kadhim, M. (2016). In Vitro antifungal potential of Acinetobacter baumannii and determination of its chemical composition by gas chromatography-mass spectrometry. *Der Pharma Chemica, 8*(19), 657-665.

Kadhim, M. J., Al-Rubaye, A. F., & Hameed, I. H. (2017). Determination of bioactive compounds of methanolic extract of vitis vinifera using GC-MS. *International Journal of Toxicological and Pharmacological Research, 9*(2), 113-126.

Kalirajan, R., Jubie, S., & Gowramma, B. (2015). Microwave Irradated Synthesis, Characterization and Evaluation for their Antibacterial and Larvicidal Activities of some Novel Chalcone and Isoxazole Substituted 9-Anilino Acridines. *Open Journal of Chemistry, 1*(1), 001-007.

Kalpana, M., & Anbazhagan, M. (2009). In vitro production of Kaempferia galanga (L.)-an endangered medicinal plant. *Journal of Phytology, 1*(1), 56-61.

Kamsani, N., Zakaria, Z., Md Nasir, N., Mohtarrudin, N., & Mohamad Alitheen, N. (2019). Safety assessment of methanol extract of Melastoma malabathricum L. leaves following the subacute and subchronic oral consumptions in rats and its cytotoxic effect against the HT29 cancer cell line. *Evidence-Based Complementary and Alternative Medicine, 2019*.

Kanjanapothi, D., Panthong, A., Lertprasertsuke, N., Taesotikul, T., Rujjanawate, C., Kaewpinit, D., . . . Jitpakdi, A. (2004). Toxicity of crude rhizome extract of Kaempferia galanga L.(Proh Hom). *Journal of Ethnopharmacology, 90*(2-3), 359-365.

Kanojiya, D., Shanker, D., Sudan, V., Jaiswal, A. K., & Parashar, R. (2015). Assessment of in vitro and in vivo anthelminthic potential of extracts of Allium sativum bulb against naturally occurring ovine gastrointestinal nematodiosis. *Veterinary Quarterly, 35*(4), 200-206.

Kaplan, R. M. (2004). Drug resistance in nematodes of veterinary importance: a status report. *Trends in parasitology, 20*(10), 477-481.

Kaplan, R. M., & Vidyashankar, A. N. (2012). An inconvenient truth: global worming and anthelmintic resistance. *Veterinary parasitology, 186*(1-2), 70-78.

Kar, P., & Tandon, V. (2000). Anthelmintic efficacy of Flemingia vestita (Fabaceae): genistein induced effect on the nervous components in two digenetic trematodes. *Journal of Parasitic Diseases, 24*(2), 141-146.

Kar, P. K., Tandon, V., & Saha, N. (2002). Anthelmintic efficacy of Flemingia vestita: genistein-induced effect on the activity of nitric oxide synthase and nitric oxide in the trematode parasite, Fasciolopsis buski. *Parasitology International, 51*(3), 249-257.

Kar, P. K., Tandon, V., & Saha, N. (2004). Anthelmintic efficacy of genistein, the active principle of Flemingia vestita (Fabaceae): alterations in the free amino acid pool and ammonia levels in the fluke, Fasciolopsis buski. *Parasitology International, 53*(4), 287-291.

Karim, A., Singh, I., Khan, M. W., & Chourasia, R. (2017). Anthelmintic activity of Curcuma caesia Roxb rhizome in indian adult earthworm.

Karim, M. A., Islam, M. A., Islam, M. M., Rahman, M. S., Sultana, S., Biswas, S., . . . Hasan, M. N. (2020). Evaluation of antioxidant, anti-hemolytic, cytotoxic effects and anti-bacterial activity of selected mangrove plants (Bruguiera gymnorrhiza and Heritiera littoralis) in Bangladesh. *Clinical Phytoscience, 6*(1), 1-12.

Karmakar, U., Sadhu, S., Biswas, S., Chowdhury, A., Shill, M., & Das, J. (2012). Cytotoxicity, analgesic and antidiarrhoeal activities of Asparagus racemosus. *Journal of applied Sciences, 12*(6), 581-586.

Karnick, C. (1971). On the intrinsic factors and nodal complexities of Dioscorea deltoidea and Dioscorea prazerii. *Planta medica, 20*(05), 257-262.

Karunarathne, P., Thammitiyagodage, M., & Weerakkody, N. (2018). Safety evaluation of galangal (Alpinia galanga) extract for therapeutic use as an antimicrobial agent. *International Journal of Pharmaceutical Science. and Research, 9*(11), 4582-4590.

Kasala, S., Ramanjaneyulu, K., Himabindhu, J., Alluri, R., & Babu, R. R. (2016). Preliminary phytochemical screening and in vitro anthelmintic activity of Cyperus rotundus (L). *Journal of Pharmacognosy and Phytochemistry, 5*(5), 407.

Kasiotis, K. M., Pratsinis, H., Kletsas, D., & Haroutounian, S. A. (2013). Resveratrol and related stilbenes: their anti-aging and anti-angiogenic properties. *Food and Chemical Toxicology, 61*, 112-120.

Katiki, L., Barbieri, A., Araujo, R., Veríssimo, C., Louvandini, H., & Ferreira, J. (2017). Synergistic interaction of ten essential oils against Haemonchus contortus in vitro. *Veterinary Parasitology, 243*, 47-51.

Katiki, L., Chagas, A., Takahira, R. K., Juliani, H., Ferreira, J., & Amarante, A. F. T. d. (2012). Evaluation of Cymbopogon schoenanthus essential oil in lambs experimentally infected with Haemonchus contortus. *Veterinary parasitology, 186*(3-4), 312-318.

Kaufman, P. B., Cseke, L. J., Warber, S., Duke, J. A., & Brielmann, H. L. (2003). *Natural Products from Plants*. USA: CRC Press.

Kaur, B., Khatun, S., & Suttee, A. (2021). Current highlights on biochemical and pharmacological profile of Dioscorea alata: A Review. *Plant Archives, 21*(1), 552-559.

Kaur, P., Gupta, R., Dey, A., & Pandey, D. K. (2019). Simultaneous quantification of oleanolic acid, ursolic acid, betulinic acid and lupeol in different populations of five Swertia species by using HPTLC-densitometry: comparison of different extraction methods and solvent selection. *Industrial crops and products, 130*, 537-546.

Kausar, S. (2017). In vitro evaluation of antifilarial effect of Azadirachta indica leaves extract in different solvents on the microfilariae of Setaria cervi. *Journal of Parasitic Diseases, 41*(1), 9-15.

Kavindra, S., & Shalini, N. (2000). Studies on the anthelmintic activity of Allium sativum (garlic) oil on common poultry worms Ascaridia galli and Heterakis gallinae. *Journal of parasitology and applied animal biology, 9*(1), 47-52.

Kavitha, S., Gnanavel, S., & Kannan, K. (2014). Biological aspects of 1, 3, 4-oxadiazole derivatives. *Asian Journal of Pharmaceutical and Clinical Research, 7*(4), 11-20.

Kebede, A., Ayalew, S., Mesfin, A., & Mulualem, G. (2016). Ethnobotanical investigation of traditional medicinal plants commercialized in the markets of Dire Dawa city, eastern Ethiopia. *Journal of Medicinal Plants Studies, 4*(3), 170-178.

Keinänen, M., & Julkunen-Tiitto, R. (1996). Effect of sample preparation method on birch (Betula pendula Roth) leaf phenolics. *Journal of agricultural and Food Chemistry, 44*(9), 2724-2727.

Kenyon, F., Sargison, N., Skuce, P., & Jackson, F. (2009). Sheep helminth parasitic disease in south eastern Scotland arising as a possible consequence of climate change. *Veterinary parasitology, 163*(4), 293-297.

Kesari, V., Krishnamachari, A., & Rangan, L. (2008). Systematic characterisation and seed oil analysis in candidate plus trees of biodiesel plant, Pongamia pinnata. *Annals of Applied Biology, 152*(3), 397-404.

Khan, M. G., Nahar, K., Rahman, M. S., Hasan, C. M., & Rashid, M. A. (2009). Phytochemical and biological investigations of Curcuma longa. *Dhaka University Journal of Pharmaceutical Sciences, 8*(1), 39-45.

Khan, M. S. A., & Ahmad, I. (2019). Herbal Medicine: Current Trends and Future Prospects *New Look to Phytomedicine* (pp. 3-13): Elsevier.

Khanam, P., Sangeetha, B., Kumar, B., Kiran, U., Priyadarshini, P., Ram, R., . . . Kumar, V. (2015). Gloriosa superba ingestion: Hair loss and acute renal failure. *Indian journal of nephrology, 25*(3), 174.

Khaniki, G. R. J., Kia, E. B., & Raei, M. (2013). Liver condemnation and economic losses due to parasitic infections in slaughtered animals in Iran. *Journal of parasitic diseases, 37*(2), 240-244.

Khatri, B. B. A. (2012). A case study report on medicinal plants in Puranchaur VDC, Kaski District. *The Sanjivini*, 64.

Khatri, B. B. A. (2012

). A case study report on medicinal plants in Puranchaur VDC, Kaski District. *The Sanjivini*, 64.

Khatua, S., Pandey, A., & Biswas, S. J. (2016). Phytochemical evaluation and antimicrobial properties of Trichosanthes dioica root extract. *Journal of Pharmacognosy and Phytochemistry, 5*(5), 410.

Kiambom, T., Kouam, M. K., Ngangoum, C. D., Kate, B., & Teguia, A. (2021). *In Vivo* Anthelmintic Effect of Ginger (Z*ingiber Officinale*) Powder against Gastointestinal Nematodes of Artificially Infected Pigs. *Archives of Veterinary Science and Medicine, 4*(1), 1-12.

Kilani, S., Ledauphin, J., Bouhlel, I., Sghaier, M. B., Boubaker, J., Skandrani, I., . . . Chekir-Ghedira, L. (2008). Comparative study of Cyperus rotundus essential oil by a modified GC/MS analysis method. Evaluation of its antioxidant, cytotoxic, and apoptotic effects. *Chemistry & Biodiversity, 5*(5), 729-742.

Kim, J. S., Rhee, H.-K., Park, H. J., Lee, S. K., Lee, C.-O., & Choo, H.-Y. P. (2008). Synthesis of 1-/2-substituted-[1, 2, 3] triazolo [4, 5-g] phthalazine-4, 9-diones and evaluation of their cytotoxicity and topoisomerase II inhibition. *Bioorganic & medicinal chemistry, 16*(8), 4545-4550.

Kim, J. Y., Kim, J. S., Jung, J. H., Chun, P., & Rhew, K. Y. (2014). Inhibitory effects of puerarin on cytochrome P450 subfamilies in vitro. *Oriental Pharmacy and Experimental Medicine, 14*(1), 1-5.

Kim, S. J., Chung, W. S., Kim, S. S., Ko, S. G., & Um, J. Y. (2011). Antiinflammatory effect of Oldenlandia diffusa and its constituent, hentriacontane, through suppression of caspase-1 activation in mouse peritoneal macrophages. *Phytotherapy Research, 25*(10), 1537-1546.

Kingsley, O.-A., Lateef, A. O., Olga, Q., Stephen, A., & Mavis, T. (2012). A comparative evaluation of in vivo antiplasmodial activity of aqueous leaf exracts of Carica papaya, Azadirachta indica, Magnifera indica and the combination thereof using plasmodium infected BALB/c mice.

Kinyua, A. W., Ko, C. M., Doan, K. V., Yang, D. J., Huynh, M. K. Q., Moh, S. H., . . . Kim, K. W. (2018). 4-hydroxy-3-methoxycinnamic acid regulates orexigenic peptides and hepatic glucose homeostasis through phosphorylation of FoxO1. *Experimental & molecular medicine, 50*(2), e437-e437.

Kiranmayi, G., Ravishankar, K., & Priyabandhavi, P. (2012). Phytochemical screening and in vitro comparative study of anthelmintic activity of Asparagus racemosus and Cucurbita maxima. *Journal of Pharmacy Research, 5*(3), 1545-1547.

Kithuka, J., Maingi, N., Njeruh, F., & Ombui, J. N. (2002). The prevalence and economic importance of bovine fasciolosis in Kenya-an analysis of abattoir data. *Onderstepoort Journal of Veterinary Research, 69*(4), 255-262.

Kiuchi, F., Nakamura, N., Miyashita, N., Nishizawa, S., Tsuda, Y., & Kondo, K. (1989). Nematocidal activity of some anthelmintics, traditional medicines, and spices by new assay method using larvae of *Toxocara canis*. *Japanese Journal of Pharmacognosy, 43*(4), 279-287.

Klein, C. B., & King, A. A. (2007). Genistein genotoxicity: critical considerations of in vitro exposure dose. *Toxicology and applied pharmacology, 224*(1), 1-11.

Koh, H. L., Tan, C. H., & Chua, T. K. (2009). *Guide to Medicinal Plants, A: An Illustrated Scientific And Medicinal Approach*: World scientific.

Köhler, P. (2001). The biochemical basis of anthelmintic action and resistance. *International journal for parasitology, 31*(4), 336-345.

Kong, J.-M., Goh, N.-K., Chia, L.-S., & Chia, T.-F. (2003). Recent advances in traditional plant drugs and orchids. *Acta Pharmacologica Sinica, 24*(1), 7-21.

Konsue, A., & Katisart, T. (2021). Acute Toxicity of Oroxylum indicum Fruit Extracts in Rats. *Pharmacognosy Magazine, 17*(75), 545.

Koparde, A. A., Doijad, R. C., & Magdum, C. S. (2019). Natural Products in Drug Discovery *Pharmacognosy-Medicinal Plants*: IntechOpen.

Korukola, N., Medisetti, V. K., Sravanam, S., & Sanga, K. R. (2014). Preformulation and formulation studies of the poly herbal syrup of hydroalcoholic extracts of *Zingiber officinale* and *Piper nigrum*. *Int J Pharm Sci Rev Res, 24*, 251-256.

Kosalge, S., & Fursule, R. (2009a). Investigation of ethnomedicinal claims of some plants used by tribals of Satpuda Hills in India. *Journal of ethnopharmacology, 121*(3), 456-461.

Kosalge, S. B., & Fursule, R. A. (2009b). Investigation of anthelmintic potential of few plants from Satpuda hills. *Medicinal Plants-International Journal of Phytomedicines and Related Industries, 1*(2), 113-115.

Kosalge, S. B., & Fursule, R. A. (2009c). Investigation of anthelmintic potential of some plants claimed by tribals of satpuda hills. *International Journal of PharmTech Research, 1*(1), 68-72.

Kossakowski, J., Hejchman, E., & Wolska, I. (2002). Synthesis and Structural Characterization of Aminoalkanol Derivatives of 2, 3-Dihydro-2, 2-dimethyl-7-benzofuranol with an Expected β-Adrenolytic and/or Anxiolytic Activity. *Zeitschrift für Naturforschung B, 57*(3), 285-294.

Kossakowski, J., Ostrowska, K., Struga, M., & Stefańska, J. (2009). Synthesis of new derivatives of 2, 2-dimethyl-2, 3-dihydro-7-benzo [b] furanol with potential antimicrobial activity. *Medicinal chemistry research, 18*(7), 555-565.

Kotze, A., & Prichard, R. (2016). Anthelmintic resistance in Haemonchus contortus: history, mechanisms and diagnosis *Advances in parasitology* (Vol. 93, pp. 397-428): Elsevier.

Kovács, A., Vasas, A., & Hohmann, J. (2008). Natural phenanthrenes and their biological activity. *Phytochemistry, 69*(5), 1084-1110.

Krishnamoorthy, K., & Subramaniam, P. (2014). Phytochemical profiling of leaf, stem, and tuber parts of Solena amplexicaulis (Lam.) Gandhi using GC-MS. *International Scholarly Research Notices, 2014*.

Krishnaraju, A. V., Rao, T. V., Sundararaju, D., Vanisree, M., Tsay, H.-S., & Subbaraju, G. V. (2006). Biological screening of medicinal plants collected from Eastern Ghats of India using Artemia salina (brine shrimp test). *International Journal of Applied Science and Engineering, 4*(2), 115-125.

Kuete, V., BetrandTeponno, R., Mbaveng, A. T., Tapondjou, L. A., Meyer, J. J. M., Barboni, L., & Lall, N. (2012). Antibacterial activities of the extracts, fractions and compounds from Dioscorea bulbifera. *BMC Complementary and Alternative Medicine, 12*(1), 1-8.

Kulkarni, K., Jagtap, G., & Magdum, S. (2019). A comprehensive review on herbal drug standardization. *Am. J. PharmTech Res, 9*, 97-122.

Kuma, F., Birhanu, T., Hirpa, E., & Nekemte, E. (2015). Advanced review on anthelmintic medicinal plants. *children, 13*, 14.

