Discovery of *Cucurbita maxima* compounds as *Schistosoma mansoni* and *Schistosoma haematobium* inhibitors.

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A thesis submitted in fulfilment of the requirements for the award of a Master of Philosophy Degree in Biology

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Declaration

I, Floryn. Mtemeli, assert except where due acknowledgement has been made, that the work entitled “Identification of *Schistosoma mansoni* and *Schistosoma haematobium* inhibitors in *Cucurbita maxima*” submitted to CUT is entirely my work, done under the principal supervision of Dr R. Shoko. The information detailed herein has never been submitted to any other institute, in whole or in part, for an award of any certificate, diploma or degree. Any editorial work, paid or unpaid, fulfilled by a third party is duly acknowledged at its point of use. A full list of the references used has also been included.

Thesis Approval

I certify that Floryn Lynorah Mtemeli was under my supervisory guidance during the entire period of her studies. I further certify that she has efficiently fulfilled all academic requirements set before her. In my judgement, her thesis entitled Identification of *Schistosoma mansoni* and *Schistosoma haematobium* Inhibitors in *Cucurbita maxima* is of adequately high quality to meet the standards of the Master of Philosophy degree awarded by Chinhoyi University of Technology (CUT).

Approved by: Main Supervisor: Dr R.Shoko

Signature: 

Date: 26 July 2023

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Table of Abbreviations

ADMET	Absorption, distribution, metabolism, elimination, and toxicity
ANOVA	Analysis of VARIANCE
ARRIVE	Animal Research: Reporting of In Vivo Experiments
CADD	Computer-aided drug design
CUT	Chinhoyi University of Technology
DALY	Disability-adjusted life years
LBDD	Ligand-based drug design
MMV	Medicines for Malaria Venture
MOHCC	Ministry of Health and Child Care
NAD	Nicotinamide Adenine Dinucleotide
NP	Natural products
NTD	Neglected tropical disease
PDB	Protein Data Bank
PNP	Purine nucleoside phosphorylase
PZQ	Praziquantel
QSAR	Quantitative structure-activity relationships
RDT	Rapid diagnostic tests
RMSD	Root mean square deviation
ROF	Rule of Five
SBDD	Structure-based drug design
SBVS	Structure-based virtual screening
SmPNP	Schistosoma mansoni purine nucleoside phosphorylase
VS	Virtual screening
WHO	World Health Organisation

Abstract

Over 200 million people worldwide suffer from schistosomiasis, a disease usually associated with poverty and poor sanitation. A single drug, praziquantel (PZQ), has been used by the medical community since the 1970s. The emerging evidence of resistance of the Schistosoma parasite to praziquantel, and the drug's ineffectiveness against juvenile stages of the parasite, calls for alternative drug. The exploration of the inhibition potential of compounds from *Cucurbita maxima* using molecular docking studies on *Schistosoma mansoni* purine nucleoside phosphorylase (SmPNP) and *Schistosoma haematobium* 28-kDa glutathione S-transferase (Sh28kDaGST) was done. Following molecular docking studies and analysis of the active sites, the primary amino acids that were observed and shown to be involved in the *Sm*PNP-ligand interaction are CYS 33, ARG 86, HIS 88, TYR 90, ALA 118, ALA 119, PRO 200, TYR 202, GLU 203, VAL 219, MET 221, THR 244, ASN 245, PRO 257 and HIS 259. For the *Sh*28dKa-ligand interaction, the primary amino acids were PHE 11, ARG 16, TRP 41, LEU 53, GLU 70 and SER 71. Momordicoside I aglycone binds to *Sm*PNP HIS88 with the lowest binding energy of -7.9kcal/mol. Balsaminoside B binds to *Sh*28kDaGST with a binding energy of −7.6kcal/mol by hydrogen bond interaction with TRP 41, LEU 53 and SER 71. The anti-schistosomal activity of *C. maxima* seeds extract alone and in combination with PZQ on *Schistosoma mansoni*-infected mice and were also done. The parasites were sampled from the Mupfure River in the Mashonaland East province of Zimbabwe from three different village sites namely Chikanza, Matimure and Mwedziwandira. Infection of guinea pigs with *Schistosoma haematobium* was attempted. Unfortunately, the infection in the guinea pigs was unsuccessful. Results indicate a statistically significant reduction in granuloma size in the *C. maxima* extract +praziquantel treated group, with a 13,24% margin compared to the *C. maxima* seeds extracts treated group. Treatment by *C. maxima* seeds extract and *C. maxima* seeds extract + PZQ significantly reduced the egg count in intestines and live ($p=0.00$; $p=0.00$ & $p=0.00$), respectively. There was no significant difference in the effect of the village sites from where the parasites were sampled ($p>0.05$). The anti-schistosomal effect of *C. maxima* extracts and their synergistic effects when in combination with praziquantel were observed. In addition to providing a predictive model for interactions between *C. maxima* ligands, *Sm*PNP and *Sh*28kDaGST, the *in vivo* work validated the *in silco* findings and confirmed that *C. maxima* does have antischistosomal properties. We propose that as future work, *in vitro* studies on the target proteins be carried out to elucidate the mechanism of action of the *C. maxima* plant. We also recommend performing selectivity tests of the best-performing compounds on the target proteins. Overall, our study lays a crucial foundation in the development of a new drug against schistosomiasis and we recommend that *C. maxima* seeds and their combination with praziquantel be considered as candidates for the development of a new drug against schistosomiasis.

Key words: Schistosomiasis, *Cucurbita maxima*, anti-schistosomal, praziquantel, *Schistosoma mansoni*.

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Chapter 1

General Introduction

1.1 Introduction

There are over 240 million people affected by schistosomiasis globally and the disease leads to around 200 000 mortalities yearly, with 95% of cases occurring in southern Africa (World Health Organisation (WHO, 2023). By long-term infection with the parasite, most of these deaths are caused by bladder cancer and liver fibrosis. This disease, also known as bilharzia, is caused by parasitic infection with blood flukes of the genus *Schistosoma*. Although most cases occur in sub-Saharan Africa, the disease also occurs in South America, the Caribbean, the Middle East, and Asia (Cando *et al.*, 2022). In addition to the direct impact on mortality, schistosomiasis also contributes to morbidity through its impact on overall health and well-being, including the disruption of daily activities, decreased quality of life, and reduced economic productivity (WHO, 2022). According to a study by (Klohe *et al.*, 2021), the morbidity rate caused by schistosomiasis is estimated to be over 1.64 million disability-adjusted life years (DALYs) globally. The study found that schistosomiasis was responsible for a significant disease burden, particularly among children and young adults, who are most vulnerable to infection and affected by the disease's negative impact on their physical and cognitive development.

Schistosoma mansoni and *Schistosoma haematobium* are the most prevalent species of parasite, accounting for approximately 90% of all infections (Mazigo, 2019). *S. mansoni* is responsible for intestinal schistosomiasis, which primarily affects the liver and the intestines, while *S. haematobium* is responsible for urinary schistosomiasis, which primarily affects the bladder and the kidneys (Rugel *et al.*, 2020). This disease can cause cognitive, physical, and child development impairments, as well as anaemia, hepatosplenomegaly, and neurological complications that could lead to death. Schistosomiasis has also been associated with anaemia, impaired development, and malnutrition among children (El-Faham *et al.*, 2017; Oettle *et al.*, 2017).

1.2 Lifecycle of the *Schistosoma* parasite

According to Siqueira *et al.* (2017), Schistosomes have complex life cycles that involve vertebrate hosts (usually mammals) and invertebrate hosts (freshwater snails). Humans are normally infected through hypodermal penetration of cercariae when they come into contact with contaminated freshwater. (De Neve *et al.*, 2018). The lifecycle of the parasite continues when humans excrete urine/faeces with schistosome eggs into water bodies where they hatch into miracidia as explained in the diagram below.