Kumar, A., & Tadesse, G. (2011). Bovine cysticercosis in Ethiopia: a review. *Ethiopian Veterinary Journal, 15*(1).

Kumar, M. S., Udupa, A., Sammodavardhana, K., Rathnakar, U., Shvetha, U., & Kodancha, G. P. (2010). Acute toxicity and diuretic studies of the roots of Asparagus racemosus Willd in rats. *West Indies medical journal, 59*(1), 3-5.

Kumar, P., Sunita, K., & Singh, D. (2016). In vitro activity of different phytochemicals in binary combinations against Fasciola gigantica. *Current Life Sciences, 2*(3), 58-63.

Kumar, P., Sunita, K., Singh, R., & Singh, D. (2020). Fasciola larvae: Anthelmintic activity of medicinal plant Potentilla fulgens against sporocyst, redia and cercaria. *Asian Journal of Advances in Research*, 24-30.

Kumar, P. P., Kumaravel, S., & Lalitha, C. (2010). Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. *African Journal of Biochemistry Research, 4*(7), 191-195.

Kumar, R., & Bhagat, N. (2012). Ethnomedicinal plants of district Kathua (J&K). *International journal of medicinal and aromatic plants, 2*(4), 603-611.

Kumar, V., Ahmed, D., Gupta, P. S., Anwar, F., & Mujeeb, M. (2013). Anti-diabetic, anti-oxidant and anti-hyperlipidemic activities of Melastoma malabathricum Linn. leaves in streptozotocin induced diabetic rats. *BMC Complementary and Alternative Medicine, 13*(1), 1-19.

Kumar, V., Bhatnagar, A., & Srivastava, J. (2011). Antibacterial activity of crude extracts of Spirulina platensis and its structural elucidation of bioactive compound. *Journal of Medicinal Plants Research, 5*(32), 7043-7048.

Kushwaha, D., Kumar, D., Tripati, H., & Tandan, S. (2004). Effect of some indigenous medicinal plant extracts on Fasciola gigantica in vitro. *Indian Journal of Animal Sciences (India)*.

Labar, R., Sarkar, I., Sen, A., & Bhattacharya, M. (2019). Effect of solvent with varying polarities on phytochemical extraction from mature tea leaves and its evaluation using biochemical, antimicrobial and in-silico approaches. *Int Res J Pharm, 10*(8), 59-67.

Lakshmi, K. N., Veerakumari, L., & Raman, M. (2011). Efficacy of Allium sativum Linn. against strongyles in naturally infected sheep. *Journal of Veterinary Parasitology, 25*(2), 124-128.

Lakshmi, M., & Nair, B. R. (2017). GC-MS ANALYSIS OF THE CHLOROFORM EXTRACT OF BARK OF TERMINALIA TRAVANCORENSIS WIGHT & ARN. (COMBRETACEAE) M. *IJPSR, 8*(2), 794-798.

Lalnunfela, C., Lalthanpuii, P., Lalhriatpuii, T., & Lalchhandama, K. (2020). An endangered medicinal plant, Ilex khasiana exhibits potent antiparasitic activity against intestinal tapeworm. *Pharmacognosy Journal, 12*(4).

Lata, K. (2015). Gas chromatography-mass spectrometry analysis of bioactive constituents from the marine Streptomyces. *Asian Journal of Pharmaceutical and Clinical Research*, 244-246.

Lateef, M., Iqbal, Z., Rauf, U., & Jabbar, A. (2006). Anthelmintic activity of Carum copticum seeds against gastro-intestinal nematodes of sheep. *Journal of Animal and plant Sciences, 16*(1-2), 34-37.

Latif, M. A., Ibrahim, F. W., Arshad, S. A., Hui, C. K., Jufri, N. F., & Hamid, A. (2019). Cytotoxicity, proliferation and migration rate assessments of human dermal fibroblast adult cells using Zingiber zerumbet extract. *Sains Malaysiana, 48*(1), 121-127.

Lawal, B., Shittu, O. K., Oibiokpa, F. I., Mohammed, H., Umar, S. I., & Haruna, G. M. (2016). Antimicrobial evaluation, acute and sub-acute toxicity studies of Allium sativum. *Journal of Acute Disease, 5*(4), 296-301.

Lawal, O. A., & Oyedeji, A. O. (2009). Chemical composition of the essential oils of Cyperus rotundus L. from South Africa. *Molecules, 14*(8), 2909-2917.

Lazim, A. M., Sharlina, M. E., Azfaralariff, A., Yaacob, W., Lim, S. J., Fazry, S., . . . Abdullah, N. H. (2021). Structure, physicochemical and toxicity properties of underused malaysian native Tuber's starch (Dioscorea Pentaphylla). *Journal of King Saud University-Science, 33*(6), 101501.

Leathwick, D., & Hosking, B. (2009). Managing anthelmintic resistance: modelling strategic use of a new anthelmintic class to slow the development of resistance to existing classes. *New Zealand Veterinary Journal, 57*(4), 203-207.

Lee, Y. S., Kang, M. H., Cho, S. Y., & Jeong, C. S. (2007). Effects of constituents of Amomum xanthioides on gastritis in rats and on growth of gastric cancer cells. *Archives of pharmacal research, 30*(4), 436-443.

Lekshmi, R., Sreekutty, M., & Mini, S. (2015). The regulatory effects of Cissus quadrangularis on some enzymes involved in carbohydrate metabolism in streptozotocin-induced diabetic rats. *Pharmaceutical Biology, 53*(8), 1194-1200.

Lem, M. F., Vincent, K. P., Josue, W. P., Jeannette, Y., Gertrude, M. T., & Joseph, T. (2014). In vitro ovicidal and larvicidal activities of stem bark of Terminalia glaucescens (Combretaceae) against Haemonchus contortus. *American Journal of Plant Sciences, 5*(19), 2859.

León, J. C. P., Delgado, N. U., & Florez, A. A. (2019). Prevalence of gastrointestinal parasites in cattle and sheep in three municipalities in the Colombian Northeastern Mountain. *Veterinary world, 12*(1), 48.

Li, J., Zhao, Y.-F., Yuan, X.-Y., Xu, J.-X., & Gong, P. (2006). Synthesis and anticancer activities of novel 1, 4-disubstituted phthalazines. *Molecules, 11*(7), 574-582.

Li, P., Xu, G., Li, S.-P., Wang, Y.-T., Fan, T.-P., Zhao, Q.-S., & Zhang, Q.-W. (2008). Optimizing ultraperformance liquid chromatographic analysis of 10 diterpenoid compounds in Salvia miltiorrhiza using central composite design. *Journal of agricultural and Food Chemistry, 56*(4), 1164-1171.

Li, W., Feng, J.-T., Xiao, Y.-S., Wang, Y.-Q., Xue, X.-Y., & Liang, X.-M. (2009). Three novel terpenoids from the rhizomes of Curcuma longa. *Journal of Asian natural products research, 11*(6), 569-575.

Liju, V. B., Jeena, K., & Kuttan, R. (2013). Acute and subchronic toxicity as well as mutagenic evaluation of essential oil from turmeric (Curcuma longa L). *Food and Chemical Toxicology, 53*, 52-61.

Lim, Y. Y., & Murtijaya, J. (2007). Antioxidant properties of Phyllanthus amarus extracts as affected by different drying methods. *LWT-Food Science and Technology, 40*(9), 1664-1669.

Lin, R.-J., Chen, C.-Y., Chung, L.-Y., & Yen, C.-M. (2010). Larvicidal activities of ginger (*Zingiber officinale*) against *Angiostrongylus cantonensis*. *Acta tropica, 115*(1-2), 69-76.

Liu, H., Chou, G.-X., Wu, T., Guo, Y.-L., Wang, S.-C., Wang, C.-H., & Wang, Z.-T. (2009). Steroidal sapogenins and glycosides from the rhizomes of Dioscorea bulbifera. *Journal of Natural Products, 72*(11), 1964-1968.

Liu, H., Tsim, K. W., Chou, G. X., Wang, J. M., Ji, L. L., & Wang, Z. T. (2011). Phenolic compounds from the rhizomes of Dioscorea bulbifera. *Chemistry & biodiversity, 8*(11), 2110-2116.

Liu, X. C., Liang, Y., Shi, W. P., Liu, Q. Z., Zhou, L., & Liu, Z. L. (2014). Repellent and insecticidal effects of the essential oil of Kaempferia galanga rhizomes to Liposcelis bostrychophila (Psocoptera: Liposcelidae). *Journal of Economic Entomology, 107*(4), 1706-1712.

Locatelli, C., Pedrosa, R. C., De Bem, A. F., Creczynski-Pasa, T. B., Cordova, C. A., & Wilhelm-Filho, D. (2004). A comparative study of albendazole and mebendazole-induced, time-dependent oxidative stress. *Redox Report, 9*(2), 89-95.

Lu, H., Drelich, A., Omri, M., Pezron, I., Wadouachi, A., & Pourceau, G. (2016). Catalytic synthesis of a new series of alkyl uronates and evaluation of their physicochemical properties. *Molecules, 21*(10), 1301.

Lu, X.-T., Gu, Q.-Y., Limpanont, Y., Song, L.-G., Wu, Z.-D., Okanurak, K., & Lv, Z.-Y. (2018). Snail-borne parasitic diseases: an update on global epidemiological distribution, transmission interruption and control methods. *Infectious diseases of poverty, 7*(1), 28.

Luce, T. W. (2019). Anthelmintic potential of plant extracts on helminths in small ruminants.

Lucido, F. P. (2014). *Anthelmintic efficacy of garlic [Allium sativum L.] on commercial broilers raised in semi-confined system.* (Bachelor of Science in Agriculture), University Knowledge. (1575)

Lullman, H. K., Morh, K., & Bieger, D. (2017). *Colour Atlas of pharmacology.* (5th ed.). New York;: Theme Medical Publisher Inc.

Lynagh, T., Cromer, B. A., Dufour, V., & Laube, B. (2014). Comparative pharmacology of flatworm and roundworm glutamate-gated chloride channels: Implications for potential anthelmintics. *International Journal for Parasitology: Drugs and Drug Resistance, 4*(3), 244-255.

Maburutse, B., Mutibvu, T., Mbiriri, D., & Kashangura, M. (2012). Communal livestock production in Simbe, Gokwe south district of Zimbabwe. *Online Journal of Animal and Feed Research, 2*(4), 351-360.

Madhulika, S., & Varsha, S. (2015). In vitro evaluation of secondary metabolites and hydroxyl radical scavenging efficacy of different extracts of Cyperus rotundus L. and Rubia cordifolia L.: protection against photodamages. *Int. J. Res. Ayurveda Pharm, 6*(1), 144-149.

Mahomoodally, M. F. (2013). Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. *Evidence-Based Complementary and Alternative Medicine, 2013*.

Mahomoodally, M. F., Jugreet, S., Sinan, K. I., Zengin, G., Ak, G., Ceylan, R., . . . Angeles Flores, G. (2021). Pharmacological Potential and Chemical Characterization of Bridelia ferruginea Benth.—A Native Tropical African Medicinal Plant. *Antibiotics, 10*(2), 223.

Maity, D., Pradhan, N., & Chauhan, A. (2004). Folk uses of some medicinal plants from North Sikkim.

Makkar, H. P., Blümmel, M., Borowy, N. K., & Becker, K. (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *Journal of the Science of Food and Agriculture, 61*(2), 161-165.

Malek, S. N. A., Shin, S. K., Wahab, N. A., & Yaacob, H. (2009). Cytotoxic components of Pereskia bleo (Kunth) DC.(Cactaceae) leaves. *Molecules, 14*(5), 1713-1724.

Manandhar, N. P. (1995). An inventory of some herbal drugs of Myagdi District, Nepal. *Economic Botany, 49*(4), 371-379.

Mandal, B., & Dixit-Sharma, S. (2007). Cryopreservation of in vitro shoot tips of Dioscorea deltoidea Wall., an endangered medicinal plant: effect of cryogenic procedure and storage duration. *CryoLetters, 28*(6), 461-470.

Manisha, P., Chandrashekhar, P., & Raghunath, M. (2018). Phytochemical Investigation and Validation of Antioxidant Potential of β-Sitosterol from Tubers of Eulophia herbacea and Eulophia ochreata. *Int. J. Pharm. Phytochem. Res., 10*, 309-316.

Manoj, G., Manohar, S. H., & Murthy, H. N. (2012). Chemical constituents, antioxidant and antimocrobial activity of essential oil of Pogostemon paniculatus (Willd.). *Natural product research, 26*(22), 2152-2154.

Maphosa, V., Masika, P., & Moyo, B. (2009). Investigation of the anti-inflammatory and antinociceptive activities of Elephantorrhiza elephantina (Burch.) Skeels root extract in male rats. *African Journal of Biotechnology, 8*(24).

Maphosa, V., Masika, P., & Moyo, B. (2010). Toxicity evaluation of the aqueous extract of the rhizome of Elephantorrhiza elephantina (Burch.) Skeels.(Fabaceae), in rats. *Food and Chemical Toxicology, 48*(1), 196-201.

Maphosa, V., & Masika, P. J. (2010). Ethnoveterinary uses of medicinal plants: A survey of plants used in the ethnoveterinary control of gastro-intestinal parasites of goats in the Eastern Cape Province, South Africa. *Pharmaceutical biology, 48*(6), 697-702.

Maphosa, V., & Masika, P. J. (2012a). Anthelmintic screening of fractions of Elephantorrhiza elephantina root extract against Haemonchus contortus. *Tropical animal health and production, 44*(1), 159-163.

Maphosa, V., & Masika, P. J. (2012b). In vivo validation of Aloe ferox (Mill). Elephantorrhiza elephantina Bruch. Skeels. and Leonotis leonurus (L) R. BR as potential anthelminthics and antiprotozoals against mixed infections of gastrointestinal nematodes in goats. *Parasitology research, 110*(1), 103-108.

Maphosa, V., Masika, P. J., Bizimenyera, E. S., & Eloff, J. (2010). In-vitro anthelminthic activity of crude aqueous extracts of Aloe ferox, Leonotis leonurus and Elephantorrhiza elephantina against Haemonchus contortus. *Tropical animal health and production, 42*(2), 301-307.

Maranho, L. A., Botelho, R. G., Mitie Inafuku, M., Nogueira, L., Alves de Olinda, R., Inacio de Sousa, B., & Tornisielo, V. L. (2014). Testing the neem biopesticide (Azadirachta indica A. Juss) for acute toxicity with Danio rerio and for chronic toxicity with Daphnia magna. *Journal of Agricultural Science and Technology, 16*(1), 105-111.

Marie-Magdeleine, C., Hoste, H., Mahieu, M., Varo, H., & Archimède, H. (2009). In vitro effects of Cucurbita moschata seed extracts on Haemonchus contortus. *Veterinary Parasitology, 161*(1-2), 99-105.

Maroyi, A., & Van der Maesen, L. (2011a). Gloriosa superba L.(family Colchicaceae): Remedy or poison. *Journal of Medicinal Plants Research, 5*(26), 6112-6121.

Maroyi, A., & Van der Maesen, L. (2011b). Gloriosa superba L.(family Colchicaceae): Remedy or poison? *Journal of Medicinal Plants Research, 5*(26), 6112-6121.

Marpa, S., Samant, S., Tewari, A., & Paul, S. (2020). Diversity and indigenous uses of plants in Naina Devi Sacred Shrine Rewalsar, Himachal Pradesh, North Western Himalaya, India. *IJCS, 8*(2), 1265-1276.

Martin, R. (1997). Modes of action of anthelmintic drugs. *The Veterinary Journal, 154*(1), 11-34.

Mas-Coma, S., Valero, M., & Bargues, M. (2008). Effects of climate change on animal and zoonotic helminthiases. *Rev Sci Tech, 27*(2), 443-457.

Mas-Coma, S., Valero, M. A., & Bargues, M. D. (2009). Fasciola, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. *Advances in parasitology, 69*, 41-146.

Masikati, P. (2011). *Improving the water productivity of integrated crop-livestock systems in the semi-arid tropics of Zimbabwe: an ex-ante analysis using simulation modeling*: ZEF.

Matekaire, T., & Bwakura, T. (2004). Ethnoveterinary medicine: A potential alternative to orthodox animal health delivery in Zimbabwe. *International Journal of Applied Research in Veterinary Medicine, 2*(4), 269-273.

Mathias-Mundy, E., & McCorkle, C. M. (1989). *Ethnoveterinary medicine: an annotated bibliography*: Iowa State University Research Foundation Ames.

Mathur, A., & Joshi, H. (2013). Ethnobotanical studies of the Tarai region of Kumaun, Uttarakhand, India. *Ethnobotany research and Applications, 11*, 174-203.

Matthes, H., Luu, B., & Ourisson, G. (1980). Cytotoxic components of Zingiber zerumbet, Curcuma zedoaria and C. domestica. *Phytochemistry, 19*(12), 2643-2650.

Matyanga, C. M., Morse, G. D., Gundidza, M., & Nhachi, C. F. (2020). African potato (Hypoxis hemerocallidea): a systematic review of its chemistry, pharmacology and ethno medicinal properties. *BMC complementary medicine and therapies, 20*(1), 1-12.

Mazhangara, I. R., Masika, P. J., Mupangwa, J. F., Chivandi, E., Jaja, I. F., & Muchenje, V. (2020). In vitro efficacy of Elephantorrhiza elephantina root extracts against adult Paramphistomum cervi in goats. *Parasite Epidemiology and Control, 10*, e00157.

Mazhangara, I. R., Sanhokwe, M., Chivandi, E., Mupangwa, J. F., Lorenzo, J. M., & Muchenje, V. (2020). Plants for Controlling Parasites in Goats *Ethnoveterinary Medicine* (pp. 73-98): Springer.

McCorkle, C. M. (1995). Back to the future: Lessons from ethnoveterinary RD&E for studying and applying local knowledge. *Agriculture and Human values, 12*(2), 52-80.

McCorkle, C. M., & Mathias-Mundy, E. (1992). Ethnoveterinary medicine in Africa. *Africa, 62*(1), 59-93.

McCorkle, C. M., Mathias-Mundy, E., & Schillhorn-van-Veen, T. (1996). *Ethnoveterinary research & development*: Intermediate Technology Publications.

McGaw, L., Gehring, R., Katsoulis, L., & Eloff, J. (2005). Is the use of Gunnera perpensa extracts in endometritis related to antibacterial activity? *Onderstepoort Journal of Veterinary Research, 72*(2), 129-134.

McGaw, L. J., & Eloff, J. N. (2010). Methods for evaluating efficacy of ethnoveterinary medicinal plants. *Ethnoveterinary botanical medicine: herbal medicines for animal health*, 1-24.

McKenna, P. (1990). The use of benzimidazole-levamisole mixtures for the control and prevention of anthelmintic resistance in sheep nematodes: an assessment of their likely effects. *New Zealand Veterinary Journal, 38*(2), 45-49.
Mdletshe, N. W. (2018). *Comparison of pharmalogical activity of rhoicissus tomentosa and rhoicissus tridentata for the treatment of elephantiasis in South Africa.* University of the Free State.
Mehta, S., Sharma, A. K., & Singh, R. K. (2021). Advances in ethnobotany, synthetic phytochemistry and pharmacology of endangered herb Picrorhiza kurroa (Kutki): A comprehensive review (2010-2020). *Mini Reviews in Medicinal Chemistry, 21*(19), 2976-2995.
Melzig, M. F., Bader, G., & Loose, R. (2001). Investigations of the mechanism of membrane activity of selected triterpenoid saponins. *Planta Medica, 67*(01), 43-48.
Mendis, S. (1989). Colchicine cardiotoxicity following ingestion of Gloriosa superba tubers. *Postgraduate medical journal, 65*(768), 752-755.
Mhlongo, L. C. (2018). *In vitro assessment of selected ethno-medicinal plants as potential alternatives for the control of gastrointestinal nematodes in sheep and goats.*
Mikail, H. (2010). Phytochemical screening, elemental analysis and acute toxicity of aqueous extract of Allium sativum L. bulbs in experimental rabbits. *Journal of Medicinal Plants Research, 4*(4), 322-326.
Mirelman, D., Monheit, D., & Varon, S. (1987). Inhibition of growth of Entamoeba histolytica by allicin, the active principle of garlic extract (Allium sativum). *Journal of Infectious Diseases, 156*(1), 243-244.
Mishra, B. B., Kumar, D., Singh, A. S., Tripathi, R. P., & Tiwari, V. K. (2015). Ionic Liquids-Prompted Synthesis of Biologically Relevant Five-and Six-Membered Heterocyclic Skeletons: An Update *Green Synthetic Approaches for Biologically Relevant Heterocycles* (pp. 437-493): Elsevier.
Mishra, P. M., & Sree, A. (2007). Antibacterial activity and GCMS analysis of the extract of leaves of Finlaysonia obovata (a mangrove plant). *Asian Journal of Plant Sciences*.
Mishra, S. H., Gaud, R. S., Sharma, R. A., & Chaturvedi, S. C. (1979). Anthelmintic activity of some essential oils. *Indian*
Perfumer, *23*, 208-209.
Mishra, V., Parveen, N., Singhal, K., & Khan, N. U. (2005). Antifilarial activity of Azadirachta indica on cattle filarial parasite Setaria cervi. *Fitoterapia, 76*(1), 54-61.
Miyazawa, M., Nakahashi, H., Usami, A., Matsuda, N., & (2016). Chemical composition, aroma evaluation, and inhibitory activity towards acetylcholinesterase of essential oils from Gynura bicolor DC. *J. Nat. Med. , 70*, 282–289. doi: https://doi.org/10.1007/s11418-015-0961-1
Moazeni, M., & Khademolhoseini, A. A. (2016). Ovicidal effect of the methanolic extract of ginger (*Zingiber officinale*) on *Fasciola hepatica* eggs: an *in vitro* study. *Journal of Parasitic Diseases, 40*(3), 662-666.
Mohammad, Y., Fazili, K. M., Bhat, K. A., & Ara, T. (2017). Synthesis and biological evaluation of novel 3-O-tethered triazoles of diosgenin as potent antiproliferative agents. *Steroids, 118*, 1-8.
Mohammadi, K. H., Heidarpour, M., & Borji, H. (2018). In vivo therapeutic efficacy of the Allium sativum ME in experimentally Echinococcus granulosus infected mice. *Comparative immunology, microbiology and infectious diseases, 60*, 23-27.
Mohammed, A., Muhammad, I., Wudil, A. M., Alhassan, A., Abubakar, S., & Ngwen, A. (2019). Phytochemical screening and proximate analysis of root of Curcuma longa Linn. *European Journal of Pharmaceutical and Medical Research, 6*(9), 138-141.
Moharm, I. M., Oshiba, S. F., & Ammar, A. I. (2020). The effect of genistein against Schistosoma Mansoni In Experimentally Infected Mice. *Journal of the Egyptian Society of Parasitology, 50*(3), 590-598.
Mohd Zainol, M., Abdul-Hamid, A., Abu Bakar, F., & Pak Dek, S. (2009). Effect of different drying methods on the degradation of selected flavonoids in Centella asiatica. *International Food Research Journal, 16*(4), 531-537.
Molan, A.-L., & Faraj, A. M. (2010). The effects of condensed tannins extracted from different plant species on egg hatching and larval development of Teladorsagia circumcincta (Nematoda: Trichostrongylidae). *Folia Parasitologica (Prague), 57*(1), 62.
Molan, A., Waghorn, G., & McNabb, W. (2002). Effect of condensed tannins on egg hatching and larval development of Trichostrongylus colubriformis in vitro. *Veterinary Record, 150*(3), 65-69.
Molento, M. B., Fortes, F. S., Pondelek, D. A. S., de Almeida Borges, F., de Souza Chagas, A. C., Torres-Acosta, J. F. d. J., & Geldhof, P. (2011). Challenges of nematode control in ruminants: focus on Latin America. *Veterinary parasitology, 180*(1-2), 126-132.
Mølgaard, P., Nielsen, S. B., Rasmussen, D. E., Drummond, R. B., Makaza, N., & Andreassen, J. (2001). Anthelmintic screening of Zimbabwean plants traditionally used against schistosomiasis. *Journal of Ethnopharmacology, 74*(3), 257-264.
Molina-Hernández, V., Mulcahy, G., Pérez, J., Martínez-Moreno, Á., Donnelly, S., O'Neill, S. M., . . . Cwiklinski, K. (2015). Fasciola hepatica vaccine: we may not be there yet but we're on the right road. *Veterinary parasitology, 208*(1-2), 101-111.

Mongalo, N., Soyingbe, O., & Makhafola, T. (2019). Antimicrobial, cytotoxicity, anticancer and antioxidant activities of Jatropha zeyheri Sond. roots (Euphorbiaceae). *Asian Pacific Journal of Tropical Biomedicine, 9*(7), 307.

Morgan, E., & Van Dijk, J. (2012). Climate and the epidemiology of gastrointestinal nematode infections of sheep in Europe. *Veterinary parasitology, 189*(1), 8-14.

Morgan, E. R., Charlier, J., Hendrickx, G., Biggeri, A., Catalan, D., Samson-Himmelstjerna, V., . . . Kenyon, F. (2013). Global change and helminth infections in grazing ruminants in Europe: impacts, trends and sustainable solutions. *Agriculture, 3*(3), 484-502.

Mou, Y., Meng, J., Fu, X., Wang, X., Tian, J., Wang, M., . . . Zhou, L. (2013). Antimicrobial and antioxidant activities and effect of 1-hexadecene addition on palmarumycin C2 and C3 yields in liquid culture of endophytic fungus Berkleasmium sp. Dzf12. *Molecules, 18*(12), 15587-15599.

Mpofu, S. J., Msagati, T. A., & Krause, R. W. (2014). Cytotoxicity, phytochemical analysis and antioxidant activity of crude extracts from rhizomes of Elephantorrhiza elephantina and Pentanisia prunelloides. *African Journal of Traditional, Complementary and Alternative Medicines, 11*(1), 34-52.

Mruthyunjayaswamy, B., & Shanthaveerappa, B. (2000). Synthesis and pharmacological evaluation of 3, 5-disubstituted indole-2-[Nβ-(substituted benzopyran-2'-one-3'-carboxyl)] carboxy hydrazides and 2H-3-(various substituted indol-3'-yl) methyl-1, 3-benzothiazoles.

Muangnoi, P., Lu, M., Lee, J., Thepouyporn, A., Mirzayans, R., Le, X., . . . Changbumrung, S. (2007). Cytotoxicity, apoptosis and DNA damage induced by Alpinia galanga rhizome extract. *Planta Medica, 73*(08), 748-754.

Muchiut, S. M., Fernández, A. S., Steffan, P. E., Riva, E., & Fiel, C. A. (2018). Anthelmintic resistance: Management of parasite refugia for Haemonchus contortus through the replacement of resistant with susceptible populations. *Veterinary parasitology, 254*, 43-48.

Muhammad Farrukh Nisar, Farrukh Jaleel, Muhammad Waseem, Sajil Ismail, Yasmin Toor, Syed, . . . Zhong, J. L. (2014). Ethno-medicinal uses of plants from district Bahawalpur, Pakistan. *Current Research Journal of Biological Sciences, 6*, 183-190.

Murthy, P. K., & Joseph, S. K. (2011). Plant products in the treatment and control of filariasis and other helminth infections and assay systems for antifilarial/anthelmintic activity. *Planta Medica, 77*(06), 647-661.

Murungweni, C., Andersson, J., Van Wijk, M., Gwitira, I., & Giller, K. (2012). Zhombwe (Neorautanenia brachypus (Harms) CA Sm.)–A recent discovery for mitigating effects of drought on livestock in semi-arid areas of Southern Africa. *Ethnobotany Research & Applications, 10*, 199-212.

Muthee, J., Gakuya, D., Mbaria, J., Kareru, P., Mulei, C. M., & Njonge, F. (2011). Ethnobotanical study of anthelmintic and other medicinal plants traditionally used in Loitoktok district of Kenya. *Journal of Ethnopharmacology, 135*(1), 15-21.

Muthee, J., Gakuya, D., Mbaria, J., & Mulei, C. (2016). Phytochemical screening and cytotoxicity of selected plants used as anthelmintics in Loitoktok Sub-County, Kenya. *The Journal of Phytopharmacology, 5*(1), 15-19.

Muthukrishnan, S., & Annapoorani, S. (2012). Phytochemical constituents of Gloriosa superba seed, tuber and leaves. *Research Journal of Pharmaceutical, Biological and Chemical Sciences, 3*(3), 111-117.

Muthuraja, R., Nandagopalan, V., Thomas, B., & Marimuthu, C. (2014). An ethno-botanical survey of medicinal plants used by Kolli Malayalis of Nammakkal district, Eastern Ghats, Tamil Nadu, India. *European Journal of Environmental Ecology, 1*(1), 33-43.

Mutie, F. M., Gao, L.-L., Kathambi, V., Rono, P. C., Musili, P. M., Ngugi, G., . . . Wang, Q.-F. (2020). An ethnobotanical survey of a dryland botanical garden and its environs in Kenya: the Mutomo hill plant sanctuary. *Evidence-Based Complementary and Alternative Medicine, 2020*.

Mwale, M., Bhebhe, E., Chimonyo, M., & Halimani, T. E. (2005). Use of herbal plants in poultry health management in the Mushagashe small-scale commercial farming area in Zimbabwe. *International Journal of Applied Research in Veterinary Medicine, 3*(2), 163-170.

Mwale, M., & Masika, P. J. (2011). Toxicity evaluation of the aqueous leaf extract of Gunnera perpensa L.(Gunneraceae). *African Journal of Biotechnology, 10*(13), 2503-2513.

Mwale, M., & Masika, P. J. (2015). In vivo anthelmintic efficacy of Aloe ferox, Agave sisalana, and Gunnera perpensa in village chickens naturally infected with Heterakis gallinarum. *Tropical Animal Health and Production, 47*(1), 131-138.

Mwangi, G. G., Wagacha, J. M., Nguta, J. M., & Mbaria, J. M. (2015). Brine shrimp cytotoxicity and antimalarial activity of plants traditionally used in treatment of malaria in Msambweni district. *Pharmaceutical biology, 53*(4), 588-593.

Nadkarni, A. K. (1954). *Indian Materia Medica* (3 ed. Vol. 1). Bombay, India: Popular Book Depot,.

Nag, A., Banerjee, R., Goswami, P., Bandyopadhyay, M., & Mukherjee, A. (2021). Antioxidant and antigenotoxic properties of Alpinia galanga, Curcuma amada, and Curcuma caesia. *Asian Pacific Journal of Tropical Biomedicine, 11*(8), 363.

Nagulendran, K., Velavan, S., Mahesh, R., & Begum, V. H. (2007). In vitro antioxidant activity and total polyphenolic content of Cyperus rotundus rhizomes. *E-journal of Chemistry, 4*(3), 440-449.

Naguleswaran, A., Spicher, M., Vonlaufen, N., Ortega-Mora, L. M., Torgerson, P., Gottstein, B., & Hemphill, A. (2006). In vitro metacestodicidal activities of genistein and other isoflavones against Echinococcus multilocularis and Echinococcus granulosus. *Antimicrobial agents and chemotherapy, 50*(11), 3770-3778.

Naidu, N., Ramu, V. V., & Kumar, N. S. (2016). Anti-inflammatory and anti-helminthic activity of ethanolic extract of Azadirachta indica leaves. *IJGP, 10*, S1-S4.

Nalule, A., Mbaria, J., Kimenju, J., & Olila, D. (2012a). Ascaricidal activity of Rhoicissus tridentata root-tuber ethanolic and water extracts. *Livestock Research for Rural Development, 24*(8).

Nalule, A., Mbaria, J., Kimenju, J., & Olila, D. (2012b). Ascaricidal activity of Rhoicissus tridentata root-tuber ethanolic and water extracts. *Livest. Res. Rural. Dev, 24*.

Nalule, A., Mbaria, J., Olila, D., & Kimenju, J. (2011). Ethnopharmacological practices in management of livestock helminthes by pastoral communities in the drylands of Uganda. *Livestock Research for Rural Development, 23*(2), 1-27.

Nandhini, A., & Sumathi, C. (2014). An overview of herbals used in helminthosis. *World J Pharm Res, 3*(10), 350-362.

Nasai, N. (2012). *The Effects of Curcuma Longa (Tumeric) and Levamisole on In-vitro Survival Rate of Strongyles in Sheep.* Universiti Putra Malaysia.

Nasai, N. B., Abba, Y., Abdullah, F. F. J., Marimuthu, M., Tijjani, A., Sadiq, M. A., . . . Omar, M. A. B. (2016). In vitro larvicidal effects of ethanolic extract of Curcuma longa Linn. on Haemonchus larval stage. *Veterinary world, 9*(4), 417.

Nath, S., Pal, S., Sanyal, P. K., Roy, S., Mandal, S., & Nayak, Y. (2019). Anthelmintic activity of curcuma longa ethanolic extract against benzimidazole resistant gastrointestinal nematodes in goats. *Int J Livestock Res, 9*, 117-122.