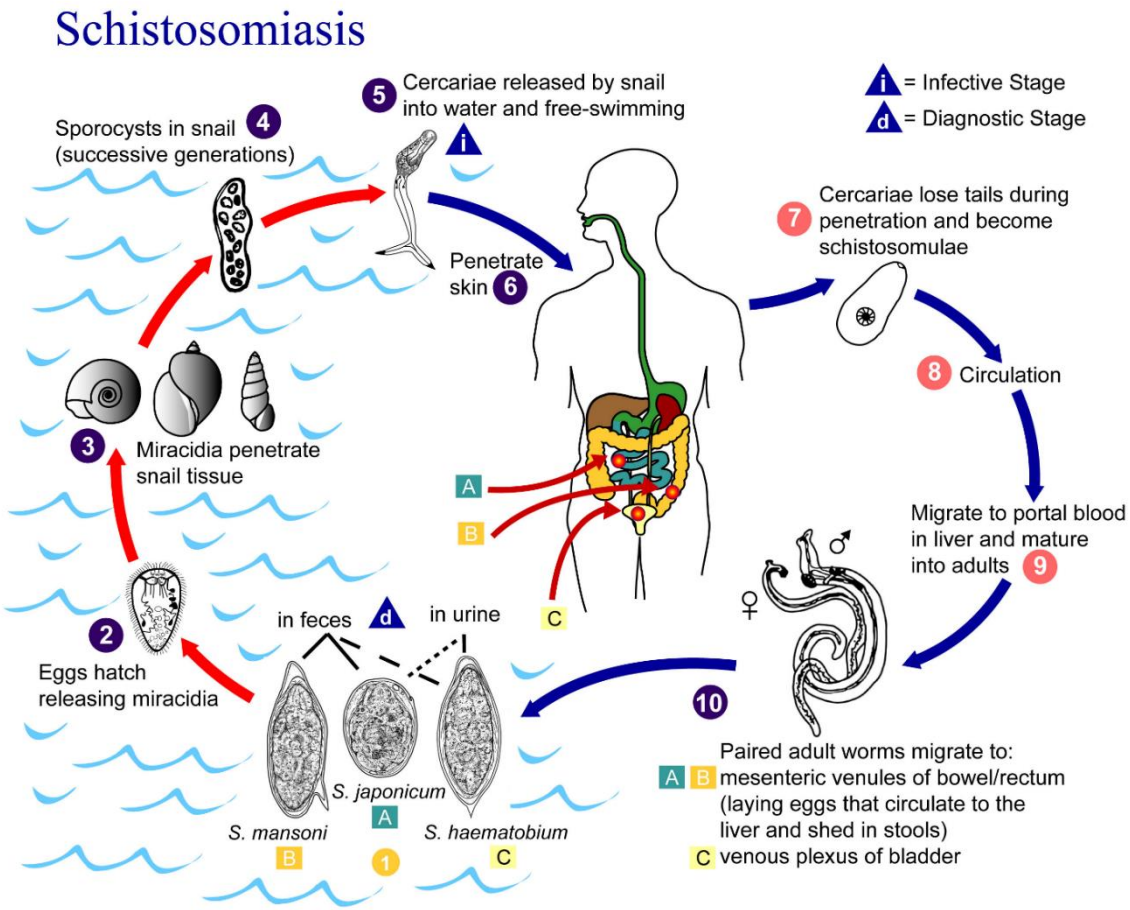

Fig 1: shows the lifecycle of the Schistosoma parasite from the infective stage (cercariae) to the diagnostic stage (eggs released in the urine of faeces) (Hane *et al.*, 2014).

1.3 Prevalence of schistosomiasis in Sub-Saharan Africa

In countries like Zimbabwe, Malawi, and Mozambique, the disease is considered to be one of the most neglected tropical diseases and a major public health concern (WHO, 2020). In Malawi, studies have shown that the disease is most prevalent in the southern regions of the country, where the majority of the population relies on freshwater sources for their daily

activities (Kamwendo *et al*., 2018). In Mozambique, the infection is widespread in the country's northern regions, where the prevalence can reach up to 70% (Secor *et al*., 2009). According to a study conducted by the WHO in 2022, Zimbabwe is among the countries with a high burden of schistosomiasis. The study found that the prevalence of schistosomiasis in Zimbabwe ranges from 20% to 50% in rural areas and up to 10% in urban areas. Another study conducted by the Ministry of Health and Child Care (MOHCC) in Zimbabwe in 2018 found that the prevalence of schistosomiasis was 27.7% among school-aged children in rural areas (Ministry of Health and Child Care, 2018).

Several factors contribute to the high prevalence of schistosomiasis in Zimbabwe, including poverty, inadequate access to safe water and sanitation, and the absence of effective control measures (Chimbari, 2018). The disease is commonly spread in areas with poor water and sanitation facilities, where people are forced to use contaminated water sources for domestic and agricultural purposes (Gomo *et al*., 2003). Additionally, the absence of effective control measures, such as the improvement of water and sanitation facilities, has contributed to the spread of the disease. Zimbabwe's health and well-being are negatively affected by schistosomiasis. It can lead to a range of health problems, including anaemia, malnutrition, and stunted growth in children. It can also increase the risk of bladder cancer and other complications (Chimbari, 2018). The disease can also significantly impact communities' economic development, as it can lead to decreased productivity and decreased school attendance among infected individuals. The mortality rate caused by schistosomiasis in Zimbabwe is difficult to determine as the disease is often asymptomatic and can go undiagnosed for long periods and many cases are not recorded or reported (WHO, 2020). However, it is estimated that the disease contributes to a significant number of deaths in the country each year.

Zimbabwe has taken some steps to control and prevent the spread of the disease despite its high burden (History, 2016). MOHCC launched a national program in 2016 to reduce the schistosomiasis burden in the country. This program provides regular treatment to school-aged children, improves access to safe water and sanitation, and raises awareness about the disease. Additionally, the MOHCC has established a national task force to oversee the implementation of the program and monitor its progress (MOHCC, 2018).

1.4 Praziquantel and its mode of action

Praziquantel is an isoquinoline-pyrazine derivative and is effective against all species of *schistosomes* infecting humans and has been the drug of choice for the last decades dating from 1970 (Fallon *et al*, 1996; LoVerde *et al.*, 2021).). It is well-tolerated, easily administered in tablet form, and affordable. PZQ chemotherapy appears to act primarily on the structure of the tegument of the worms and thus generates an influx of Ca^{2+} ions, which leads to muscular contraction (Dupré *et al*, 1999). This leads to alteration of the schistosome calcium transport channels in the schistosome adult worm tegument, increasing cellular ion permeability, and thus inducing spastic muscular paralysis (da Silva *et al.*, 2017). Schistosome teguments undergo morphological changes that expose parasite antigens to greater amounts, resulting in stronger schistosome-specific antibodies. Due to this damage to the tegument, the host's immune system attacks the worms, leading to their death (Mutapi *et al.*, 2017). However, despite this success, the list of its side effects and the outlook of relying on a single drug to treat millions of people is of concern. The major intrinsic downside of the use of the drug praziquantel includes the potential for the emergence of drug-resistant *schistosome s*trains and the fact that cured individuals are fully susceptible to a new infection (Eissa *et al.*, 2020).

The first account of possible PZQ resistance was observed after an exhaustive study in northern Senegal, where the drug was producing poor treatment rates (18–39%) and snails in the area were carrying *Schistosoma* strains. After they had been tested in the laboratory, these snails had reduced susceptibility to PZQ. Additional PZQ-resistant evidence was collected in Egypt (Ismail *et al*, 1999; Padraic *et al*, 2009& Bergquist *et al.*, 2017). Another rising public health issue, parasite hybridization, has the potential to have a substantial global impact on parasite development and epidemiology and provides a variety of control issues for parasitic diseases (Borlase *et al.*, 2018). Human populations were observed in Senegal and increased hepatic but decreased urogenital morbidity was noted together with reduced improvement following treatment with praziquantel, in those infected with zoonotic hybrids compared to non-hybrids (Fall *et al.*, 2021). A case study of the *S. haematobium* group of zoonotic hybrid *schistosomes* in West Africa offered a singular chance for empirical research into the potential effects of hybridization and introgressions in a multi-host system which is important for human and veterinary health (Borlase *et al.*, 2021). Therefore, existing intervention models should be reconsidered for their convenience in achieving a compelling approach to reducing disease transmission. (Gurarie and King, 2014).

1.5 Progress made on the discovery of new drugs

Over the past few decades, there has been significant interest in the development of new drugs against schistosomiasis (Mtemeli *et al.*, 2022). One of the key areas of research has been the development of new anti-schistosomal compounds (Sorokina & Steinbeck, 2020). In 2000, the WHO launched the Global Plan to Combat schistosomiasis, which aimed to reduce the disease burden by 50% by 2020. Several new anti-schistosomal drugs have been studied and tested in clinical trials in this context. One of the tested drugs is tribendimidine. This compound is highly effective against *Schistosoma mansoni*, one of the major causative agents of schistosomiasis (Li *et al*., 2017). Tribendimidine acts by blocking the absorption of glucose by the parasite, thereby leading to its death. Clinical trials of tribendimidine have demonstrated its efficacy and safety, with a cure rate of over 90% (Li *et al*., 2017). Another new drug that has been studied is oxamniquine. Oxamniquine is a modified form of PZQ and is highly effective against *S mansoni* (Guevara *et al*., 2020). In a clinical trial conducted in sub-Saharan Africa, oxamniquine was shown to have a cure rate of over 90% (Guevara *et al*., 2020). It is however, crucial to develop drugs that target all the species of the parasite and in this light, new derivatives of PZQ are being studied with the hope that they will be more potent with a broader spectrum of activity against the parasite (Al-Mekhlafi *et al*., 2018).

In addition to the vast research on new anti-schistosomal drug development, there has also been a significant advancement in the development of new tools for diagnosing and monitoring schistosomiasis. For example, new rapid diagnostic tests (RDTs) have been developed that can quickly and accurately detect the presence of the parasite in patients (Al-Mekhlafi *et al*., 2018). These RDTs are simple to use, do not require laboratory facilities, and can provide results in less than 15 minutes (Al-Mekhlafi *et al*., 2018). Regardless of the strides that have been made, none of the new drugs has reached the market yet.

1.6 Natural products as anti-schistosomal agents

Natural products have been used to treat schistosomiasis for centuries. Plant-derived compounds such as artemisinin (ART) and berberine (BBR) have shown promise in reducing worm burdens and egg counts and reducing symptoms of the disease (Zhou *et al*., 2020). In addition to ART and BBR, other natural products such as compounds derived from the plants *Alchornea cordifolia*, *Melia azedarach*, and *Casimiroa edulis* have also been investigated for their potential use in treating schistosomiasis. (Obasi *et al*., 2016; Kone *et al*., 2018 & Mbonye *et al*., 2019).