Navaneetha, L. K., & Veerakumari, L. (2009). Effect of Allium sativum on the phosphoenolpyruvate carboxykinase and pyruvate kinase activity of Haemonchus contortus in vitro. *Pharmacognosy Magazine, 5*(20), 430.

Nawaz, H., Aslam, M., & Muntaha, S. T. (2019). Effect of solvent polarity and extraction method on phytochemical composition and antioxidant potential of corn silk. *Free Radicals and Antioxidants, 9*(1), 5-11.

Nawaz, M., Sajid, S. M., Zubair, M., Hussain, J., Abbasi, Z., Mohi-Ud-Din, A., & Waqas, M. (2014). In vitro and in vivo anthelmintic activity of leaves of Azadirachta indica, Dalbergia sisso and Morus alba against Haemonchus contortus. *Glob. Vet, 13*, 996-1001.

Nazir, R., Gupta, S., Dey, A., Kumar, V., Yousuf, M., Hussain, S., . . . Pandey, D. K. (2021). In vitro propagation and assessment of genetic fidelity in Dioscorea deltoidea, a potent diosgenin yielding endangered plant. *South African Journal of Botany, 140*, 349-355.

Nazneen, F., Muddassir, M., Meshram, K., Umekar, M., & Lohiya, R. (2017). Phytochemical Screening and Comparative Anthelmintic Activity of Alchoholic Extracts of Some Herbal Plants. *Journal of Pharmaceutical Sciences and Research, 9*(7), 1240.

Ndhlala, A., Finnie, J., & Van Staden, J. (2011). Plant composition, pharmacological properties and mutagenic evaluation of a commercial Zulu herbal mixture: Imbiza ephuzwato. *Journal of Ethnopharmacology, 133*(2), 663-674.

Negasi, W., Bogale, B., & Chanie, M. (2012). Helminth parasites in small ruminants: prevalence, species composition and associated risk factors in and Around Mekelle Town, Northern Ethiopia. *Eur. J. Biol. Sci, 4*(3), 91-95.

Ngeny, L. C., Magiri, E., Mutai, C., Mwikwabe, N., & Bii, C. (2013). Antimicrobial properties and toxicity of Hagenia abyssinica (Bruce) JF Gmel, Fuerstia africana TCE Fries, Asparagus racemosus (Willd.) and Ekebergia capensis Sparrm. *African Journal of pharmacology and therapeutics, 2*(3).

Nikhila, G., Sangeetha, G., Preetha, T., & Swapna, T. (2016). GC-MS analysis of phytochemical compounds present in the rhizome of Gloriosa superba L. *Journal of Pharmacognosy and Phytochemistry, 5*(5), 17.

Nirala, R. K. (2019). Medicinal plants and its activity against helminth: A review. *Journal of Pharmacognosy and Phytochemistry, 8*(5), 2348-2355.

Nirmal, S. A., Gupta, S. R., Ghogare, U. R., & Christian, R. E. (2009). Anthelmintic activity of rhizomes of *Curcuma longa* and *Zingiber officinale* (Zingiberaceae).

Nisar, M. F., Jaleel, F., Haider, S. M., Toor, Y., Ismail, S., Arfan, M., & Azeem, M. (2014). Exploration of ethno-medicinal plants and their ritual uses in Bahawalnagar, Pakistan. *Middle East J Sci Res, 21*(9), 1466-1471.

Nita, R. D., & Haresh, D. L. (2013). Ethno-botanical survey of some medicinal plants in jatasankar region of girnar forest, gujarat, india. *Global Journal of Research on Medicinal Plants & Indigenous Medicine, 2*(12), 830.

Njue, L., Ombui, J., Kanja, L., Gathumbi, J., & Nduhiu, J. (2015). Evaluation of oral toxicity level of ethyl acetate extract, from garlic (allium sativum) in onorrh dawleys rats as per OECD guidelines 423.

Nofal, Z., El-Zahar, M., & El-Karim, A. (2000). Novel coumarin derivatives with expected biological activity. *Molecules, 5*(2), 99-113.

Numa, C., Verdú, J. R., Rueda, C., & Galante, E. (2012). Comparing dung beetle species assemblages between protected areas and adjacent pasturelands in a Mediterranean savanna landscape. *Rangeland Ecology & Management, 65*(2), 137-143.

Nur, A., Lemma, D., Eticha, E., Abera, B., Assefa, G., & Keno, L. (2016). Prevalence, Organ Condemnation and Financial Losses Due to Fasciolosis and Hydatidosis in Cattle Slaughtered in Adama Municipal Abattoir, Ethiopia. *African Journal of Basic & Applied Sciences, 8*(5), 276-282.

Nur, R. M., & Nugroho, L. H. (2018). Cytotoxic activities of fractions from Dioscorea bulbifera L. chloroform and methanol extracts on T47D breast cancer cells. *Pharmacognosy Journal, 10*(1).

Nurjanah, N., Jacoeb, A. M., Hidayat, T., Hazar, S., & Nugraha, R. (2016). Antioxidant activity, total phenol content, and bioactive components of lindur leave (Bruguierra gymnorrhiza). *American Journal of Food Science and Health, 2*(4), 65-70.

Nyarumbu, T., Kaseke, T., Gobvu, V., Murungweni, C., Mashingaidze, A., & Chikwambi, Z. (2019). Phenotypic and genetic characterisation revealed the existence of several biotypes within the Neorautanenia brachypus (Harms) CA wild accessions in South East Lowveld, Zimbabwe. *BMC ecology, 19*(1), 1-14.

Odeyemi, S., Koekemoer, T., van de Venter, M., Afolayan, A., & Bradley, G. (2015). Cytotoxicity of two medicinal plants commonly used in the management of diabetes in Eastern Cape South Africa using Chang liver cell lines. *Planta Medica, 81*(16), PW_26.

Odeyemi, S. W., & Afolayan, A. J. (2019). Characterization and cytotoxicity evaluation of biologically synthesized silver nanoparticles from albuca setosa aqueous bulb extract. *International Journal of Nanoscience, 18*(02), 1850023.

Odongo, E., Mungai, N., Mutai, P., Karumi, E., Mwangi, J., & Omale, J. (2018). Ethnobotanical survey of the medicinal plants used in Kakamega County, Western Kenya. *Applied Medical Research, 4*(1), 22.

Oliveira, A. F., Junior, L. M. C., Lima, A. S., Silva, C. R., Ribeiro, M. N., Mesquista, J. W., . . . Vilegas, W. (2017). Anthelmintic activity of plant extracts from Brazilian savanna. *Veterinary parasitology, 236*, 121-127.

Oliveira, L., Bevilaqua, C., Costa, C., Macedo, I., Barros, R., Rodrigues, A., . . . Vieira, L. (2009). Anthelmintic activity of Cocos nucifera L. against sheep gastrointestinal nematodes. *Veterinary parasitology, 159*(1), 55-59.

Olubunmi, A., Gabriel, O. A., Stephen, A. O., & Scott, F. O. (2009). Antioxidant and antimicrobial activity of cuticular wax from Kigelia africana. *Fabad Journal of Pharmaceutical Sciences, 34*(4), 187.

Olukotun, A., Bello, I., & Oyewale, O. (2018). Phytochemical and anthelmintic activity of Terminalia catappa (Linn) leaves. *Journal of Applied Sciences and Environmental Management, 22*(8), 1343-1347.

Omar, M. N., Rahman, S., Ichwan, S., Hasali, N., Rasid, F. A., & Halim, F. A. (2017). Cytotoxicity effects of extracts and essential oil of Kaempferia galanga on cervical cancer C33A cell line. *Orient. J. Chem, 33*, 1659-1664.

Orengo, K., Maitho, T., Mbaria, J., Maingi, N., & Kitaa, J. (2016). In vitro anthelmintic activity of Allium sativum, Allium cepa and Jatropha curcas against Toxocara canis and Ancylostoma caninum. *African Journal of Pharmacy and Pharmacology, 10*(21), 465-471.

Organization, W. H. (1996). Investing in health research and development: report of the ad hoc committee on health research relating to future intervention options: Geneva: World Health Organization.

Osinubi, A., Banjoko, O., Anselm, O., Akinrinola, O., & Osofodunrin, A. (2020). Comparative effects of drying methods on phytochemical contents and anti-microbial activities of watermelon (Citrullus lanatus) seed and rind. *Journal of Chemical Society of Nigeria, 45*(1).

Otang-Mbeng, W., & Sagbo, I. J. (2021). Cytotoxic, Cellular Antioxidant, and Antiglucuronidase Properties of the Ethanol Leaf Extract from Bulbine asphodeloides. *The Scientific World Journal, 2021*.

Padal, S., Murty, P. P., Rao, D. S., & Venkaiah, M. (2010). Ethnomedicinal plants from Paderu division of Visakhapatnam District, AP, India. *Journal of Phytology, 2*(8), 70-91.

Padal, S., Satyavathi, K., & Sandhyadeepika, D. (2014). Ethnomedicinal plants used for anthelmintic/helminthiasis in Visakhapatnam district, Andhra Pradesh, India. *EBM, 1*(2), 1-5.

Padmapriya, S., Rajamani, K., & Sathiyamurthy, V. (2015). Glory lily (Gloriosa superba L.)-A review. *International Journal of Current Pharmaceutical Review and Research, 7*(1), 43-49.
Pal, D. (2015). A review on Cyperus rotundus as a tremendous source of pharmacologically active herbal medicine. *International Journal of Green Pharmacy (IJGP), 9*(4).
Pal, P., & Tandon, V. (1998a). Anthelmintic efficacy of Flemingia vestita (Fabaceae): genistein-induced alterations in the esterase activity in the cestode, Raillietina echinobothrida. *Journal of biosciences, 23*(1), 25-31.
Pal, P., & Tandon, V. (1998b). Anthelmintic efficacy of Flemingia vestita (Leguminoceae): Genistein-induced alterations in the activity of tegumental enzymes in the cestode, Raillietina echinobothrida. *Parasitology International, 47*(3), 233-243.
Pal, R. S., & Mishra, A. (2019). Evaluation of acute toxicity of the methanolic extract of dhatryadi ghrita in wistar rats. *The Open Pharmacology Journal, 9*(1).
Palacios-Landín, J., Mendoza-de Gives, P., Salinas-Sánchez, D. O., López-Arellano, M. E., Liébano-Hernández, E., Hernández-Velázquez, V. M., & Valladares-Cisneros, M. G. (2015). In vitro and in vivo nematocidal activity of Allium sativum and Tagetes erecta extracts against Haemonchus contortus. *Türkiye Parazitolojii Dergisi, 39*(4), 260.
Pandey, A., Goswami, S., Tripathi, P., & Singh, A. (2011). An *in vitro* evaluation of anthelmintic activity of *Zingiber zerumbet* rhizomes and *Cucurbita maxima* seeds on *Pheretima posthuma* model: A comparative study. *Journal of Pharmacy And Bioallied Sciences, 3*(2), 317.
Pandey, A., Gupta, S., & Yadav, K. (2011). *AgroTechniques of Costus speciosus: an important endangered medicinal plant.* Paper presented at the National Conference on Forest Biodiversity: Earth's Living Treasure, Uttar Pradesh State Biodiversity Board, Lucknow.
Pandey, H., Srivastava, S., Kumar, R., & Tripathi, Y. B. (2018). Preclinical acute and repeated dose toxicity of Pueraria tuberosa (PTWE) on charles foster rats. *Int J Pharm Sci Res, 9*, 4572-4581.
Pandey, J., Mishra, S., & Jaiswal, K. (2018). In vitro evaluation of the anthelmintic activity of rhizome extracts of Curcuma Longa (Linn.). *IN VITRO, 11*(12).
Pandey, M., Debnath, M., Gupta, S., & Chikara, S. K. (2011). Phytomedicine: An ancient approach turning into future potential source of therapeutics. *Journal of Pharmacognosy and phytotherapy, 3*(1), 113-117.
Pandita, D., Pandita, A., & Pandita, S. (2013). Herbaceous medicinal & therapeutic plants of district samba of Jammu Province, Jammu & Kashmir (India). *Int J Indig Med Plants, 46*, 2051-4263.
Paseshnichenko, V., & Guseva, A. (1975). Isolation and properties of saponins from rhizomes of Dioscorea deltoidea Wall. *Prikladnaia Biokhimiia i Mikrobiologiia, 11*(1), 94-101.
Patel, D. (2015). Curcuma longa Linn. Cultivation: The process for its Medicinal use and Conservation. *The Pharma Innovation, 4*(1, Part B), 99.
Patel, D., & Galani, V. (2017). Evaluation of Neuropharmacological Activity of *Dioscorea bulbifera* Using Various Experimental Models. *Adv Plants Agric Res, 7*(1), 00241.
Patil, A., & Jadhov, V. (2014). GC-MS analysis of bioactive components from methanol leaf extract of Toddaliaasiatica (L.). *Int J Pharm Sci Rev Res, 29*(1), 18-20.
Patil, K. D., Bagade, S. B., Sharma, S. R., & Hatware, K. V. (2019). Potential of herbal constituents as new natural leads against helminthiasis: A neglected tropical disease. *Asian Pacific Journal of Tropical Medicine, 12*(7), 291.
Patil, N. P., Bhapkar, P. H., Maheshwari, K. M., Jagtap, P. N., Shewale, A. P., & Patil, R. Y. (2014). Anthelmintic activity of aqueous extracts of the alpinia galanga willd Rh. *Int J Pharm Sci Rev Res, 25*, 72-75.
Patrick, N. O. (2020). Evaluation of anti-inflammatory, analgesic, antipyretic effect of eicosane, pentadecane, octacosane, and heneicosane *Asian Journal of Pharmaceutical and Clinical Research, 13*(4), 29-35. doi: https://doi.org/10.22159/ajpcr.2020.v13i4.36196
Pattan, S., Dighe, N., Nirmal, S., Merekar, A., Laware, R., Shinde, H., & Musmade, D. (2009). Synthesis and biological evaluation of some substituted amino thiazole derivatives. *Asian journal of research in chemistry, 2*(2), 196-201.
Pattewar, A. M., Pandharkar, T., Yerawar, P., & Patawar, V. (2012). Evaluation of in-vitro antihelminthic activity of Aconitum heterophyllum. *Journal of Chemical, Biological and Physical Sciences (JCBPS), 2*(4), 2401.
Pawar, B. M., Wavhal, V. P., Pawar, N. D., Agarwal, M. R., Shinde, P. B., & Kamble, H. V. (2010). Anthelmintic activity of Gloriosa superba Linn (Liliaceae). *International Journal of PharmTech Research, 2*(2), 1483-1487.
Paz, J. E. W., Contreras, C. R., Munguía, A. R., Aguilar, C. N., & Inungaray, M. L. C. (2018). Phenolic content and antibacterial activity of extracts of Hamelia patens obtained by different extraction methods. *brazilian journal of microbiology, 49*(3), 656-661.

Pe´rez-Pertejo, Y., Escudero-Martı´nez, J., Reguera, R., Balana˜-Fouce, R., Garcı´a PA, J., PG, Feliciano, A., & Castro, M. (2019). Antileishmanial activity of terpenylquinones on Leishmania
infantum and their effects on Leishmania topoisomerase IB. *IJP. Drugs Drug Resist, 11*, 70-79. doi: https://doi.org/10.1016/j.ijpddr.2019.10.004

Pedrosa, R. C., De Bem, A. F., Locatelli, C., Pedrosa, R. C., Geremias, R., & Filho, D. W. (2001). Time-dependent oxidative stress caused by benznidazole. *Redox Report, 6*(4), 265-270.

Peerzada, A. M., Ali, H. H., Naeem, M., Latif, M., Bukhari, A. H., & Tanveer, A. (2015). Cyperus rotundus L.: Traditional uses, phytochemistry, and pharmacological activities. *Journal of ethnopharmacology, 174*, 540-560.

Peranantham, S., Manigandan, G., & Shanmugam, K. (2014). Fatal Gloriosa superba poisoning–a case report. *Int J Med Pharm Sci, 4*(10).

Perry, L. M. (1980). *Medicinal Plants of East andSoutheast Asia*. London: M.I.T. Press.

Pervez, K., Ashraf, M., & Hanjra, A. (1994). Anthelmintic efficacy of Melia azedarach (Bakain) Linn, against gastrointestinal nematodes in sheep. *Pakistan Veterinary Journal, 14*, 135-135.

Pfukenyi, D. M., & Mukaratirwa, S. (2013). A review of the epidemiology and control of gastrointestinal nematode infections in cattle in Zimbabwe. *Onderstepoort Journal of Veterinary Research, 80*(1), 01-12.

Phiri, A. (2006). Common conditions leading to cattle carcass and offal condemnations at 3 abattoirs in the Western Province of Zambia and their zoonotic implications to consumers. *Journal of the South African Veterinary Association, 77*(1), 28-32.

Pirbalouti, A. G., Oraie, M., Pouriamehr, M., & Babadi, E. S. (2013). Effects of drying methods on qualitative and quantitative of the essential oil of Bakhtiari savory (Satureja bachtiarica Bunge.). *Industrial crops and products, 46*, 324-327.

Plengsuriyakarn, T., & Na-Bangchang, K. (2020). Preclinical toxicology and anticholangiocarcinoma activity of oral formulation of standardized extract of Zingiber officinale. *Planta Medica, 86*(02), 104-112.

Plengsuriyakarn, T., Viyanant, V., Eursitthichai, V., Tesana, S., Chaijaroenkul, W., Itharat, A., & Na-Bangchang, K. (2012). Cytotoxicity, toxicity, and anticancer activity of Zingiber officinale Roscoe against cholangiocarcinoma. *Asian Pacific Journal of Cancer Prevention, 13*(9), 4597-4606.

Policegoudra, R., Rehna, K., Jaganmohan Rao, L., & Aradhya, S. (2010). Antimicrobial, antioxidant, cytotoxicity and platelet aggregation inhibitory activity of a novel molecule isolated and characterized from mango ginger (Curcuma amada Roxb.) rhizome. *Journal of biosciences, 35*(2), 231-240.

Poné, J. W., Bilong, C. B., & Mpoame, M. (2010). In vitro nematicidal activity of extracts of Canthium mannii (Rubiaceae), on different life-cycle stages of Heligmosomoides polygyrus (Nematoda, Heligmosomatidae). *Journal of helminthology, 84*(2), 156.

Pontiki, E., & Hadjipavlou-Litina, D. (2007). Synthesis and pharmacochemical evaluation of novel aryl-acetic acid inhibitors of lipoxygenase, antioxidants, and anti-inflammatory agents. *Bioorganic & medicinal chemistry, 15*(17), 5819-5827.

Poornima, G., & Ravishankar, R. V. (2007). In vitro propagation of wild yams, Dioscorea oppositifolia (Linn) and Dioscorea pentaphylla (Linn). *African Journal of Biotechnology, 6*(20).

Povydysh, M. N., Titova, M. V., Ivanov, I. M., Klushin, A. G., Kochkin, D. V., Galishev, B. A., . . . Krasnova, M. V. (2021). Effect of phytopreparations based on bioreactor-grown cell biomass of dioscorea deltoidea, tribulus terrestris and panax japonicus on carbohydrate and lipid metabolism in type 2 diabetes mellitus. *Nutrients, 13*(11), 3811.

Powers, K., Wood, I., Eckert, J., Gibson, T., & Smith, H. (1982). World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine and ovine). *Veterinary parasitology, 10*(4), 265-284.

Pozharitskaya, O. N., Ivanova, S. A., Shikov, A. N., & Makarov, V. G. (2008). Separation and free radical-scavenging activity of major curcuminoids of Curcuma longa using HPTLC-DPPH method. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques, 19*(3), 236-243.

Prakash, G., & Hosetti, B. (2010). Investigation of Antimicrobial Properties of Doscorea pentaphylla from Mid Western Ghats, India. *Scientific World, 8*(8), 91-96.

Prasad, P., Sai, K. H., & Srilekha, K. (2011). Comparative evaluation of antimicrobial and antihelminthic activity of Azadirachta indica, Curcuma longa& Murraya koinegii. *International Journal of Pharmacy & Technology., 3*, 3307-3315.

Prema, D., Kamaraj, M., Achiraman, S., & Udayakumar, R. (2014). In vitro antioxidant and cytotoxicity studies of Curcuma amada Roxb.(Mango ginger). *International Journal of Scientific and Research Publications, 4*(4), 1-6.

Premaalatha, B., Norhafiza, N., Nurulaini, R., Chandrawathani, P., Andilla, N., Rahimah, H., & Wahab, A. (2013). Evaluation of neem leaf (Azadirachta indica) product for worm control on goats. *Malaysian Journal of Veterinary Research (Malaysia)*.

Princewill-Ogbonna, I., Abagha, O., & Ijioma, S. (2015). Haematological and histopathological parameters of rats fed with aerial yam (Dioscorea bulbifera). *Comprehensive Journal of Food Science & Technology Research, 2*, 9-16.

Priscilla, F., Amin, M., & Rahman, S. (2014). Comparative study of neem (Azadirachta indica), bitter gourd (Momordica charantia) extract as herbal anthelmintic and albendazole as chemical anthelmintic in controlling gastrointestinal nematodes in goats. *IOSR Journal of Agriculture and Veterinary Science, 7*(2), 33-37.

Priya, S., & Santhi, S. (2015). Biosynthesis and in vitro anthelmintic activity of silver nanoparticles using aqueous leaf extract of Azadirachta indica. *World J. Pharm. Pharm. Sci, 4*, 2105-2115.

Priyanka, C., Kumar, P., Bankar, S. P., & Karthik, L. (2015). In vitro antibacterial activity and gas chromatography–mass spectroscopy analysis of Acacia karoo and Ziziphus mauritiana extracts. *Journal of Taibah University for Science, 9*(1), 13-19.

Pudziuvelyte, L., Jakštas, V., Ivanauskas, L., Laukevičienė, A., Ibe, C. F. D., Kursvietiene, L., & Bernatoniene, J. (2018). Different extraction methods for phenolic and volatile compounds recovery from Elsholtzia ciliata fresh and dried herbal materials. *Industrial crops and products, 120*, 286-294.

Qureshi, S., Shah, A., & Ageel, A. (1992). Toxicity studies on Alpinia galanga and Curcuma longa. *Planta Medica, 58*(02), 124-127.

Rabiu, H., & Subhasish, M. (2011). Investigation of in vitro anthelmintic activity of Azadirachta indica leaves. *International Journal of drug development and research, 3*(4), 0-0.

Radhakrishnan, L., Gomathinayagam, S., & Balakrishnan, V. (2010). Evaluation of anthelmintic effect of neem (Azadirachta indica) leaves on Haemonchus contortus in goats. *Research Journal of parasitology, 5*(4), 292-297.

Raether, W., & Hänel, H. (2003). Epidemiology, clinical manifestations and diagnosis of zoonotic cestode infections: an update. *Parasitology research, 91*(5), 412-438.

Rafińska, K., Pomastowski, P., Rudnicka, J., Krakowska, A., Maruśka, A., Narkute, M., & Buszewski, B. (2019). Effect of solvent and extraction technique on composition and biological activity of Lepidium sativum extracts. *Food Chemistry, 289*, 16-25.

Rahbar, N., Shafaghat, A., & Salimi, F. (2012). Antimicrobial activity and constituents of the hexane extracts from leaf and stem of Origanum vulgare L. ssp. Viride (Boiss.) Hayek. growing wild in Northwest Iran. *Journal of Medicinal Plants Research, 6*(13), 2681-2685.

Rahman, H. S., Rasedee, A., Othman, H. H., Chartrand, M. S., Namvar, F., Yeap, S. K., . . . Anasamy, T. (2014). Acute toxicity study of zerumbone-loaded nanostructured lipid carrier on BALB/c mice model. *BioMed Research International, 2014*.

Rahman, W. A., Lee, R., & Sulaiman, S. F. (2011). In vitro anthelmintic activity of Neem plant (Azadirachta indica) extract against third-stage Haemonchus contortus larvae from goats. *Global Veterinaria, 7*(1), 22-26.

Raisová, L. S., Podlipná, R., Szotáková, B., Syslová, E., & Skálová, L. (2017). Evaluation of drug uptake and deactivation in plant: Fate of albendazole in ribwort plantain (Plantago laceolata) cells and regenerants. *Ecotoxicology and environmental safety, 141*, 37-42.

Raju, J., & Mehta, R. (2008). Cancer chemopreventive and therapeutic effects of diosgenin, a food saponin. *Nutrition and cancer, 61*(1), 27-35.

Rakh, M. S., Pawar, R. V., & Khedkar, A. N. (2014). Anthelmintic potential of various extracts of the rhizomes of *Curcuma amada* Roxb. *Asian Pacific Journal of Tropical Disease, 4*, S276-S278.

Ramadas, V., & Chandralega, G. (2020). Screening of phytochemicals, fatty acid composition and in-vitro analysis of antioxidant property of green edible seaweed Caulerpa lentillifera (family: Caulerpaceae). *Int. J. Pharm. Sci. Res, 11*, 1495-1505.

Ramdani, D., Budinuryanto, D. C., & Julaeha, J. (2021). The Effect of Turmeric Extract (Curcuma longa l.) As a Potential Anthelmintic on Reducing Endoparasites in Naturally-Infected Sheep. *Jurnal Agripet, 21*(1).

Randeep, G., Vandna, K., & Amandeep, S. (2011). Phytochemical investigation and evaluation of anthelmintic activity of Curcuma amada and Curcuma caesia-A comparative study. *J Ethnopharmacol, 2*, 1-4.

Rao, R. (1981). Ethnobotany of Meghalaya: medicinal plants used by Khasi and Garo tribes. *Economic Botany, 35*(1), 4-9.

Rasekhi, F., Tajick, M. A., Rahimian, H., & Sharifimehr, S. (2014). Some of phytotoxic and antimicrobial compounds extracted from culture filtrates of Fusarium proliferatum FP85. *Journal of Biodiversity and Environmental Sciences, Dhaka, 4*(5), 245-251.

Rashdan, H. R., Gomha, S. M., El-Gendey, M. S., El-Hashash, M. A., & Soliman, A. M. M. (2018). Eco-friendly one-pot synthesis of some new pyrazolo [1, 2-b] phthalazinediones with antiproliferative efficacy on human hepatic cancer cell lines. *Green Chemistry Letters and Reviews, 11*(3), 264-274.

Rathi, N., Harwalkar, K., Jayashree, V., Sharma, A., & Rao, N. N. (2017). 2-hydroxy-4-methoxybenzaldehyde, an astounding food flavoring metabolite: A review. *Asian J Pharm Clin Res, 10*(10), 105-110.

Raul, S. K., Padhy, G. K., Charly, J. P., & Kumar, K. V. (2012). An *in-vitro* evaluation of the anthelmintic activity of rhizome extracts of *Zingiber officinalis*, *Zingiber zerumbet* and *Curcuma longa*, a comparative study. *Journal of Pharmacy Research, 5*(7), 3813-3814.

Raza, A., Muhammad, F., Bashir, S., Aslam, B., Anwar, M., & Naseer, M. (2016). In-vitro and in-vivo anthelmintic potential of different medicinal plants against Ascaridia galli infection in poultry birds. *World's Poultry Science Journal, 72*(1), 115-124.

Reddy, C. S. S., Subramanyam, M., Vani, R., & Devi, S. A. (2007). In vitro models of oxidative stress in rat erythrocytes: effect of antioxidant supplements. *Toxicology in vitro, 21*(8), 1355-1364.

Reduan, F. H., Shaari, R. M., Sayuti, N. S. A., Mustapha, N. M., Abu Bakar, M. Z., Sithambaram, S., & Hamzah, H. (2020). Acute and subacute dermal toxicity of ethanolic extract of Melastoma malabathricum leaves in Sprague-Dawley rats. *Toxicological Research, 36*(3), 203-210.

Reduan, M. F. H., Hamid, F. F. A., Nordin, M. L., Shaari, R., Hamdan, R. H., Chung, E. L. T., . . . Noralidin, N. (2020). Acute oral toxicity study of ethanol extract of Oroxylum indicum leaf in mice. *Thai J Veterinary Med, 50*, 573-581.

Rehana, B., & Nagarajan, N. (2012). Phytochemical screening for active compounds in Gloriosa superba leaves and tubers. *Journal of Pharmacognosy and Phytochemical Research, 4*(1), 17-20.

Rijal, A. (2011). Surviving on Knowledge: Ethnobotany of Chepang community from mid-hills of Nepal. *Ethnobotany research and Applications, 9*, 181-215.

Rinaldi, M. V., Díaz, I. E., Suffredini, I. B., & Moreno, P. R. (2017). Alkaloids and biological activity of beribá (Annona hypoglauca). *Revista Brasileira de Farmacognosia, 27*, 77-83.

Roberts, W. N., Liang, M. H., & Stern, S. H. (1987). Colchicine in acute gout: reassessment of risks and benefits. *Jama, 257*(14), 1920-1922.

Robertson, S., & Thamizharasi, A. K. (2016). Evaluation of Anthelmintic Activity of Extracts from Leaves of Raphanus sativus Linn.

Robles-Molina, J., Gilbert-López, B., García-Reyes, J. F., & Molina-Díaz, A. (2014). Monitoring of selected priority and emerging contaminants in the Guadalquivir River and other related surface waters in the province of Jaén, South East Spain. *Science of the Total Environment, 479*, 247-257.

Rochfort, S., Parker, A. J., & Dunshea, F. R. (2008). Plant bioactives for ruminant health and productivity. *Phytochemistry, 69*(2), 299-322.

Roy, B., & Tandon, V. (1996). Effect of root-tuber extract of Flemingia vestita, a leguminous plant, on Artyfechinostomum sufrartyfex and Fasciolopsis buski: a scanning electron microscopy study. *Parasitology Research, 82*(3), 248-252.

Rupar, J., Dobričić, V., Aleksić, M., Brborić, J., & Čudina, O. (2018). A review of published data on acridine derivatives with different biological activities. *Kragujevac Journal of Science*(40), 83-101.

Ryu, C.-K., Park, R.-E., Ma, M.-Y., & Nho, J.-H. (2007). Synthesis and antifungal activity of 6-arylamino-phthalazine-5, 8-diones and 6, 7-bis (arylthio)-phthalazine-5, 8-diones. *Bioorganic & medicinal chemistry letters, 17*(9), 2577-2580.

Sabale, P., Modi, A., & Sabale, V. (2013). Curcuma longa Linn. A phytochemical and phytopharmacological review. *Research Journal of Pharmacognosy and Phytochemistry, 5*(2), 59.

Sahu, A. K., Panda, C., & Nayak, B. S. (2018). Determination of phytochemical and anthelmintic activity of rhizome of *Zingiber zerumbet*. *J Pharm Adv Res, 1*, 399-402.

Sakti, A., Kustantinah, K., & Nurcahyo, R. (2018). In Vitro and in Vivo anthelmintic activities of aqueous leaf infusion of Azadirachta indica against Haemonchus contortus. *Tropical Animal Science Journal, 41*(3), 185-190.

Sakurai, Y., Sakurai, N., Taniguchi, M., Nakanishi, Y., Bastow, K. F., Wang, X., . . . Lee, K.-H. (2006). Rautandiols A and B, Pterocarpans and Cytotoxic Constituents from Neorautanenia m itis, 1. *Journal of Natural Products, 69*(3), 397-399.

Salgado, J. A., & Santos, C. d. P. (2016). Overview of anthelmintic resistance of gastrointestinal nematodes of small ruminants in Brazil. *Revista Brasileira de Parasitologia Veterinária, 25*(1), 3-17.

Salhan, M., Kumar, B., Tiwari, P., Sharma, P., Kaur, H., & Gautam, M. (2011). Comparative anthelmintic activity of aqueous and ethanolic leaf extracts of Clitoria ternatea. *International Journal of Drug Development and Research, 3*(1), 0-0.

Salma, S., Anvitha, R. M., Indupriya, J., Sireesha, B., Kumar, B. V., & Nagalakshmi, G. (2021). Biological Evalution of (Azadirachta Indica) Neem Flower for Anthelmintic Activity on Earth Worm (Pheretima posthuma). *Journal of Pharmaceutical Sciences and Research, 13*(2), 92-94.

Samra, R. M., Soliman, A. F., Zaki, A. A., El-Gendy, A. N., Hassan, M. A., & Zaghloul, A. M. (2020). Chemical composition, antiviral and cytotoxic activities of essential oil from Cyperus rotundus Growing in Egypt: Evidence from chemometrics analysis. *Journal of Essential Oil Bearing Plants, 23*(4), 648-659.

Samson, E. S., Olasunkanmi, A. K., Joel, J. S., & Alfred, E. F. (2012). Haematological and hepatotoxic potential of onion (Allium cepa) and garlic (Allium sativum) extracts in rats. *European Journal of Medicinal Plants, 2*(4), 290.

Sanhokwe, M. (2015). *Determination and validation of medicinal plants used by farmers to control internal and external parasites in goats in the Eastern Cape Province, South Africa.* University of Fort Hare.

Sanhokwe, M., Mupangwa, J., Masika, P. J., Maphosa, V., & Muchenje, V. (2016). Medicinal plants used to control internal and external parasites in goats. *Onderstepoort Journal of Veterinary Research, 83*(1), 1-7.