Cucurbita plants, commonly known as pumpkins belong to the family *Cucurbitaceae* family. In recent years, there has been a growing interest in the probable use of *Cucurbita* plants as anthelminthic agents. For example, a study by (Abdel Aziz *et al*., 2018) showed that an ethanolic extract of *C. maxima* seeds had significant anthelmintic activity against the parasitic worm *Ascaridia galli*. Similarly, another study showed that the extracts of *C. moschata* seeds had significant anthelmintic activity against the parasitic worm *Fasciola gigantica* (Efektifitas *et al*., 2017). However, data on the anti-schistosomal properties of the *C. maxima* plant remains scarce.

Problem Statement

Praziquantel has been the drug of choice against schistosomiasis since 1970. There has been laboratory-based evidence showing that schistosomes are becoming less susceptible to PZQ. Furthermore, zoonotic hybridization events such as those involving *S. haematobium* and *S. bovis* strains in West Africa pose a threat to human health.

Justification

The reliance of the medical sector on a single drug (PZQ) poses a threat to health of many as selective pressure could lead to schistosomes gaining resistance to the drug.

Aim

This study was aimed at exploring the inhibition potential of compounds from *C. maxima* on *S. mansoni* purine nucleoside phosphorylase (*Sm*PNP) and *S. haematobium* 28-kDa glutathione S-transferase (*Sh*28kDaGST) using molecular docking studies. *In vivo,* studies were also performed to assess the anti-schistosomal activity of *C. maxima* seed extracts on *S. mansoni* using murine models. The study also aimed at assessing the *in vivo* anti-schistosomal potential of *C. maxima* extracts in combination with praziquantel.

1.7 Objectives

- To determine the inhibition potential of compounds from *C. maxima* on *S. mansoni* purine nucleoside phosphorylase (*Sm*PNP) and *S. haematobium* 28-kDa glutathione S-transferase (*Sh*28kDaGST) using molecular docking studies.
- To assess the anti-schistosomal activity of *C. maxima* seed extracts on *S. mansoni* and *S. haematobium* using murine models *in vivo.*
- To assess the synergistic anti-schistosomal activity of *C. maxima* seed extracts and praziquantel on *S. mansoni* and *S. haematobium* using murine models *in vivo.*

1.8 Thesis structure

This thesis's main part was determining the anti-schistosomal activity of *C. maxima* on *S. mansoni* and *S. haematobium in silico* and *in vivo*. Chapter 1 outlines the prevalence of schistosomiasis and its debilitating effects, making the discovery of new drugs necessary. Chapter 2 explores the advances made so far in the discovery of new drugs against schistosomiasis based on natural products. Chapters 3 and 4 aim to determine the anti-schistosomal activity of *C. maxima* against *S. mansoni* and *S. haematobium*. In Chapter 3, we perform an exploration of *C. maxima* compounds as potential therapeutics against *S. mansoni* purine nucleoside phosphorylase and *S. haematobium* 28Kda glutathione S-transferase using molecular docking studies. Chapter 4 aims to assess the anti-schistosomal activity of crude *C. maxima* seed extracts on *S. mansoni in vivo*. The chapter also aims to assess the activity of the combination of crude extracts and PZQ in comparison to the extracts alone. Bulb C mice are used as models in this chapter. Chapter 5 discusses the main findings of our work. We hypothesise the mechanism of synergistic action of the *C. maxima* seeds with praziquantel. A summary of our findings is presented in Chapter 6 as well as recommendations for future work.

The flow chart below describes the sequence in which the study was carried out.

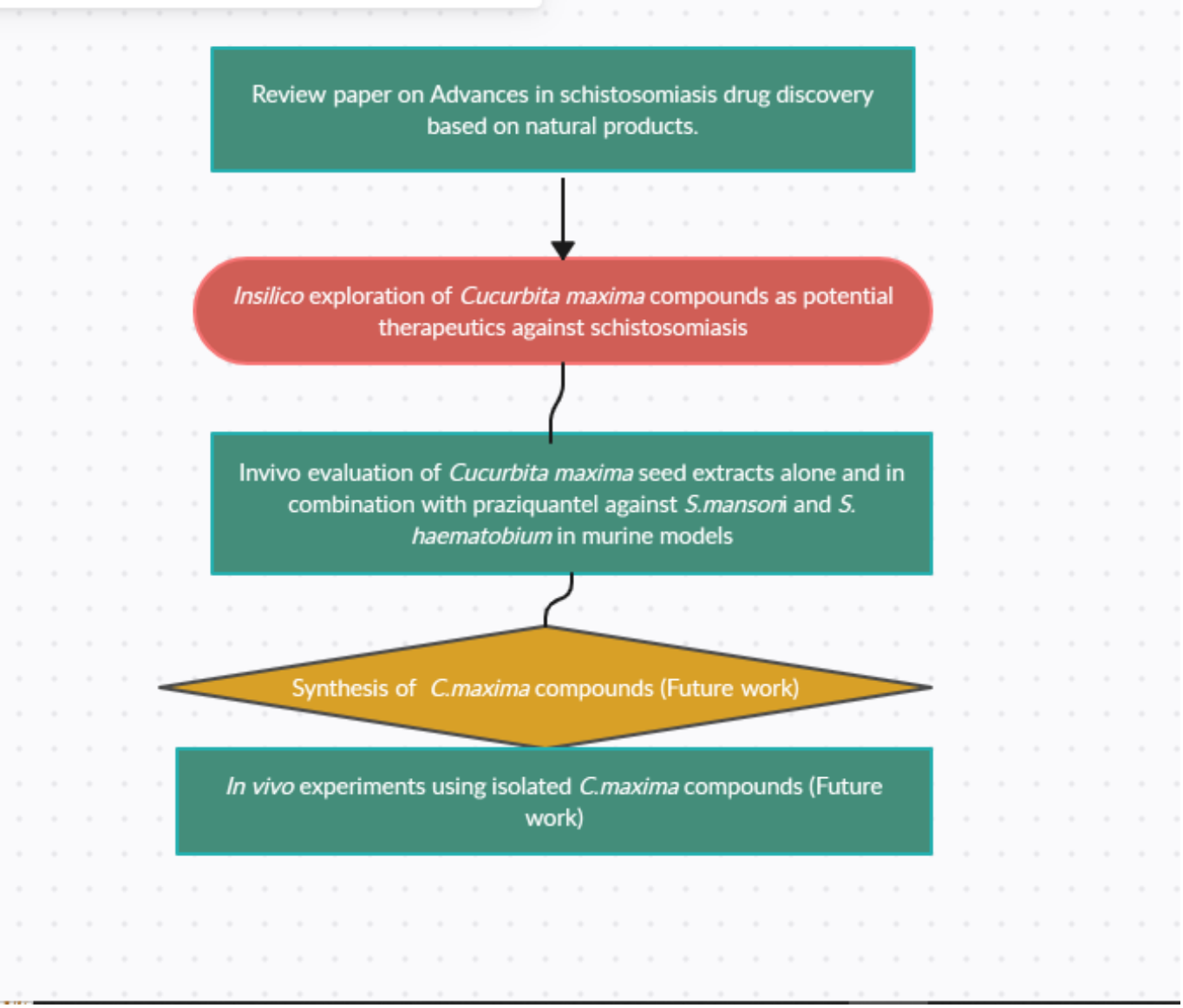

Fig2: shows the outline and the sequence in which the study was carried out.

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Chapter 2

Chapter 2

A Review of the Advances Made in the Discovery of Drugs for Schistosomiasis Based on Natural Products

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Abstract

Schistosomiasis is a neglected tropical disease affecting over 250 million people worldwide. The disease is the second most prevalent neglected tropical disease after malaria. Treatment of schistosomiasis relies on the administration of praziquantel (also known as biltricide). Reliance on a single drug poses a threat to the public health system as the parasite may become resistant as shown by some laboratory findings. The possibility of the resistance rising to clinically significant levels has motivated the scientific community to search for new drug nominees. For a long time, natural products have always been a foundation for the identification of drug leads in the pharmaceutical industry. This paper reviews the progress made in the discovery of natural anti-schistosomal agents in the field of drug discovery. We focus mainly on natural products that have been tested on the schistosome parasite and exhibited potency. We also highlight applications of advanced techniques in drug discovery with a major focus on computer-aided drug discovery methods. Specifically, we discuss structure-based drug discovery and ligand-based drug design approaches with an emphasis on virtual screening.

Keywords

schistosomiasis, praziquantel, schistosomicidal, virtual screening, natural products

Introduction

Schistosomiasis is a water-borne parasitic neglected tropical disease (NTD) and is endemic in sub-Saharan Africa's poverty-stricken parts. Of the world's estimated 240 million cases of the disease in 2020, 90% of the cases were recorded in sub-Saharan Africa (Aula *et al*., 2021; Abe *et al*., 2020; Fuss *et al*., 2020). The distribution of the disease is influenced by the low-income status of the regions characterised by poor sanitation, lack of effective health policies and generally poor living conditions (Onasanya *et al*., 2021). Africa, generally, depends on herbal medicines with over 80% of the population, particularly in rural areas, depending on herbal medicines (Cam *et al*., 2005). It is, therefore, important to understand how these communities use traditional herbal medicines given the prevalence of schistosomiasis.

Schistosomiasis is caused by six species of schistosomes; *Schistosoma mansoni, Schistosoma intercalatum, Schistosoma japonicum, Schistosoma mekongi Schistosoma guineensis* and *Schistosoma haematobium. S. haematobium* causes urogenital schistosomiasis whereas the other species cause intestinal schistosomiasis. Of the species, *S. mansoni* and *S. haematobium* are the most common species in Africa (Oyeyemi *et al*., 2020). Cercariae are subsequently shed by the vector snails into freshwater and penetrate the intact skin of humans who get in contact with contaminated water (Mbereko *et al*., 2020; Caffrey *et al*., 2019). After penetrating the human skin, the maturing larvae become adults that produce eggs in about 5-7 weeks. The eggs produced are usually released into the environment through faeces or urine to complete the lifecycle. However, in some cases, the eggs remain in host tissues where they induce inflammation and then die (Neves *et al*., 2015).

Over the past few years, natural products (NPs) and compounds derived from NPs are gaining popularity as a foundation for the development of new schistosomiasis drugs (Ndegwa *et al*., 2022). The efficiency of novel compounds against schistosomes is well-defined using various approaches like prophylactic strategies, killing the adult parasite, the cercariae and schistosomula and suppressive tactics such as hindering worm egg-laying (Tekwu *et al*., 2017). Regardless of all the studies that are underway, in the absence of a vaccine, the treatment of schistosomiasis greatly depends on PZQ, which has been the drug of choice since the 1970s (Gönnert and Andrews, 1977). The other two drugs oxamniquine and metrifonate ceased to be used clinically because of various reasons such as the toxicity of metrifonate to humans and the selective effectiveness of oxamniquine on *S. mansoni* only (Cheuka *et al*., 2017).

The primary mechanism of PZQ remains unknown. However, the drug acts on the tegument of mature worms only. It is hypothesised that PZQ interrupts calcium ion homeostasis in the parasite which results in an uncontrolled influx of calcium ions causing muscle contraction and paralysis (Thomas and Timson, 2020). The drug's easy administration, safety, tolerance, affordability and effectiveness against all five species of schistosomes have made its use advantageous (Chisango *et al*., 2019). The disadvantage of PZQ is that if a patient harbours parasites at various life stages, the patient will still show symptoms of the disease regardless of treatment (Caffrey *et al.*, 2019). The reliance by the medical sector on a single drug for the treatment of schistosomiasis and the failure of existing measures to eliminate the disease has led to intensified efforts to find new anti-schistosomiasis drug leads (Mtemeli *et al*., 2021; Lombardo *et al*., 2019; Gouveia *et al*., 2018). Here, we review the progress that has been made in the development of new drugs used for treating schistosomiasis based on NPs.

The Schistosomiasis Drug Discovery Journey

Drugs have assumed critical roles in preventing and treating several diseases including schistosomiasis. The historical framework of the use of drugs in treating infections and alleviating symptoms goes back to ancient times (Lesser, 2021). At present, traditional target-based and phenotype-based screening assays are frequently employed in drug discovery; predominantly for NTDs (Moreira-Filho *et al*., 2021).

The conventional process used to discover and develop an effective drug is expensive and time-consuming. The process usually consists of six main steps *viz*; disease selections, target hypothesis, lead identification, lead optimisation, preclinical trial and clinical trial (Kumar, 2017). These steps were devised to ensure that drugs delivered to patients are safe and efficacious.

NPs have attracted interest in therapeutic use for a long time. 64% of all marketed drugs originate from NPs (Ferreira *et al.*, 2018). Some of the NP-derived drugs that are trademark of today's pharmaceutical care include quinine, theophylline, penicillin G, morphine, paclitaxel, digoxin, vincristine, doxorubicin and cyclosporine *etc* (Cragg and Newman, 2013; Shelar and Shirote, 2011).

NPs are considered to be a significant foundation for drug discovery because they have diverse chemical components and biomedical activities (Süntar, 2020). Also, NPs are exceptional in

that they are often rich in stereogenic centres and cover portions of chemical space that are usually not occupied by a greater part of synthetic drugs and medications (Marxer *et al*., 2012).

The investigation of medicinal plants as an innovative strategy for the tentative control of schistosomiasis is one of the sustainable and encouraging research leads. Since schistosomiasis is one of the NTDs, the drug discovery channel is, by all means, underfunded (Lombardo *et al*., 2019). However, the academic research side has explored a great number of bioactive ingredients from plants with schistosomicidal properties; specifically, those used in traditional herbal medicine (Bergquist *et al*., 2017; Tung, 2014). Some of the plants with anti-schistosomal activities that have been studied are discussed below.

Zingiber officinale

The anti-schistosomal activity of *Z. officinale* has been evaluated against *S. mansoni* and the potency of the plant has been demonstrated (Mostafa and Eid, 2011; Sanderson *et al*., 2002). Successful *in vitro* studies of the anti-schistosomal activity of *Z. officinale* were done although the researchers observed no significant difference in treated and untreated mice for the *in vivo* experiments (Sanderson *et al*., 2002). Other studies revealed anti-schistosomal effects of *Z. officinale* with regard to the histopathological changes observed in the small intestines of infected mice (El-Sameih *et al*., 2017). A dramatic decrease in the number of granulomas and retracted granulomal development resulting in the majority of eggs being trapped within intestinal compartments not inducing an inflammatory response was observed (Aly and Mantawy, 2013). Additionally, *Z. officinale* also facilitated the restoration of the appearance of normal hepatocytes and normal hepatic strand organisation by scavenging free radicals with its strong antioxidant effect (Abd El Wahab *et al*., 2021). The treatment of *S. mansoni*-infected mice with aqueous ginger extract loaded on chitosan nanoparticles led to an apparent decrease in elevated liver peroxidation and improved liver function that reflects the antioxidant defence system (El-derbawy and Kholy, 2019). At a maximum nontoxic dose of the extract, the cytotoxicity assay showed that treated Vero cells did not display any morphological differences when compared to the control, at the value of 250 μl/ml (El-Nour *et al*., 2021). Another study observed the significant synergistic effect of ginger-derived nanoparticles when used in combination with PZQ or mefloquine (Abd El Wahab *et al*., 2021).

Anonidium mannii

The presence of alkaloids, phenols, polyphenols, saponins, tannins and steroids in *A. manni* has been demonstrated (Ngangoue *et al*., 2020). Two compounds from the plant, aristolactam A-II and piperolactam D, had good activity (IC_{50} of 10–20 μM) on adult *S. mansoni in vitro* and demonstrated effective inhibition of the recently discovered, Nicotinamide Adenine Dinucleotide catabolizing enzyme in *S. mansoni* (*Sm*NACE) (Toussi Matchi *et al*., 2020). Schistosomes are unable to produce Nicotinamide Adenine Dinucleotide (NAD) - a vital cellular metabolite. The parasites salvage NAD by using vitamins from the host. Interruption of the parasite's NAD salvage pathway adversely impacts both the mature and immature worms' metabolism, reproduction and survival (Schultz *et al*., 2020). Cytotoxicity tests were performed on Huh7 and A549 cells at the concentration of 100 μM and the cell viability of Huh7 cells was found to be ≈15% and 80% for A549 cells (Toussi Matchi *et al*., 2020).

Rauwolfia vomitoria

Ethanolic crude extracts of *R. vomitoria* stem bark and root have been shown to have moderate anti-schistosomal properties against the cercariae and adult worms of *S. mansoni*. In a study by Tekwu *et al.* (2017), the stem bark and root displayed half-maximal inhibitory concentration (IC_{50}) values of 77.5 μg/mL and 112 μg/mL, respectively. Their results suggested that the stem bark is comparatively more toxic than the roots. Using this criterion, all the IC_{50} values that were defined in their study were much greater than 20 μg/mL. This indicates that both plant parts are not intensely cytotoxic and can be used in the treatment of schistosomiasis. HepG2 and Chang liver cells were assessed using the3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay and the extracts were found to be safe at $IC_{50} > 20$ μg/mL (Tekwu *et al*., 2017).

Cucurbita pepo

Studies have shown that *Cucurbita pepo* seed oil is effective against *S. mansoni* schistosomula, in juveniles and adults *in vitro* (Ammar *et al*., 2020; Beshay *et al*., 2018). The mortality rate of the worms, the effect on motility, morphological integument changes and *C. pepo* seed oil-induced DNA instability are indications that the seed oil is a promising anti-schistosomal product (Ammar *et al*., 2020). In older studies, the administration of seed oil to treat *S. mansoni*-infected mice led to significant reductions in liver and intestinal egg burden, with a noteworthy increase in the percentage of dead eggs in the oogram pattern. The seed oil also led to an improved granuloma size, structure and fibrosis (Beshay *et al*., 2018).

Pulsatilla chinensis

It has been shown that Hederacolchiside A1 (HSA) found in the plant *P. chinensis* has anti-schistosomal properties (Kang *et al*., 2018). In tests done on mice infected with *S. japonicum*, female worm recovery and egg load in treated mice were reduced. The schistosomicidal activity of HSA against both juvenile and adult *S. japonicum* with a dose-response relationship was observed (Kang *et al*., 2018).