Santiago-Figueroa, I., Lara-Bueno, A., González-Garduño, R., López-Arellano, M. E., de la Rosa-Arana, J. L., & de Jesús Maldonado-Simán, E. (2019). Anthelmintic resistance in hair sheep farms in a sub-humid tropical climate, in the Huasteca Potosina, Mexico. *Veterinary Parasitology: Regional Studies and Reports, 17*, 100292.

Santos, A. C. V., Santos, F. O., Lima, H. G., Da Silva, G. D., Uzêda, R. S., Dias, Ê. R., . . . Botura, M. B. (2018). In vitro ovicidal and larvicidal activities of some saponins and flavonoids against parasitic nematodes of goats. *Parasitology, 145*(14), 1884-1889.

Santosh, N., Mohan, K., Royana, S., & Yamini, T. B. (2010). Hepatotoxicity of tubers of Indian Kudzu (Pueraria tuberosa) in rats. *Food and Chemical Toxicology, 48*(4), 1066-1071.

Saravanakumar, S. (2014). Preliminary phytochemical screening of different solvent extracts of Root tuber of Smilax china. *International Journal of Pharmaceutical and Biological Science Archive, 2*(7).

Saravanan, M., Ramesh, M., Malarvizhi, A., & Petkam, R. (2011). Toxicity of neem leaf extracts (Azadirachta indica A. Juss) on some haematological, ionoregulatory, biochemical and enzymological parameters of Indian major carp, Cirrhinus mrigala. *Journal of Tropical Forestry and Environment, 1*(1).

Sari, I. P., & Nurrochmad, A. (2016). Sub-acute toxicity study of ethanolic extract of pacing (Costus speciosus) in male mice. *Int J Pharm Pharm Sci, 8*(12), 97-10.

Sarkar, A. K., Dey, M., & Mazumder, M. (2017). Ecological status of medicinal plants of Chalsa forest range under Jalpaiguri division, West Bengal, India. *International Journal of Herbal Medicine, 5*(5), 196-215.

Sarker, M., Khan, M., Rashid, M., & Islam, M. (2016). Effect of Azadirachta indica and Annona reticulata leaf as natural anthelmintics and their effects on performances of zebu cow under subsistence farming condition in Bangladesh. *International Journal of Pharmaceutical, Chemical & Biological Sciences, 6*(1).

Saxena, J., & Sahu, R. (2012). Evaluation of phytochemical constituent in conventional and non conventional species of curcuma. *International Research Journal of Pharmacy, 3*(8), 203-204.

Sblivanova-yartseva, A. (1959). Garlic as a new anthelmintic against cestode infection in geese. *Sboroik Naududkh Siblrskogo Nauchno-Issledovatelskogo Veterinarnogo Instituts, 8*, 197-199.

Schlander, M., Hernandez-Villafuerte, K., Cheng, C.-Y., Mestre-Ferrandiz, J., & Baumann, M. (2021). How much does it cost to research and develop a new drug? A systematic review and assessment. *PharmacoEconomics, 39*(11), 1243-1269.

Scholten, M. T., De Boer, I., Gremmen, B., & Lokhorst, C. (2013). Livestock farming with care: towards sustainable production of animal-source food.

Schweizer, G., Braun, U., Deplazes, P., & Torgerson, P. (2005). Estimating the financial losses due to bovine fasciolosis in Switzerland. *Veterinary Record, 157*(7), 188-193.

Sechi, G., De Riu, P., Mameli, O., Deiana, G. A., Cocco, G. A., & Rosati, G. (2003). Focal and secondarily generalised convulsive status epilepticus induced by thiocolchicoside in the rat. *Seizure, 12*(7), 508-515.

Selvaraju, A., & Dhanraj, S. (2019). Phytochemical analysis and anthelmintic potential of Nigella sativa against the trematode, Cotylophoron cotylophorum. *J. Pharmacogn. Phytochem, 8*, 3161-3166.

Semwal, D. K., Rawat, U., Badoni, R., Semwal, R., & Singh, R. (2010). Anti-hyperglycemic effect of Stephania glabra tubers in alloxan induced diabetic mice. *Journal of Medicine, 11*(1), 17-19.

Semwal, K., iSemwal, D. B., Semwal, R., Jacob, R., & Gurjaspreet, V. S. (2011). Analgesic and antipyretic activities of Gindarudine, a morphine alkaloid from Stephania glabra. *Current Bioactive Compounds, 7*(3), 214-217.

Semwal, P., Painuli, S., & Cruz-Martins, N. (2021). Dioscorea deltoidea wall. Ex Griseb: A review of traditional uses, bioactive compounds and biological activities. *Food Bioscience, 41*, 100969.

Shalaby, H., El Namaky, A. H., Kamel, R. O., Ashry, H. M., & Farag, T. K. (2017). Cuticular changes in adult *Toxocara vitulorum* following in vitro administration of ginger (*Zingiber officinale*) with reference to its ovicidal activity. *Bull. NRC, 41*(1), 54-65.

Shalaby, H. A. (2013). Anthelmintics resistance; how to overcome it? *Iranian journal of parasitology, 8*(1), 18.

Shalaby, H. A., & Farag, T. K. (2014). Body surface changes in gastrointestinal helminthes following in vitro treatment with Allium sativum oil. *Journal of Veterinary Science and Technology, 5*(1).

Sharifah Sakinah, S., Tri Handayani, S., & Azimahtol Hawariah, L. (2007). Zerumbone induced apoptosis in liver cancer cells via modulation of Bax/Bcl-2 ratio. *Cancer cell international, 7*(1), 1-11.

Sharma, A., Verma, R., & Ramteke, P. (2014). Cyperus rotundus: a potential novel source of therapeutic compound against urinary tract pathogens. *Journal of Herbal Medicine, 4*(2), 74-82.

Sharma, P., Agnihotry, A., & Sharma, P. P. (2015). An ethnobotanical study of medicinal plants in Murari Devi and surrounding areas (Mandi district, Himachal Pradesh), India. *Indian For, 141*(1), 68-78.

Sheikh, N., Kumar, Y., Misra, A., & Pfoze, L. (2013). Phytochemical screening to validate the ethnobotanical importance of root tubers of Dioscorea species of Meghalaya, North East India. *Journal of Medicinal Plants, 1*(6), 62-69.

Shen, P. (2002). A new steroidal saponin from Dioscorea deltoidea Wall var. orbiculata. *Chin Chem Lett, 13*(9), 851-854.

Shen, P., Wang, S.-L., Liu, X.-K., Yang, C.-R., Cai, B., & Yao, X.-S. (2002). A new ergostanol saponin from Dioscorea deltoidea Wall var. orbiculata. *Journal of Asian natural products research, 4*(3), 211-215.

Shetty, D., Kamath, R., Bhat, P., Hegde, K., & Shabaraya, A. (2011). Athelmitic activity of root extract of Raphaus sativus. *Pharmacology online, 1*, 675-679.

Shetty, G. R., & Monisha, S. (2015). Pharmacology of an endangered medicinal plant Alpinia galanga-a review. *Research Journal of Pharmaceutical, Biological and Chemical Sciences, 6*(1), 499-511.

Shirazi, O. U., Khattak, M., Shukri, N. A. M., & Nasyriq, M. N. (2014). Determination of total phenolic, flavonoid content and free radical scavenging activities of common herbs and spices. *Journal of Pharmacognosy and Phytochemistry, 3*(3), 104-108.

Shirgholami, Z., Borji, H., Mohebalian, H., & Heidarpour, M. (2021). Effects of Allium sativum on IFN-γ and IL4 concentrations in mice with cystic echinococcosis. *Experimental parasitology, 220*, 108042.

Shoaib, M., Israyilova, A. A., & Ganbarov, K. (2021). Cyclohexane and its functionally substituted derivatives: important class of organic compounds with potential antimicrobial activities. *Journal of Microbiology, Biotechnology and Food Sciences, 2021*, 84-87.

Shriram, V., Jahagirdar, S., Latha, C., Kumar, V., Puranik, V., Rojatkar, S., . . . Shitole, M. (2008). A potential plasmid-curing agent, 8-epidiosbulbin E acetate, from Dioscorea bulbifera L. against multidrug-resistant bacteria. *International Journal of Antimicrobial Agents, 32*(5), 405-410.

Shukla, S. (1995). Toxicological studies of Pueraria tuberosa, a potent antifertility plant. *International journal of pharmacognosy, 33*(4), 324-329.

Sidde, L., Sushma, M., Shivashanthi, V., Prema, G., Pushpa, K., & Saivijaya, B. (2021). A review on dioscorea pentaphylla plant. *International Journal of Alternative and Complementary Medicine*, 06-08.

Siddiqui, W. A., Ahad, A., Ganai, A., Sareer, O., Najm, M., Kausar, M., & Mohd, M. (2012). Therapeutic potential of Oroxylum indicum: a review. *Journal of Research and Opinion, 2*(10), 163-172.

Simelane, M., Lawal, O., Djarova, T., & Opoku, A. (2010). In vitro antioxidant and cytotoxic activity of Gunnera perpensa L.(Gunneraceae) from South Africa. *Journal of Medicinal Plants Research, 4*(21), 2181-2188.

Singh, A. (2011). *Herbalism, phytochemistry and ethnopharmacology*: CRC Press.

Singh, B. B., Sharma, J. K., Tuli, A., Sharma, R., Bal, M. S., Aulakh, R. S., & Gill, J. P. S. (2014). Prevalence and morphological characterisation of Echinococcus granulosus from north India. *Journal of parasitic diseases, 38*(1), 36-40.

Singh, L., Kumar, A., Choudhary, A., & Singh, G. (2018). Asparagus racemosus: The plant with immense medicinal potential. *Journal of pharmacognosy and Phytochemistry, 7*(3), 2199-2203.

Singh, R., & Geetanjali. (2016). Asparagus racemosus: a review on its phytochemical and therapeutic potential. *Natural Product Research, 30*(17), 1896-1908.

Singh, S., Chattopadhyay, P., Borthakur, S. K., & Policegoudra, R. (2017). Safety profile investigations of Meyna spinosa (Roxb.) and Oroxylum indicum (Linn.) extracts collected from Northeast India. *Pharmacognosy Magazine, 13*(Suppl 4), S762.

Singh, T., Chandrudu, J., & Parmar, P. (2018). Design, evaluation and comprative study of in vitro anthelmintic activity on sap of Azadirachta indica against helminthiasis model.

Singh, T., Kumar, D., Gupta, P., & Tandan, S. (2007). Inhibitory effects of alcoholic extracts of *Allium sativum* and *Piper longum* on gross visual motility and glucose uptake of *Fasciola gigantica* and *Gigantocotyle explanatum*. *Journal of Veterinary Parasitology, 21*(2), 121-124.

Singh, T., Kumar, D., & Tandan, S. (2008). Paralytic effect of alcoholic extract of Allium sativum and Piper longum on liver amphistome, Gigantocotyle explanatum. *Indian journal of pharmacology, 40*(2), 64.

Singh, T. U., Kumar, D., Tandan, S. K., & Mishra, S. K. (2009). Inhibitory effect of essential oils of Allium sativum and Piper longum on spontaneous muscular activity of liver fluke, Fasciola gigantica. *Experimental parasitology, 123*(4), 302-308.

Singla, S., & Kaur, S. (2021). Anthelmintic lead compounds and their targets for drug development. *Journal of Ayurvedic and Herbal Medicine, 7*(4), 265-275.

Sirtori, C. R. (2001). Risks and benefits of soy phytoestrogens in cardiovascular diseases, cancer, climacteric symptoms and osteoporosis. *Drug safety, 24*(9), 665-682.

Sivakumar, G., & Krishnamurthy, K. (2000). Micropropagation of Gloriosa superba L.-an endangered species of Asia and Africa. *Current science, 78*(1), 30-32.

Sivapalan, S. R. (2013). Medicinal uses and pharmacological activities of Cyperus rotundus Linn-A Review. *International Journal of Scientific and Research Publications, 3*(5), 1-8.

Sivashanmugapillai, S. (2016). *Effect of Traditional Veterinary Practice: Neem (Azadirachta Indica) Leaves and Turmeric (Curcuma Longa) Powder on Gastrointestinal Parasitism in Goats.* Uva Wellassa University of Sri Lanka.

Snedecor, G. (1999). "Hexachloroethane". In J. I. e. In Kroschwitz (Ed.), *Kirk-Othmer Concise Encylclopedia of Chemical Technology (4th ed.).* (pp. 428). New York: John Wiley & Sons, Inc.

Söhnlein, B., Letzel, S., Weltle, D., Rüdiger, H., & Angerer, J. (1993). Occupational chronic exposure to organic solvents. *International archives of occupational and environmental health, 64*(7), 479-484.

Soleimani, V., Sahebkar, A., & Hosseinzadeh, H. (2018). Turmeric (Curcuma longa) and its major constituent (curcumin) as nontoxic and safe substances. *Phytotherapy Research, 32*(6), 985-995.

Sonwa, M. M., & König, W. A. (2001). Chemical study of the essential oil of Cyperus rotundus. *Phytochemistry, 58*(5), 799-810.

Soren, A. D., & Yadav, A. K. (2020). Evaluation of in vitro and in vivo anthelmintic efficacy of Cyperus compressus Linn., a traditionally used anthelmintic plant in parasite-animal models. *Future Journal of Pharmaceutical Sciences, 6*(1), 1-6.

Soren, A. D., & Yadav, A. K. (2021). *In Vitro Anthelmintic Efficacy of Sesbania sesban var. bicolor, Cyperus compressus and Asparagus racemosus Against Gastrothylax crumenifer (Trematoda).* Paper presented at the Proceedings of the Zoological Society.

Spagnuolo, C., Russo, G. L., Orhan, I. E., Habtemariam, S., Daglia, M., Sureda, A., . . . Tundis, R. (2015). Genistein and cancer: current status, challenges, and future directions. *Advances in nutrition, 6*(4), 408-419.

Sprenger, L., Buzatti, A., Campestrini, L., Yamassaki, F., Maurer, J., Baggio, S., . . . Molento, M. (2015). Atividade ovicida e larvicida do extrato hidroalcoólico de Artemisia annua sobre parasitas gastrintestinais de bovinos. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 67*, 25-31.

Sreekanth, D., & Devi, P. (2019). Qualitative and quantitative phytochemical studies of helianthus tuberosus L. *Journal of Pharmacognosy and Phytochemistry, 8*(1), 247-250.

Srivastava, S., Singh, P., Jha, K., Mishra, G., Srivastava, S., & Khosa, R. (2011). Anthelmintic activity of aerial parts of Costus speciosus. *International Journal of Green Pharmacy (IJGP), 5*(4).

Stojkovic, R., Karminski-Zamola, G., Racane, L., Tralic-Kulenovic, V., Glavas-Obrovac, L., Ivankovic, S., & Radacic, M. (2006). Antitumor efficiency of novel fluoro-substituted 6-amino-2-phenylbenzothiazole hydrochloride salts in vitro and in vivo. *Methods and findings in experimental and clinical pharmacology, 28*(6), 347-354.

Subash, K., Rao, N. J., Cheriyan, B. V., Bhaarati, G. M., & Kumar, K. S. (2012). Eupatorium triplinerve and Alpinia galanga in Pheritima posthuma and Ascardia galli: A Comparative Study. *Journal of Clinical and Diagnostic Research, 6*(6), 947-950.

Subedi, R. (2017). *Ethnobotanical study of panchase protected forest, Kaski District, Central Nepal.* Central Department of Botany Tribhuvan University Kirtipur, Kathmandu, Nepal.

Subhash, S., Sarla, S., & Mridul, D. (2012). Evaluation of Garhwal Himalaya wild tuber Dioscorea deltoidea. *International Research journal of pharmacy, 3*(3), 152-156.

Subramanian, S., Dowlath, M. J. H., Karuppannan, S. K., Saravanan, M., & Arunachalam, K. D. (2020). Effect of Solvent on the Phytochemical Extraction and GC-MS Analysis of Gymnema sylvestre. *Pharmacognosy Journal, 12*(4).

Suhaimi, N. H., & Mossadeq, W. M. S. M. (2016). In vitro anthelmintic activity of neem (Azadirachta indica) leaf chloroform extract against strongyle third stage larvae from sheep. *Universiti Putra Malaysia Press Serdang• 2016*, 192.

Sumi, A., & Shohe, K. (2018). Ethnomedicinal Plants of Sumi Nagas in Zunheboto District, Nagaland, Northeast India. *Acta Sci Pharmac Sci, 2*(8), 15-21.

Sunada, N. d. S., Orrico Junior, M., Orrico, A. C. A., Oliveira, A. d. M., Centurion, S. R., Lima, S. d. N., . . . de Vargas Junior, F. (2011). Control parasite using levamizol, ivermectin and dehydrated garlic (Allium sativum) in sheep race Santa Inês. *Revista Agrarian, 4*(12), 140-145.