Artemisia annua

Since the 1980s, artemisinins, a family of sesquiterpene trioxane lactones, *A. annua* (sweet wormwood) derivatives, are anti-schistosomal agents (Neves *et al*., 2015). Artemisinins appear to be more effective on juvenile worms than adult worms (Liu *et al*., 2014). This may be due to artemisinins' mechanism of action on the schistosomes as the artemisinins react with the iron ions of haeme. The iron ions of haeme, are derived from haemoglobin digestion. Reactive oxygen species and chelates are produced in the process (Xiao *et al*., 2001). Several clinical studies have commenced, repositioning artemisinins as schistosomicidal drugs (Panic *et al*., 2014; Liu *et al*., 2014).

In addition to the few studies discussed above, many other studies have been carried out on plants with schistosomicidal properties. Table 1 presents a summary of other plants that have shown potency against schistosomes.

Table 1: *In vivo* studies of selected traditionally employed plant species against *S. mansoni*

Plant species	**Part used**	**Lethal doses**	**Model (*In vitro/in vivo*)**	**Main observations,**	**References**
***Abrus precatorius* L subsp. AfricanusVerdc**	Stem Root	LC_{50} = 1.50.6 mg/mL (T=1h)	*In vitro*	Active concentration of the roots ranged from 0.6 mg/ml to 33.8 mg/ml and	(Mølgaard *et al*., 2001)

				1.5 mg/ml for the stem on *S mansoni*	
Anonidium mannii	Root	IC_{50} =10–20 µM *Sm*NAD^+ catabolizing enzyme (T=6h)	*In vitro*	Compounds from the plant *A.manni* namely Aristolactam A-II and piperolactam D had good activity on adult *S. mansoni* and its NAD^+ catabolizing enzyme respectively	(Toussi *et al*., 2020)
Artemisia annua ***Artemisia afra***	Leaves	33,5-55.9 mg 1.26mg (T=7d)	*In vivo* (clinical trial)	96.4% cure rate for *A. annua* and 88.8% cure rate for *A. afra* with average *S. mansoni* parasite clearance of 1/day	(Panic *et al*., 2014; Liu *et al*., 2014; Munyangi *etal*., 2018; Gruessner *et al*., 2019)
Berkheya speciosa **(DC.)** **O. Hoffm**	Aqueous plant extracts	LC_{50}>6.25 mg/mL (T=1h)	*In vitro*	Crude plant extracts were lethal against the schistosomules of both *S. mansoni* and *S. haematobium*	(Sparg *et al*., 2000)

Cucurbita pepo	Seed oil	100% mortality at 10 µg/mL (T=24h)	*In vitro* *In vivo*	All stages of *S. mansoni* that were tested were susceptible to *C. pepo* seed oil. The seed oil was more effective than PZQ on juvenile worms and schistosomula	(Ammar *et al*., 2020; Beshay *et al*., 2019)
***Euclea divinorum* Hiem**	Aqueous plant extracts	$LC_{50=}$ 50 mg/mL (T=1h)	*In vitro*	Significant anti-schistosomal activity on adult and juvenile *S. mansoni* at 50 mg/ml,	(Sparg *et al*., 2000)
***Euclea natalensis* A. (DC)**	Aqueous plant extracts	LC_{50}>3.13 mg/mL (T=1h)	*In vitro*	66.7% of the *S. haematobium* schistosomula worms were killed at a concentration of 3.13 mg/ml	(Sparg *et al*., 2000)
***Maytenus senegalensis* (lam) Excell.**	Leaves & stem Root Root &bark	25 mg/mL 25 mg/mL 2.5 mg/mL (T=1h)	*In vitro*	A significant effect was observed on schistosomules of *S. mansoni*	(Mølgaard *et al*., 2001)
***Ocimum americanum* hexane, *Ocinmum americanum* water**	Whole plant	Worm reduction in mice (68.7	*In vivo*	68.7% *S. mansoni* worm reduction exhibited by *O.*	(Waiganjo *et al*.,2016)

		and 63.4%) vs praziquantel (75.2%) (T=1h)		*americunum* hexane; 63.4%, *O. americanum* water and 61.6%, *O. americanum* crude 52%	
Origanum majorana	Aqueous extract	90% mortality at 500, 250 μg/ml (T=6 and 12 h)	*In vitro* *In vivo*	100% mortality of schistosomula and adult worms (Egyptian *Schisto soma* strains) of *S. haematobium* at 500, 250, and 125 µg/ml concentrations	(Fadladdin, 2021)
***Pterocarpus angolensis* DC**	Leaves Stem bark	LC_{50}=102 mg/mL LC_{50}=33.8 mg/mL LC_{50}=51.3 mg/mL (T=1h)	*In vivo*	The efficacy of *P. angolensis* was comparable to that of praziquantel on *S. haematobium*	(Cock *et al*., 2018; Ndamba *et al*., 1994)
***Pulsatilla chinensis* (Hederacolchiside A1)**	Whole plant	LD_{50} = 21.05 mg·kg−1 (T=1h)	*In vitro* *In vivo*	HSA had high activity against *S. japonicum* and *S. mansoni* less in 11h days old parasites *in vivo*	(Kang *et al*., 2018)
Rauwolfia vomitoria	Root and stem bark	<1000 μg/mL. (cecarie)	*In vitro*	All *S. mansoni* cercariae were	(Tekwu *et al*.,2017)

		<1000 μg/mL. (Inhibition value on adults) (T=2h)		killed within 2 h of exposure at a concentration range of 62.5–1000 μg/mL and 250–1000 μg/mL of stem bark and roots, respectively	
***Scierocarya birrea* (A. Rich.) Hochest. Subsp, Caffra**		LC_{50}>25 mg/mL (T=1h)	*In vivo* *In vitro*	Lethal at 50 mg/ml, killing either 66.7 or 100% of the *S. mansoni* schistosomula worms	(Sparg *et al*., 2000).
Zingiber officinale	Root/tuber	500, 250, and 125 μg/ml l ed to 100% mortality (T=6h)	*In vitro* &i*n vivo*	The crude aqueous extract at a dose of 500 mg/kg body weight exhibited anti-schistosomal activity on *S. mansoni*	(El-derbawy and Kholy, 2019; El-Nour *et al*., 2021)
Ziziphus spina-christi	Aqueous extract	18.90ppm (T=25h)	*In vivo*	100% mortality of *S. mansoni* schistosomule and adult worms observed at 500, 250, and 125 µg/ml concentrations	(Fadladdin, 2021; Yousif *et al*., 2017)

Current Drug Nominees for Schistosomiasis

Only a limited number of plant species traditionally used against *S. mansoni* have undergone scientific screening. Primarily, *in vitro* assessments were made on these plants and the most significant results were obtained from the *Abrus precatorius* L. subsp. *Africanus* v*erdc* root extract which, however, still needs to be further assessed *in vivo* and in randomized clinical trials. The most progressive agents that have been tried for potential use in the treatment of schistosomiasis are quinine analogues and artemisinin-based antimalarial drugs (Duarte Galhardo de Albuquerque *et al*., 2020). The trials were motivated mostly by their proven usefulness in treating malaria. The effective clinical trials by these agents give assurance for the use of NPs in this infectious disease (Cheuka, *et al*., 2017). Some chemical structures of anti-schistosomal bioactive molecules are cardoldiene (18), 2-methyl cardoldiene (19) (both derived from *Anacardium occidentale)*, berberine (20) (derived from plants in the Berberidaceae family in the genus *Berberis*), furoxan (21), and artemether (22) (derived from *Artemisia annua*) shown in (Fig 1).

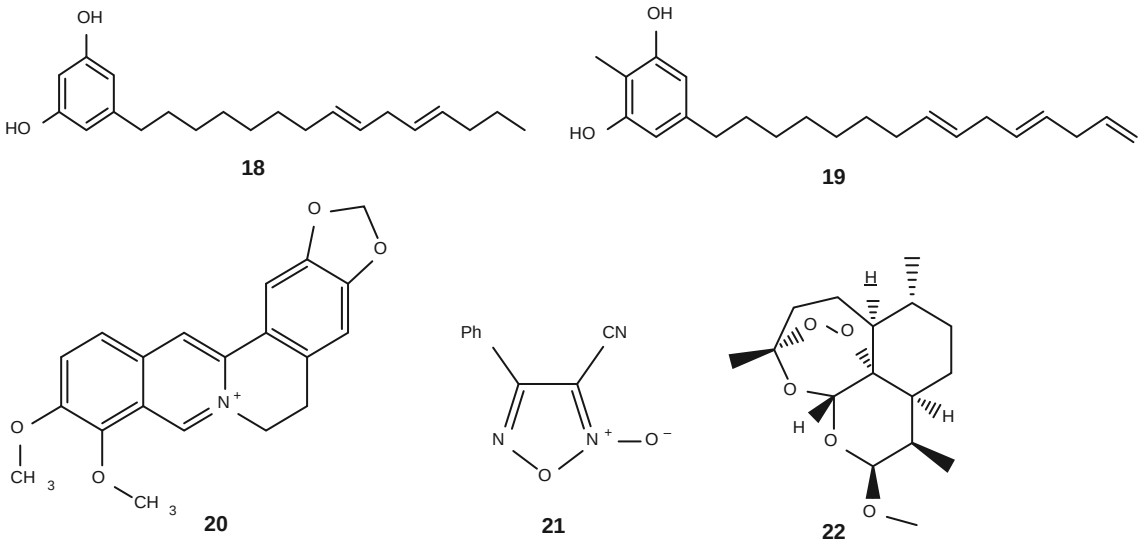

*Fig1***:** Structures of various bioactive molecules against schistosomiasis (Ghosh *et al*., 2019).

Nanoparticles and Nanocarriers

Some researchers have discovered that nanoparticles such as nanoselenium and nanogold have therapeutic properties against intestinal disorders induced by *S. mansoni* (Adekiya *et al*., 2020 Dkhil *et al*., 2019). In these studies, the nanoparticles caused a reduction in the intensity of oxidative stress, changes in body weight, histological damage and the number of goblet cells in the jejunum. It was proposed that the therapeutic effects of nanoparticles are associated with their antioxidant activities (Dkhil *et al*., 2019). It was also revealed that the particular physical structure of nanoparticles, such as having large surface areas, permits the nanoparticles to interact with microorganisms (Ghosh *et al*., 2019). Their size gains them access to the cells. In another study that investigated the *in vitro* cytotoxicity and schistosomicidal activity of oleic acid-loaded nanocapsules, a 100% concentration-dependent mortality was observed from 200 μg/mL in 4 h of incubation and 50 μg/mL in 24 h (Nunes *et al*., 2021). Curcumin-loaded gold nanoparticles were tested on *S. mansoni in vivo* and displayed a synergistic anti-schistosomal effect when combined with PZQ. A 97.4% reduction in worm burden in the 3rd week was observed (Mokbel *et al*., 2020). The studies showed that the nanocapsules and curcumin-loaded gold nanoparticles led to severe damage to the tegument dorsal surface, making it a promising new treatment alternative.

Marine Organisms

The potency of 13 macroalgae extracts from the Gracilaria, Dictyota and Laurencia genera was tested against *S. mansoni in vitro* (Stein *et al*., 2015). The extracts of *Dictyota dichotoma, Dictyota menstrualis, Dictyota mertensii, Plocamium brasiliense, Spyridia hypnoides, Gracilaria ornate, Chondria littoralis and Laurencia dendroidea* led to a100% mortality of adult worms at a concentration of 500 g/ml after 2h. When the concentration was lowered to 100g/ml and 100% mortality was observed after 24-72h. In another trial, 45 crude extracts obtained from 37 species of Brazilian macroalgae were screened for schistosomicidal and molluscicidal activity *in vitro* on *S. mansoni*. Of the 37 species, 21 displayed schistosomicidal activity. Worm reproduction was notably inhibited by most of the species as observed by egg counting (Stein *et al*., 2021). Given that there is a dearth of literature on the potential of marine organisms in schistosomiasis drug discovery, these few studies may generate increased interest to characterise more seaweeds.

Progress Made in Anti-schistosomal Lead Compound Optimisation and Development

Academic and pharmaceutical research groups working on drug discovery have made significant progress in identifying a range of novel leads, which still stand as the foundation for anti-schistosomal drug discovery (Bergquist *et al*., 2017). Many experiments have been done *in vitro* on larval, juvenile and adult *S. mansoni*. To date, more than 500 000 compounds have been screened in high-throughput screening projects (Hernandez *et al*., 2019; Guidi *et al*., 2017; Neves *et al*., 2016; Kuhn *et al*., 2010). One of the latest studies reported an *in vitro* and *in vivo* screening of the Medicines for Malaria Venture (MMV) Pathogen Box containing 400 compounds. The study revealed that MMV022029 and MMV022478 had the highest worm burden reduction and granted a series of new potent scaffolds and pharmacophores that could be used to design and develop appropriate alternative(s) to PZQ (Pasche *et al*., 2019). The pharmacophores' structures are shown in Figure 2.

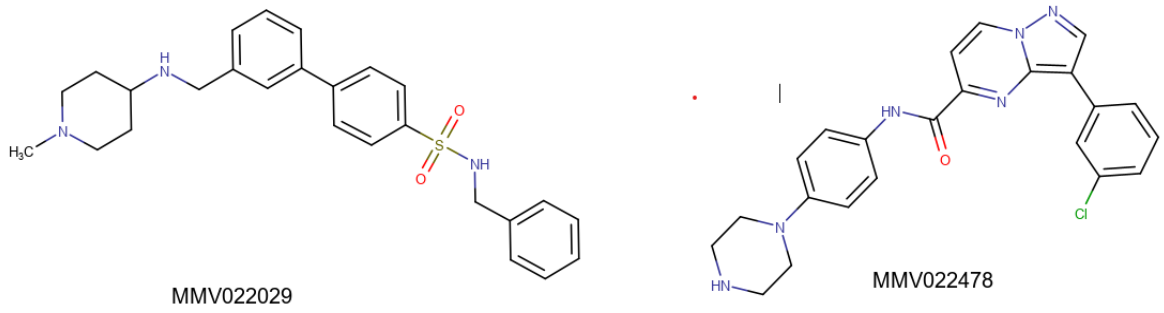

Fig 2: The structures of the MMV pharmacophores with schistosomicidal properties.

Some of the leads are currently used in structure-activity relationships in various ongoing experiments and quantitative structure-activity relationships (QSAR) (Melo-Filho *et al*., 2016). Despite the urgent need to find new schistosomiasis drugs, there is no single drug candidate that is close to reaching the market at present (Lombardo *et al*., 2019). Of the 402 new chemical entities that were registered between 2000 and 2013, none targeted schistosomiasis or NTDs (Ferreira *et al*., 2015).

Application of Advanced Techniques in Schistosomiasis Drugs Discovery

Currently, drug design and development are driven by the modernisation and knowledge of a combination of experimental and computational approaches (Neves *et al*., 2016). The integration of molecular and CADD in drug development alongside organic synthesis has led to increased availability of biological, structural and chemical data (Ferreira *et al*., 2018; Herrera Acevedo *et al*., 2017; Neves *et al*., 2015). Many molecular targets from the *Schistosoma* species have been evaluated (Mafud *et al*., 2016). The disclosure of the genomes of the three species of schistosomes that are usually implicated in infection has been a breakthrough in the field as it led to increased comprehension of the molecular machinery involved in the parasite-host relationship and, therefore, in the disease pathophysiology (Ferreira *et al*., 2018). Various proteins are emerging as cutting-edge potential drug targets. Examples of the proteins are; G-protein-coupled receptors, kinases, ion channels, reductases, acetylases and proteases. Presently, 238 schistosomes' protein structures are registered in the Protein Data Bank and the majority of the proteins were obtained through X-ray crystallography (RCSB PDB, 2020). The existence of large virtual libraries of small molecules has assisted medicinal chemists in virtually screening possible drug candidates for NTDs. Libraries such as the ZINC, GDB-17, ChemSpiderr, ChemMine, Drug Bank and PubChem contain small molecules ranging between 5000 and 100 billion and are readily available online (Lin *et al*., 2020; Banegas-Luna *et al*., 2018). This information has brought schistosomiasis drug discovery into the molecular age and opened various new possibilities in the field. Currently, schistosomiasis drug discovery programs are carried out through the implementation of unprecedented molecular modelling coupled with drug repositioning research, structure-based drug design and target-focused compound screening (Ferreira *et al*., 2018). Computational techniques used in schistosomiasis drug discovery are generally categorised under structure-based drug design (SBDD) and ligand-based drug design (LBDD) (Wilson and Lill, 2011).

SBDD is the design and optimisation of a chemical structure to identify a compound suitable for clinical testing of a drug lead (Batool *et al*., 2019). SBDD is based on knowledge of the drug's three-dimensional structure and how the shape of the drug and its charge causes it to interact with its biological target. In SBDD, large databases or libraries of 3D structures of small molecules can virtually be screened on a 3D structure of schistosomiasis protein (Batool *et al*., 2019). Molecular docking is a process commonly used in screening good drug-like

compounds. Through the application of specialised docking programs, the process searches the possible conformation or poses of molecules inside the binding pocket of drug targets and scores them according to their stability energy-wise (Xu *et al*., 2017). LBDD is an approach commonly used in default of the receptor 3D structure. LBDD is rooted in the form of established molecules that bind to the biological target of interest. 3D QSAR and pharmacophore modelling are the most essential and extensively used tools in LBDD. They can offer predictive models well-suited for lead identification and optimisation.

One refined application that can speed up the discovery of drugs with schistosomicidal activity is virtual screening (VS). VS is the use of computational filters in a database of chemical structures to predict the bioactivity of a compound concerning a specific target. The major benefit obtained from VS is the reduced time and resources required for an *in vitro* screen of a chemical library of known compounds (Nunes *et al*., 2016).

Efforts have been made in using computational methods to develop new schistosomicidal agents or drugs. In this section (Table 2), we summarise the general categories of CADD methods used in the discovery of novel schistosomicidal drugs.

Table 2: The general categories of CADD methods used in the discovery of novel schistosomicidal drugs.