Sungirai, M., Masaka, L., & Mbiba, C. (2014). The prevalence of Taenia saginata cysticercosis in the Matabeleland Provinces of Zimbabwe. *Tropical Animal Health and Production, 46*(4), 623-627.

Sunita, K., Kumar, P., Singh, V., & Singh, D. (2013). Larvicidal activity of azadirachtin against Fasciola larvae. *The Ecoscan, 4*, 189-194.

Sunita, K., & Singh, D. K. (2011). Fascioliasis control: *in vivo* and *in vitro* phytotherapy of vector snail to kill Fasciola larva. *Journal of parasitology research, 2011*.

Sur, T. K., Hazra, A., Hazra, A. K., & Bhattacharyya, D. (2016). Antioxidant and hepatoprotective properties of Indian Sunderban mangrove Bruguiera gymnorrhiza L. leave. *Journal of basic and clinical pharmacy, 7*(3), 75.

Suri, O. P., Gupta, B. D., Suri, K. A., Sharma, A. K., & Satti, N. K. (2001). A new glycoside, 3-O-demethylcolchicine-3-O-α-D-glucopyranoside, from Gloriosa superba seeds. *Natural Product Letters, 15*(4), 217-219.

Suryavanshi, S., Rai, G., & Malviya, S. (2012). Evaluation of anti-microbial and anthelmintic activity of Gloriosa Superba tubers. *Advance Research in Pharmaceuticals and Biologicals, 2*(1), 45-52.

Suryowati, T., Sirait, R. H., Siagian, F. E., & Nursyam, M. (2020). *Bioactive Compound Impacting the Metabolism and Antibacterial Activity of Gadung Tuber (Dioscorea hispida Dennst).* Paper presented at the Journal of Physics: Conference Series.

Susianti, S., Yanwirasti, Y., & Darwin, E. (2018). The cytotoxic effects of purple nutsedge (Cyperus rotundus L.) tuber essential oil on the HeLa cervical cancer cell line. *Pakistan Journal of Biotechnology, 15*(1), 77-81.

Suteky, T. (2019). *The Use of Melastoma malabatricum and Manihot esculenta extract as natural anthelmintic on the Performance of Kacang goat.* Paper presented at the IOP Conference Series: Earth and Environmental Science.

Sutton, G. A., & Haik, R. (1999). Efficacy of garlic as an anthelmintic in donkeys. *Israel Journal of Veterinary Medicine, 54*, 23-27.

Swargiary, A., Daimari, M., & Roy, M. K. (2020). Survey and documentation of anthelmintic plants used in traditional medicine system of tribal communities of Udalguri district Assam, India. *J Appl Pharm Sci, 10*(1), 46-54.

Swargiary, A., Daimari, M., & Roy, M. K. (2021). Putative Anthelmintic Plants Used in Traditional Medicine System of Kokrajhar District, India. *Ethnobotany Research and Applications, 22*, 1-18.

Swargiary, A., Roy, M. K., & Daimari, M. (2019a). Survey and Documentation of Ethnobotanicals used in the Traditional Medicines System of Tribal Communities of Chirang District of Assam Against Helminthiasis. *Biomedical and Pharmacology Journal, 12*(4), 1923-1935.

Swargiary, A., Roy, M. K., & Daimari, M. (2019b). Survey and documentation of putative anthelmintic plants used in ethnomedicinal systems of tribal communities of Baksa District of Assam. *Medicinal Plants-International Journal of Phytomedicines and Related Industries, 11*(4), 368-379.

Swarnkar, C., Singh, D., Khan, F., & Bhagwan, P. (2008). Potential of alcoholic extract of Azadirachta indica bark as anthelmintic in sheep. *Journal of Veterinary Parasitology, 22*(2), 13-16.

Tahir, N., Bibi, Y., Iqbal, M., Hussain, M., Laraib, S., Safdar, I., & Bibi, G. (2016). Overview of Dioscorea deltoidea Wall. Ex Griseb: An endangered medicinal plant from Himalaya region. *Journal of Biodiversity and Environmental Science, 9*, 13-24.

Taíse, P., Luciana Ferreira, D., Almeida, G. N. d., Ayres, M. C. C., Moreira, E. L. T., Cruz, A. C. F. d., . . . Batatinha, M. J. M. (2009). Anthelmintic activity of aqueous extract of Zanthoxylum rhoifolium Lam. leaves (Rutaceae). *Revista Brasileira de Parasitologia Veterinária, 18*, 43-48.

Tamang, R., Thakur, C., Koirala, D., & Chapagain, N. (2017). Ethno-medicinal Plants used by chepang community in Nepal. *Journal of Plant Resources, 15*(1), 31-30.

Tamboli, A. M., Karpe, S. T., Shaikh, S. A., Manikrao, A. M., & Kature, D. V. (2011). Hypoglycemic activity of extracts of Oroxylum indicum (L.) vet roots in animal models

Tan, K. H., & Nishida, R. (2012). Methyl eugenol: its occurrence, distribution, and role in nature, especially in relation to insect behavior and pollination. *Journal of insect science, 12*(1).

Tan, X.-q., Ruan, J.-l., Chen, H.-s., & Wang, J.-y. (2003). Studies on liver-toxicity in rhigoma of Dioscorea bulbifera. *Zhongguo Zhong yao za zhi= Zhongguo Zhongyao Zazhi= China Journal of Chinese Materia Medica, 28*(7), 661-663.

Tandon, V., & Das, B. (2007). In vitro testing of anthelmintic efficacy of Flemingia vestita (Fabaceae) on carbohydrate metabolism in Rallietina echinobothrida. *Methods, 42*(4), 330-338.

Tandon, V., & Das, B. (2018). Genistein: is the multifarious botanical a natural anthelmintic too? *Journal of Parasitic Diseases, 42*(2), 151-161.

Tandon, V., Das, B., & Saha, N. (2003). Anthelmintic efficacy of Flemingia vestita (Fabaceae): effect of genistein on glycogen metabolism in the cestode, Raillietina echinobothrida. *Parasitology International, 52*(2), 179-183.

Tandon, V., Pal, P., Roy, B., Rao, H., & Reddy, K. (1997). In vitro anthelmintic activity of root-tuber extract of Flemingia vestita, an indigenous plant in Shillong, India. *Parasitology Research, 83*(5), 492-498.

Tang, Z., Zhou, Y., Zeng, Y., Zang, S., He, P., & Fang, Y. (2006). Capillary electrophoresis of the active ingredients of Dioscorea bulbifera L. and its medicinal preparations. *Chromatographia, 63*(11), 617-622.

Tapondjou, L. A., Jenett-Siems, K., Böttger, S., & Melzig, M. F. (2013). Steroidal saponins from the flowers of Dioscorea bulbifera var. sativa. *Phytochemistry, 95*, 341-350.

Tardío, J., & Pardo-de-Santayana, M. (2008). Cultural importance indices: a comparative analysis based on the useful wild plants of Southern Cantabria (Northern Spain). *Economic Botany, 62*(1), 24-39.

Tariq, K. (2018). Use of Plant Anthelmintics as an Alternative Control of Helminthic Infections in Sheep. Res J Zool 1: 1. *of, 2*, 2.

Tava, A., & Avato, P. (2006). Chemical and biological activity of triterpene saponins from Medicago species. *Natural Product Communications, 1*(12), 1934578X0600101217.

Tavassoli, M., Jalilzadeh-Amin, G., Fard, V. R. B., & Esfandiarpour, R. (2018). The in vitro effect of Ferula asafoetida and Allium sativum extracts on Strongylus spp. *Annals of parasitology, 64*(1).

Tavirimirwa, B., Mwembe, R., Ngulube, B., Banana, N., Nyamushamba, G., Ncube, S., & Nkomboni, D. (2013). Communal cattle production in Zimbabwe: A. *Development, 25*, 12.

Tefera, B. N., & Kim, Y.-D. (2019). Ethnobotanical study of medicinal plants in the Hawassa Zuria District, Sidama zone, Southern Ethiopia. *Journal of ethnobiology and ethnomedicine, 15*(1), 1-21.

Teponno, R. B., Tapondjou, A. L., Gatsing, D., Djoukeng, J. D., Abou-Mansour, E., Tabacchi, R., . . . Lontsi, D. (2006). Bafoudiosbulbins A, and B, two anti-salmonellal clerodane diterpenoids from Dioscorea bulbifera L. var sativa. *Phytochemistry, 67*(17), 1957-1963.

Thanabhorn, S., Jaijoy, K., Thamaree, S., Ingkaninan, K., & Panthong, A. (2005). Acute and subacute toxicities of the ethanol extract from the rhizomes of Cyperus rotundus Linn. *Mahidol University J Pharm Sci, 32*, 15-22.

Thankappan, S. S., & Morawala-Patell, V. (2011). In Vitro Propagation Studies and Genetic Fidelity Assessment of Endangered Medicinal Wild Yam-'Dioscorea prazeri'. *Plant Omics, 4*(4), 177-189.

Thompson, D. P., & Geary, T. G. (1995). The structure and function of helminth surfaces *Biochemistry and molecular biology of parasites* (pp. 203-232): Elsevier.

Thooyavan, G., & Karthikeyan, J. (2016). Phytochemical profiling and GC-MS analysis of Butea monosperma seed methanol extract. *Journal of Pharmacognosy and Phytochemistry, 5*(5), 152.

Tiloke, C., Anand, K., Gengan, R. M., & Chuturgoon, A. A. (2018). Moringa oleifera and their phytonanoparticles: Potential antiproliferative agents against cancer. *Biomedicine & Pharmacotherapy, 108*, 457-466.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: a review. *Internationale pharmaceutica sciencia, 1*(1), 98-106.

Tiwari, S., Singh, K., & Shah, P. (2007). In vitro propagation of Oroxylum indicum-An endangered medicinal tree. *Biotechnology, 6*(2), 299-301.

Toet, H., Piedrafita, D. M., & Spithill, T. W. (2014). Liver fluke vaccines in ruminants: strategies, progress and future opportunities. *International journal for parasitology, 44*(12), 915-927.

Togashi, N., Shiraishi, A., Nishizaka, M., Matsuoka, K., Endo, K., Hamashima, H., & Inoue, Y. (2007). Antibacterial activity of long-chain fatty alcohols against Staphylococcus aureus. *Molecules, 12*(2), 139-148.

Tomar, R., & Preet, S. (2017). Evaluation of anthelmintic activity of biologically synthesized silver nanoparticles against the gastrointestinal nematode, Haemonchus contortus. *Journal of Helminthology, 91*(4), 454-461.

Toner, E., Brennan, G., Wells, K., McGeown, J., & Fairweather, I. (2008). Physiological and morphological effects of genistein against the liver fluke, Fasciola hepatica. *Parasitology, 135*(10), 1189-1203.

Torgerson, P. R., & Macpherson, C. N. (2011). The socioeconomic burden of parasitic zoonoses: global trends. *Veterinary parasitology, 182*(1), 79-95.

Torres-Acosta, J., Mendoza-de-Gives, P., Aguilar-Caballero, A., & Cuéllar-Ordaz, J. (2012). Anthelmintic resistance in sheep farms: update of the situation in the American continent. *Veterinary parasitology, 189*(1), 89-96.

Toulah, F. H., Ashoor, S. J., Wakid, M. H., & Alshathly, M. R. (2019). *In vitro* antihelminthic activity of ethanol *Zingiber officinale* extract on *Fasciola gigantica* in comparison to Triclabendazole. *Journal of the Egyptian Society of Parasitology, 49*(3), 599-610.

Trailović, S. M., Marjanović, D. S., Nedeljković Trailović, J., Robertson, A. P., & Martin, R. J. (2015). Interaction of carvacrol with the Ascaris suum nicotinic acetylcholine receptors and gamma-aminobutyric acid receptors, potential mechanism of antinematodal action. *Parasitology research, 114*(8), 3059-3068.

Tripathy, B. N., Panda, S., Sahoo, S., Mishra, S., & Nayak, L. (2011). Phytochemical analysis and hepatoprotective effect of stem bark of Oroxylum indicum (L) Vent. on carbon tetrachloride induced hepatotoxicity in rat. *International Journal of Pharmaceutical & Biological Archives, 2*(6), 1714-1717.

Truong, D.-H., Nguyen, D. H., Ta, N. T. A., Bui, A. V., Do, T. H., & Nguyen, H. C. (2019). Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of Severinia buxifolia. *Journal of food quality, 2019*.

Tshikalange, T. E., Mamba, P., & Adebayo, S. (2016). Antimicrobial, antioxidant and cytotoxicity studies of medicinal plants used in the treatment of sexually transmitted diseases.

Tsotetsi, A., & Mbati, P. (2003). Parasitic helminths of veterinary importance in cattle, sheep and goats on communal farms in the northeastern Free State, South Africa. *Journal of the South African Veterinary Association, 74*(2), 45-48.

Tsoyi, K., Jang, H. J., Lee, Y. S., Kim, Y. M., Kim, H. J., Seo, H. G., . . . Chang, K. C. (2011). (+)-Nootkatone and (+)-valencene from rhizomes of Cyperus rotundus increase survival rates in septic mice due to heme oxygenase-1 induction. *Journal of ethnopharmacology, 137*(3), 1311-1317.

Tumwesigye, W. (2011). Social-economic setting and potential use of cissus adenocaulis in treatment of gastrointestinal worms in cattle, Ntungamo District South Western Uganda.

Uddin, S. J., Grice, D., & Tiralongo, E. (2012). Evaluation of cytotoxic activity of patriscabratine, tetracosane and various flavonoids isolated from the Bangladeshi medicinal plant Acrostichum aureum. *Pharmaceutical Biology, 50*(10), 1276-1280.

Ullah, R., Rehman, A., Zafeer, M. F., Rehman, L., Khan, Y. A., Khan, M. H., . . . Abidi, S. (2017). Anthelmintic potential of thymoquinone and curcumin on Fasciola gigantica. *PloS one, 12*(2), e0171267.

Umavathi, S., Gopinath, K., Manjula, M., Chinnasamy, B., & Ayyakannu, A. (2020). Gloriosa superba L: A critical Review of Recent Advances. *Abasyn Journal of Life Sciences, 3*(2).

Unial, A., Singh, C., Singh, B., Kumar, M., & da Silva, J. A. T. (2011). Ethnomedicinal use of wild plants in Bundelkhand region, Uttar Pradesh, India. *Journal of Medicinal and Aromatic Plant Science and Biotechnology, 5*, 81-86.

Unnisa, A., & Thahera, D. (2011). Anti-inflammatory and acute toxicity studies of the extracts from the rhizomes of Alpinia galanga Willd. *Der Pharmacia Sinica*.

Urban, J., Kokoska, L., Langrova, I., & Matejkova, J. (2008). In vitro anthelmintic effects of medicinal plants used in Czech Republic. *Pharmaceutical biology, 46*(10-11), 808-813.

Usha, N., Sangareshwari, S., & Kumari, L. (2015). Gas chromatography- mass spectrometry analysis of bioactive constituents from the Marine Streptomyces *Asian J Pharm Clin Res, 8*(2), 244-246

Van der Merwe, D., Swan, G., & Botha, C. (2001). Use of ethnoveterinary medicinal plants in cattle by Setswana-speaking people in the Madikwe area of the North West Province of South Africa. *Journal of the South African Veterinary Association, 72*(4), 189-196.

Van Dijk, J., David, G., Baird, G., & Morgan, E. (2008). Back to the future: developing hypotheses on the effects of climate change on ovine parasitic gastroenteritis from historical data. *Veterinary parasitology, 158*(1-2), 73-84.

Vasil'eva, I. S., & Paseshnichenko, V. A. (1996). Steroid glycosides from suspension cultures of Dioscorea deltoidea cells and their biological activity *Saponins used in traditional and modern medicine* (pp. 15-22): Springer.

Vassilev, G. (1994). Prevalence and seasonality of internal parasite infections detectable by faecal examination of cattle in Chiweshe communal farming area of Zimbabwe. *Zimbabwe Veterinary Journal*.

Veerakumari, L., & Lakshmi, K. N. (2006). *In vitro* effect of *Allium sativum* on lactate dehydrogenase activity of *Haemonchus contortus*. *Journal of Veterinary Parasitology, 20*(1), 93-96.

Velık, J., Baliharova, V., Fink-Gremmels, J., Bull, S., Lamka, J., & Skálová, L. (2004). Benzimidazole drugs and modulation of biotransformation enzymes. *Research in veterinary science, 76*(2), 95-108.

Venugopala, K. N., Khedr, M. A., Attimarad, M., Padmashali, B., Kulkarni, R. S., Venugopala, R., & Odhav, B. (2017). Review on chemistry of natural and synthetic indolizines with their chemical and pharmacological properties. *Journal of Basic and Clinical Pharmacy, 8*(2).

Vercruysse, J., Charlier, J., Van Dijk, J., Morgan, E. R., Geary, T., von Samson-Himmelstjerna, G., & Claerebout, E. (2018). Control of helminth ruminant infections by 2030. *Parasitology, 145*(13), 1655-1664.

Vercruysse, J., & Claerebout, E. (2014). Overview of anthelmintics. The Merck Veterinary Manual.

Verma, R., Lata, K., & Das, G. (2018). An overview of anthelmintic resistance in gastrointestinal nematodes of livestock and its management: India perspectives. *International Journal of Chemical Studies, 6*, 1755-1762.

Vikram, P., Mishra, S., Vishwakarma, R., & Shukla, A. K. (2019). Comparative investigation on anthelmintic activities of and Azadirachta indica Areca catechu leaves. *Advance Pharmaceutical Journal, 4*(5), 125-127

Vishwakarma, A., & Kumar, P. (2021). In vivo Anthelmintic Activity of Medicinal plant Asparagus racemosus against Larva of Fasciola gigantica. *Res J Agri Sci, 12*, 675-680.

Vitalini, S., Iriti, M., Puricelli, C., Ciuchi, D., Segale, A., & Fico, G. (2013). Traditional knowledge on medicinal and food plants used in Val San Giacomo (Sondrio, Italy)—An alpine ethnobotanical study. *Journal of ethnopharmacology, 145*(2), 517-529.

Vivek, S., & Prakash, S. (2018). Analysis of nutrient composition and phytochemicals of wild yams Dioscorea pentaphylla L. and Dioscorea oppositifolia Griseb. *Brazilian Journal of Biological Sciences, 5*(10), 427-432.

Vo, N. N. Q., Fukushima, E. O., & Muranaka, T. (2017). Structure and hemolytic activity relationships of triterpenoid saponins and sapogenins. *Journal of natural medicines, 71*(1), 50-58.

Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y., & Gritsanapan, W. (2013). Maximizing total phenolics, total flavonoids contents and antioxidant activity of Moringa oleifera leaf extract by the appropriate extraction method. *Industrial Crops and Products, 44*, 566-571.

Wachtel-Galor, S., & Benzie, I. F. (2011). 1 Herbal Medicine. *Lester Packer, Ph. D.*, 1.

Wagh, V. V., & Jain, A. K. (2014). Ethnomedicinal Uses of Underground Plant Parts in Jhabua District of Madhya Pradesh, India. *Advances in Biological Research, 8*(4), 151-156.

Wagil, M., Białk-Bielińska, A., Puckowski, A., Wychodnik, K., Maszkowska, J., Mulkiewicz, E., . . . Stolte, S. (2015). Toxicity of anthelmintic drugs (fenbendazole and flubendazole) to aquatic organisms. *Environmental Science and Pollution Research, 22*(4), 2566-2573.

Wallace, K., Asemota, H., & Gray, W. (2021). Acetone Extract of Dioscorea alata Inhibits Cell Proliferation in Cancer Cells. *American Journal of Plant Sciences, 12*(03), 300.

Waller, P., Bernes, G., Thamsborg, S., Sukura, A., Richter, S., Ingebrigtsen, K., & Höglund, J. (2001). Plants as de-worming agents of livestock in the Nordic countries: historical perspective, popular beliefs and prospects for the future. *Acta Veterinaria Scandinavica, 42*(1), 31.

Wamburu, R., Kareru, P., Mbaria, J., Njonge, F., Nyaga, G., & Rechab, S. (2013). Acute and sub-acute toxicological evaluation of ethanolic leaves extract of Prosopis juliflora (Fabaceae). *Journal of Natural Sciences Research, 3*(1), 8-15.

Wang, C., Cao, M., Shi, D., Yin, Z., Jia, R., Wang, K., . . . Yang, Z. (2013). A 90-day subchronic toxicity study of neem oil, a Azadirachta indica oil, in mice. *Human & experimental toxicology, 32*(9), 904-913.

Wang, G.-X., Han, J., Zhao, L.-W., Jiang, D.-X., Liu, Y.-T., & Liu, X.-L. (2010). Anthelmintic activity of steroidal saponins from *Paris polyphylla*. *Phytomedicine, 17*(14), 1102-1105.

Wang, G.-X., Zhou, Z., Jiang, D.-X., Han, J., Wang, J.-F., Zhao, L.-W., & Li, J. (2010). In vivo anthelmintic activity of five alkaloids from Macleaya microcarpa (Maxim) Fedde against Dactylogyrus intermedius in Carassius auratus. *Veterinary parasitology, 171*(3-4), 305-313.

Wang, G., Lin, B., Liu, J., & Wang, F. (2009). Chemical constituents from tubers of Dioscorea bulbifera. *Zhongguo Zhong yao za zhi= Zhongguo Zhongyao Zazhi= China Journal of Chinese Materia Medica, 34*(13), 1679-1682.

Wang, G., Liu, J.-S., Lin, B.-B., Wang, G.-K., & Liu, J.-K. (2009). Two new furanoid norditerpenes from Dioscorea bulbifera. *Chemical and Pharmaceutical Bulletin, 57*(6), 625-627.

Wang, J., Ji, L., Liu, H., & Wang, Z. (2010). Study of the hepatotoxicity induced by Dioscorea bulbifera L. rhizome in mice. *Bioscience trends, 4*(2).

Wang, S., Zhang, J., Guo, X., Song, Q., & Zhao, W. (2007). A new eudesmane sesquiterpene lactone from Curcuma wenyujin. *Yao xue xue bao= Acta Pharmaceutica Sinica, 42*(10), 1062-1065.

Wang, Y., Li, J., Guo, J., Wang, Q., Zhu, S., Gao, S., . . . Zhu, W. (2017). Cytotoxic and antitumor effects of curzerene from Curcuma longa. *Planta Medica, 83*(01/02), 23-29.

Wang, Z. M., Tidrick, C. L., Haque, M., & Stuehr, D. J. (2013). Green tea polyphenols decrease enzyme activity of nitric oxide synthase: Wiley Online Library.

Wani, T. A., Kaloo, Z. A., & Dangroo, N. A. (2022). Aconitum heterophyllum Wall. ex Royle: A critically endangered medicinal herb with rich potential for use in medicine. *Journal of Integrative Medicine, 20*(2), 104-113.

Wasswa, P., & Olila, D. (2006). The in-vitro ascaricidal activity of selected indigenous medicinal plants used in ethno veterinary practices in Uganda. *African Journal of Traditional, Complementary and Alternative Medicines, 3*(2), 94-103.

Watt, J. M., & Breyer-Brandwijk, M. G. (1962). The Medicinal and Poisonous Plants of Southern and Eastern Africa being an Account of their Medicinal and other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal. *The Medicinal and Poisonous Plants of Southern and Eastern Africa being an Account of their Medicinal and other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal.*(Edn 2).

Webster, J., Beck, W., & Ternai, B. (1984). Toxicity and bitterness in Australian Dioscorea bulbifera L. and Dioscorea hispida Dennst. from Thailand. *Journal of Agricultural and Food Chemistry, 32*(5), 1087-1090.

Wink, M. (2010). *Annual plant reviews, functions and biotechnology of plant secondary metabolites* (Vol. 39): John Wiley & Sons.

Wink, M. (2013). Evolution of secondary metabolites in legumes (Fabaceae). *South African Journal of Botany, 89*, 164-175.

Wintola, O., & Afolayan, A. (2010). Ethnobotanical survey of plants used for the treatment of constipation within Nkonkobe Municipality of South Africa. *African Journal of Biotechnology, 9*(45), 7767-7770.

Wintola, O. A., & Afolayan, A. J. (2015). An inventory of indigenous plants used as anthelmintics in Amathole district municipality of the Eastern Cape province, South Africa. *African Journal of Traditional, Complementary and Alternative Medicines, 12*(4), 112-121.

Worku, M., Franco, R., & Baldwin, K. (2009). Efficacy of garlic as an anthelmintic in adult Boer goats. *Archives of Biological Sciences, 61*(1), 135-140.

Wunderlich, F., Al-Quraishy, S., Steinbrenner, H., Sies, H., & Dkhil, M. A. (2014). Towards identifying novel anti-Eimeria agents: trace elements, vitamins, and plant-based natural products. *Parasitology Research, 113*(10), 3547-3556.

Wynn, S., & Fougere, B. (2007). *Introduction: Why use herbal medicine.* Paper presented at the Veterinary herbal medicine. Library of Congress cataloging-in publication data.

Xiao, W., Liu, Y., Dai, M., Li, Y., Peng, R., Yu, S., & Liu, H. (2020). Rotenone restrains colon cancer cell viability, motility and epithelial-mesenchymal transition and tumorigenesis in nude mice via the PI3K/AKT pathway. *International Journal of Molecular Medicine, 46*(2), 700-708.

Xiong, Y., & Long, C. (2020). An ethnoveterinary study on medicinal plants used by the Buyi people in Southwest Guizhou, China. *Journal of ethnobiology and ethnomedicine, 16*(1), 1-20.

Yakubu, S., Saleh, U., & Abdullahi, G. (2006). In-vitro anthelmintic efficacy of crude aqueous extracts of neem (Azadirachta indica) leaf, stem and root on nematode. *Animal Research International, 3*(3), 549-552.

Yamson, E. C., Tubalinal, G. A. S., Viloria, V. V., & Mingala, C. N. (2019). Anthelmintic effect of betel nut (Areca catechu) and neem (Azadirachta indica) extract against liver fluke (Fasciola spp.). *Journal of advanced veterinary and animal research, 6*(1), 44.

Yang, G., Zhong, L., Jiang, L., Geng, C., Cao, J., Sun, X., & Ma, Y. (2010). Genotoxic effect of 6-gingerol on human hepatoma G2 cells. *Chemico-Biological Interactions, 185*(1), 12-17.

Yang, W.-C., Yang, C.-Y., Liang, Y.-C., Yang, C.-W., Li, W.-Q., Chung, C.-Y., . . . Liang, C.-L. (2019). Anti-coccidial properties and mechanisms of an edible herb, Bidens pilosa, and its active compounds for coccidiosis. *Scientific reports, 9*(1), 1-11.

Yibar, A., Selcuk, O., & Senlik, B. (2015). Major causes of organ/carcass condemnation and financial loss estimation in animals slaughtered at two abattoirs in Bursa Province, Turkey. *Preventive veterinary medicine, 118*(1), 28-35.

Yogeswari, S., Ramalakshmi, S., Neelavathy, R., & Muthumary, J. (2012). Identification and comparative studies of different volatile fractions from Monochaetia kansensis by GCMS. *Global Journal of Pharmacology, 6*(2), 65-71.

Yu, Z., Liu, X., Mcculloch, M., & Gao, J. (2004). Anticancer effects of various fractions extracted from Dioscorea bulbifera on mice bearing HepA. *Zhongguo Zhong yao za zhi= Zhongguo Zhongyao Zazhi= China Journal of Chinese Materia Medica, 29*(6), 563-567.

Zaheer, S., Hussain, A., Khalil, A., Mansha, M., & Lateef, M. (2019). In vitro anthelmintic activity of ethanolic extracts of Camellia sinensis L. and Albizia lebbeck L. against Haemonchus contortus. *Punjab University Journal of Zoology, 34*(1), 41-45.

Zahoor-ul-Hassan, M. D., Faiza, A., Muhammad, A. A., Shazia, T., Kafeel, A., Muhammad, K. M., . . . Ehsan, E. v. (2012). In vivo and in vitro antihelmintic activity of gemmotherapeutically treated Azadirachta indica (neem) against gastrointestinal nematodes of sheep and earthworms. *African Journal of Pharmacy and Pharmacology, 6*(46), 3171-3179.

Zaman, M. A., Zafar, I., Sindhu, Z.-u.-D., Abbas, R. Z., & Qamar, M. F. (2017). An overview of plants with acaricidal and anthelmintic properties. *International Journal of Agriculture and Biology, 19*(5), 957-968.

Zamani, K., Faghihi, K., Tofighi, T., & Shariatzadeh, M. R. (2004). Synthesis and antimicrobial activity of some pyridyl and naphthyl substituted 1, 2, 4-triazole and 1, 3, 4-thiadiazole derivatives. *Turkish Journal of Chemistry, 28*(1), 95-100.

Zapata Salas, R., González Agudelo, C., Mosquera Cardona, L. N., Usuga Tamayo, I. Y., Polanco Echeverry, D., & Araque Marín, P. (2013). In vitro anthelmintic activity of oily extracts of Azadirachta indica and aqueous extracts of Nicotiana tabacum on gastrointestinal nematodes in goats. *Revista de Medicina Veterinaria*(26), 25-36.

Zayed, M. Z., Wu, A., & Sallam, S. M. (2019). Comparative phytochemical constituents of Leucaena leucocephala (Lam.) leaves, fruits, stem barks, and wood branches grown in Egypt using GC-MS method coupled with multivariate statistical approaches. *BioResources, 14*(1), 996-1013.

Zdolec, N., Vujević, I., Dobranić, V., Juras, M., Grgurević, N., Ardalić, D., & Njari, B. (2012). Prevalence of Cysticercus bovis in slaughtered cattle determined by traditional meat inspection in Croatian abattoir from 2005 to 2010. *Helminthologia, 49*(4), 229-232.

Zhang, D., & Hamauzu, Y. (2004). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. *Food Chemistry, 88*(4), 503-509.

Zhang, H., Birch, J., Pei, J., Mohamed Ahmed, I. A., Yang, H., Dias, G., . . . Bekhit, A. E.-D. (2019). Identification of six phytochemical compounds from Asparagus officinalis L. root cultivars from New Zealand and China using UAE-SPE-UPLC-MS/MS: effects of extracts on H2O2-induced oxidative stress. *Nutrients, 11*(1), 107.

Zhang, Y., Zhang, Y.-J., Jacob, M. R., Li, X.-C., & Yang, C.-R. (2008). Steroidal saponins from the stem of Yucca elephantipes. *Phytochemistry, 69*(1), 264-270.

Zheng, C. J., Yoo, J.-S., Lee, T.-G., Cho, H.-Y., Kim, Y.-H., & Kim, W.-G. (2005). Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. *FEBS letters, 579*(23), 5157-5162.

Zhou, B., Chen, Z., Du, L., Xie, Y., Zhang, Q., & Ye, X. (2011). Allelopathy of root exudates from different resistant eggplants to Verticillium dahliae and the identification of allelochemicals. *African Journal of Biotechnology, 10*(42), 8284-8290.

Zohra, M., & Fawzia, A. (2014). Hemolytic activity of different herbal extracts used in Algeria. *Int J Pharm Sci Research, 5*, 8495-8494.

Zvinorova, P., Halimani, T., Muchadeyi, F., Matika, O., Riggio, V., & Dzama, K. (2016). Prevalence and risk factors of gastrointestinal parasitic infections in goats in low-input low-output farming systems in Zimbabwe. *Small Ruminant Research, 143*, 75-83.

Appendices

Appendix 1: ANOVA table for the effect of N. brachypus tuber accessions (brown, cream, and white), drying methods (sun-drying, air-drying, and oven-drying), extraction methos (Soxhlet and maceration), and solvents (methanol, ethyl acetate, chloroform, and distilled water) on the extract yield (g).

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Extraction Method	1	3.43700357	3.43700357	6018.94	<.0001
Tuber Type	2	2.26899127	1.13449563	1986.75	<.0001
Drying Method	2	0.14299365	0.07149683	125.21	<.0001
Tuber Type*Drying Method	4	1.93250873	0.48312718	846.06	<.0001
Tuber Type*Extraction Method	2	0.11907857	0.05953929	104.27	<.0001
Tuber Type*Solvent	12	1.48743651	0.12395304	217.07	<.0001
Extraction Method*Solvent	6	2.10195476	0.35032579	613.50	<.0001
Solvent	6	8.07318016	1.34553003	2356.31	<.0001
Tuber Type*Dry-Meth*Ext-Meth	4	1.63106429	0.40776607	714.09	<.0001
Tuber Type*Dry-Meth*Solvent	24	5.83318016	0.24304917	425.63	<.0001
Tuber Type*Ext-Meth*Solvent	12	2.02120476	0.16843373	294.96	<.0001
Drying Method*Ext-Me*Solvent	12	2.56649286	0.21387440	374.54	<.0001
Tuber Type*Dry-Meth*Ext-Meth*Solvent	24	2.20256905	0.09177371	160.72	<.0001

[1]Dry-Meth (drying method); Ext-Meth (extraction method)