Techniques of CADD used	*S. mansoni* proteins targeted	Proposed drugs/molecules	References
Structure-based virtual screening (SBVS), Homology modelling	Dihydroorotate dehydrogenase (DHODH)	manitimus, capecitabine, brequinar analogue and leflunomide.	(Otarigho, 2019)
Molecular docking, homology modelling	*Sm* ATPase	Licofavone B	(Aleixo de Carvalho *et al*., 2015)
Molecular docking	*Sm* TGR	8-hydroxyquinoline-5-sufonyl 1,4-diazepine derivative (Compound 7b)	(Allam*et al*., 2013)

QSAR-based virtual screening	*Sm* TGR	sorangicin A, A-349079-S1 and amphidinolide H1	(Mendonça*et al*., 2019)
Combi-QSAR-based virtual screening	*Sm* TGR	4-nitro-3,5-bis(1-nitro-1H-pyrazol-4-yl)-1H-pyrazole (LabMol-17) and 3-nitro-4-{[(4-nitro-1,2,5-oxadiazol-3-yl) oxy] methyl}-1,2,5-oxadiazole (LabMol-19)	(Melo-Filho *et al*., 2016)
Molecular docking, homology modelling	*S. mansoni* phosphofructokinase	C_2H_5 analogue of 6-gingerol	(Durojaye *et al*., 2019)
Molecular docking, VS, molecular dynamics and homology modelling	*Sm* HDAC1	(N,8-dihydroxy-8-(naphthalen-2-yl) octanamide zinc (ZINC13474421)	(Singh and Pandey, 2015)
Molecular docking	*Sm* TGR	8-hydroxyquinoline-5-sulfonamido derivatives compound 3 and 4	(Eweas *et al*., 2012)

Most CADD efforts in schistosomiasis drug discovery have focused on the design and synthesis of schistosomicidal agents followed by VS. Although the chemical space of current NPs databases is huge enough to attract VS projects, there are very few studies that have been done to advance the discovery of schistosomicidal agents. In a 2017 study (Akachukwu *et al*., 2017), African-originated plant metabolites were evaluated for 'drug-likeness' and docked toward four selected validated *Schistosoma* drug targets; Glutathione S-transferase, Thioredoxinglutathione reductase, Histone deacetylase and *S. mansoni*. Out of a total of 27 bioactive compounds with anti-*Schistosoma* history, one of the compounds emerged as the most interesting candidate by both being drug-like and inhibiting the activities of the studied enzyme targets at the micromole arrangement.

For the past decade, African NP medicinal chemists and database developers have progressed much in the development of small compound NPs databases and libraries. Currently, there are more than ten NP libraries across the African continent, with 20000 chemical entries in total (Sorokina and Steinbeck, 2020). Most of the libraries are not yet available online except for the Northern African NPs Database (NANPDB) (Ntie-Kang *et al*., 2017), Integrated Ethiopian traditional herbal medicine and phytochemicals database (ETM-DB) (Bultum *et al*., 2019) and South African natural compound database (SANCDB) (Hatherley *et al*., 2015). The development of libraries allows medicinal chemists and drug developers to conduct in-depth *in silico* studies that can advance the discovery of schistosomicidal drugs.

Structure-based Virtual Screening

SBVS explores information about a 3D structure of targets that are either determined experimentally by, for example, X-ray crystallography and nuclear magnetic resonance or are computationally predicted through homology modelling to select suitable ligands (Neves *et al*., 2015).

In one of the most recent studies, an ecto-enzyme called *Sm*NACE in *S. mansoni* was discovered. *Sm*NACE has an encouraging topology because it is one of the uncommonly recognised and characterised targets on the tegument of adult schistosomes that causes serious clinical problems and could be considered a prospective target for drug nominees (Toussi Matchi *et al*., 2020). The enzyme purine nucleoside phosphorylase (PNP), one of the explored targets, is a crucial element in the purine recovery biochemical route (Pereira *et al*., 2010). Given that *Schistosoma* worms do not have a *de novo* purine biosynthesis pathway, they solely rely on the salvage pathway to fulfil their requirement for purines, as they are essential for the biosynthesis of nucleic acids (Torini *et al*., 2018). Based on these findings, PNP could be considered a pharmacological target for developing new schistosomicidal agents (Ferreira *et al*., 2018). Drug discovery studies that use diverse methods for drug screening are more likely to find new lead compounds (Tavares *et al*., 2016).

Drug Repurposing

Drug repurposing/repositioning of approved drugs has been one of the starting points in identifying new schistosomiasis drug leads. Various drugs that have been FDA-approved were assessed for schistosomicidal potency (Gouveia *et al*., 2018). The initial and most extensively investigated drug class are the semisynthetic artemisinins, together with dihydroartemisinin, artemether, and artesunate, which are efficient against juvenile schistosomes but less so against mature worms (Conor *et al*., 2019; Gouveia *et al*.,2018).

Other drugs that have been investigated and exhibited potency include mefenamic acid which was shown to be effective against *S. mansoni* (Lago *et al.*, 2019); promethazine on *S. mansoni* (Roquini *et al*., 2019)*;* spironolactone on *S. mansoni* (Guerra *et al.*, 2019); single oral fixed-dose of praziquantel-miltefosine-nano combination on *S. mansoni* (Eissa *et al*., 2020); miltefosine on *S. mansoni* (El-Faham *et al.*, 2017); perhexiline maleate (Guidi *et al*., 2016); clofazimine and doramectin have also displayed schistosomicidal activity (Gemma *et al*., 2019). In these studies, it is evident that repurposing approaches are useful as starting points in the discovery of novel drugs against *Schistosoma*.

Major Hindrances in Drug Development and Prospects

As the term implies, NTDs are neglected by the pharmaceutical production sector since the diseases affect mainly the poorest societies (Kefalidou *et al*., 2016; Moon *et al*., 2012). In these countries, the majority of people can barely afford a balanced diet, accommodation, clothes and formal health care. Thus, many resort to alternative traditional medicines (Length, 2017). Firstly, major pharmaceutical companies' investment in therapeutic areas is not financially appealing because of poor financial returns. It is also known that the drug discovery process is costly and with a high failure rate (Weng *et al*., 2018) thus, drug discovery against parasitic diseases, in general, has not been inspired by profit-making motives (Cheuka *et al.*, 2017). As a result, pharmaceutical companies rather develop drugs that target chronic diseases, ensuring long-term sales (Kefalidou *et al*., 2016). Further challenges also include ensuring the right to use sufficient plant resources, licensed property rights concerns and the complications of NP chemistry with related incompetence of working with NPs (Cheuka *et al*., 2017). The number of well-validated molecular drug targets for tropical diseases is very negligible, partly because the detailed biology of many of the pathogens is yet to be understood (De Rycker *et al*., 2018). In light of these hindrances, the application of *in silico* methods in the discovery of novel schistosomiasis drugs is also slackened.

VS has arisen in drug discovery as a robust computational method of screening huge libraries of small molecules for new hits with preferred properties that would then be experimentally tried (Neves *et al*., 2018). Computational simulations used in VS strategies are very proficient in the identification of pharmacologically dynamic compounds or novel indications for drugs already used to treat other diseases. The use of this technology may speed up the pace at which schistosomiasis drug discovery is moving. Testing the candidate compounds *in silico* and selecting those with a high chance of binding to the target in the parasite is vital as it lowers

the costs of drug discovery (Pereira *et al*., 2020). Political leaders and governments need to identify schistosomiasis as a public health problem that needs to be given greater consideration in terms of funding and setting up effective monitoring and surveillance systems (Abe *et al*., 2010). More investigation of the therapeutic impact of a wide range of natural antioxidants and herbal extracts against these diseases may prove to be of great assistance for the poorest among the human population living in the tropical nations of Asia, Africa and North and South America where schistosomiasis is endemic (Ghosh *et al*., 2019). It is vital to employ CADD methods to minimise the costs involved in the discovery of new schistosomiasis drugs considering that the most affected regions are low-income countries (Zorn *et al*., 2021).

Conclusions

In conclusion, we would like to highlight that the recent advances in CADD represent a new era in the discovery of anti-schistosomal drugs. While many plants have been tested against schistosomiasis and proved to be potent, none of them is close to reaching the market as new drugs. We propose using *in silico* methods to accelerate this process as they are more accurate and more efficient than the conventional approaches. In consideration of the already underfunded schistosomiasis drug discovery process, the use of CADD methods will lead to the identification of hit and lead compounds at a lesser cost.

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Conflicts of Interest

No potential conflict of interest was reported by the authors.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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Chapter 3

In Silico Study of *Cucurbita maxima* Compounds as Potential Therapeutics against Schistosomiasis.

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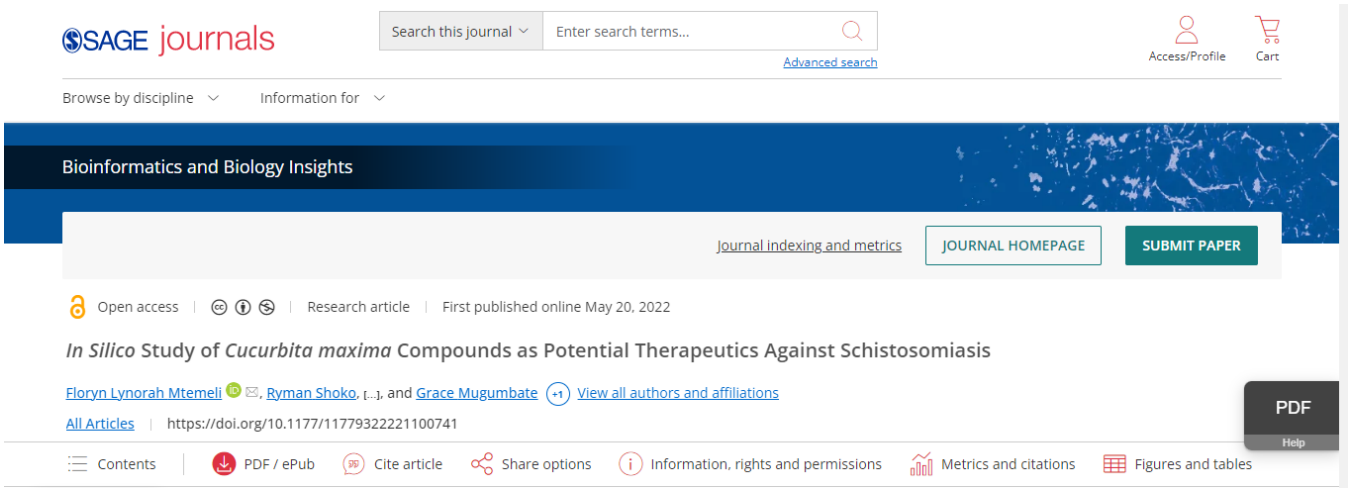


Abstract

Schistosomiasis, a disease usually related to poverty and poor sanitation, affects over 200 million people worldwide. Since the 1970s, the medical sector has depended on a single drug, praziquantel, for the treatment of the disease. The emerging evidence of resistance of the Schistosoma parasite to praziquantel and the drug's inefficacy against juvenile stages of the parasite makes the need to find alternative drugs an urgent matter. In this study, we explored the inhibition potential of compounds from *Cucurbita maxima* using molecular docking studies on *SmPNP* and *Schistosoma haematobium* 28 kDa glutathione S-transferase (*Sh*28kDaGST). Following molecular docking studies and analysis of the active sites, the primary amino acids that were observed and shown to be involved in the *Sm*PNP-ligand interaction are CYS 33, ARG 86, HIS 88, TYR 90, ALA 118, ALA 119, PRO 200, TYR 202, GLU 203, VAL 219, MET 221, THR 244, ASN 245, PRO 257 and HIS 259. For the *Sh*28dKa-ligand interaction, the primary amino acids were PHE 11, ARG 16, TRP 41, LEU 53, GLU 70 and SER 71. Momordicoside I aglycone binds to *Sm*PNP with the lowest binding energy of -7.9 kcal/mol by pi sigma bond interactions with HIS 88. Balsaminoside B binds to *Sh*28kDaGST with a binding energy of -7.6 kcal/mol by hydrogen bond interaction with TRP 41, LEU 53 and SER 71. Pharmacokinetic studies showed favourable drug-like properties for the ten compounds that exhibited the lowest binding energies. Therefore, we propose that bioactive compounds from *C. maxima* be considered as potential novel drug hits in the treatment of schistosomiasis.

Keywords: Schistosomiasis, *Cucurbita maxima*, purine nucleoside phosphorylase, 28kDa glutathione S-transferase, pharmacokinetics.

Introduction

Schistosoma species are digenetic blood trematodes and are the causal agents of schistosomiasis (Mawa *et al.*, 2021). The annual estimated number of deaths due to the disease varies between 24 000 and 200 000 globally (Mostafa & Eid, 2011). The six species responsible for morbidity are *Schistosoma mansoni*, *Schistosoma haematobium*, *Schistosoma japonicum*, *Schistosoma guineensis*, *Schistosoma mekongi* and *Schistosoma interlacum* (Mawa *et al.*, 2021). The most common species in sub-Saharan Africa are *S. mansoni* and *S. haematobium*. Since 1970, the treatment of schistosomiasis has greatly relied on the drug praziquantel (Gönnert & Andrews, 1977). The reliance on a single drug for the treatment of the disease poses a threat to the medical sector as this can lead to drug resistance. Reduced efficacy of the drug following mass drug administration programmes and the reported laboratory-based resistance (Wang *et al.*, 2012) necessitates the need for the search for potential novel drug candidates.

Research in drug discovery based on natural products has been practised for a long time (Ferreira *et al.*, 2018). Various plants have been investigated for anti-schistosomal activity *in vitro* and *in vivo*. These include *Zizinger officinale* (Mostafa & Eid, 2011)**,** *Anonidium manni i*(Toussi Matchi *et al.*, 2020), *Rauwolfia vomitoria* (Tekwu *et al.*, 2017), *Pulsatilla chinensis* (Kang *et al.*, 2018) and *Artemisia annua* (Neves *et al.*, 2015). *Cucurbita* cultivars such as *Cucurbita pepo* and *Cucurbita moschata* have also been tested for anti-schistosomal activity. *C. pepo* seed oil has been shown to induce microsatellite instability and tegumental damage to *S. mansoni in vitro* (Ammar *et al.*, 2020), while the curative effect of *C. moschata* was observed when patients infected with schistosomiasis were treated with daily doses of the powdered seeds(Kang *et al.*, 2018).

Cucurbita maxima are rich in alkaloids, flavanoids, phenolics, carbohydrates, tannins, saponins, terpenoids and proteins. The plant is cultivated for nutritional and medicinal purposes (Kujawska & Pieroni, 2015). For centuries, the plant has been used to treat intestinal infections (Kujawska & Pieroni, 2015), renal failure (Mahomoodally *et al.*, 2016), constipation, hyperplasia and parasite infestation (Menendez-Baceta *et al.*, 2014). Oral consumption of seeds has also been used for blood pressure regulation (Roy, S., Datta, 2015). The molluscicidal

potential and potency of *C. maxima* have been studied and successfully determined (Mtemeli *et al.*, 2020). However, there is a dearth of literature on the plant's anti-schistosomal properties. Currently, 17 compounds from the plant are available on online databases such as PubChem (https://pubchem.ncbi.nlm.nih.gov/) and CHEMBL (https://www.ebi.ac.uk/chembl/). However, data on *in silico* studies on the anti-schistosomal activities of the plant is currently unavailable in the public domain.

Various schistosome proteins and kinetic parameters have been studied as potential drug targets. Presently, 238 schistosome protein structures are registered in the Protein Data Bank and the majority of the proteins were obtained through X-ray crystallography (H.M. Berman *et al.*, 2000). *S. mansoni* purine nucleoside phosphorylase (*Sm*PNP) and *S. haematobium* 28-kDa Glutathione S-transferases (*Sh*28GST) are crucial targets in schistosomes.

PNP also known as inosine phosphorylase (Rama *et al.*, 2015), plays a fundamental role in the maintenance of proper cellular function and metabolism, acting both in the *de novo* purine synthesis and the purine nucleotide salvage pathway (Romanello *et al.*, 2017). One crucial component of the salvage pathway is the catalysis of the reversible phosphorolysis of the N-ribosidic bond of 6-oxopurine deoxynucleosides and nucleosides delivering their correspondent base and ribose-1-phosphate (Torini *et al.*, 2018). PNP facilitates the metabolism of inosine into hypoxanthine, adenosine into adenine and guanosine into guanine, and in each case, a ribose phosphate is created. Mutations in the PNP enzyme lead to severe combined immunodeficiency (SCID)(Singh *et al.*, 2013).

The *Sh*28GST are enzymes associated with parasite metabolic cycles and host immune adjustment (Molehin, 2020). In schistosomes, 28GST has been shown to revoke the development of host epidermal Langerhans cells to the depleting lymph nodes (Johnson *et al.*, 2003). The protein is uncovered on the outer layer of the cercaria in the same manner as in adult worms, suggesting its inclusion in the parasite-host communication. First discovered in the 1980s, the protein is considered a promising candidate for a schistosomiasis vaccine, having undergone successful phases 1 and 2 clinical trials (McManus, 2021). It is hypothesised that the enzymes assist the schistosomes by protecting them from membrane damage and toxins circulating in the host blood (Johnson *et al.*, 2003). This is achieved through immune-effector cells at the parasite surface, yielding lipid peroxidation products. Also, the increase in the solubility of haematin in the schistosome gut aids in the reduction of the "constipation" of worms (Johnson *et al.*, 2003).

There is a need to control morbidity and eventually eliminate schistosomiasis as well as to attain Sustainable Development Goals three "achieve health for all" (Klohe *et al.*, 2021). To achieve this goal, computational biology studies can be carried out to speed up drug discovery efforts against schistosomiasis. In this work, we screened the library of 17 *Cucurbita maxima* bioactive compounds to determine their anti-schistosomal properties using molecular docking against *Sm*PNP and *Sh*28GST. Our results show that Momordicoside I aglycone and Balsaminoside B have the lowest binding affinity of -7.9 kcal/mol and -7.6 kcal/mol respectively.

Materials and Methods

Protein Preparation

The crystal structures of *Sm*PNP (3FAZ) and *Sh*28GST (1OE7) were retrieved from the PDB (https://www.rcsb.org/) in a complex with co-crystallised ligands. The proteins were prepared using Biovia Discovery Studio Visualiser v21.1.0.20298 (http://www.accelrys.com) through the deletion of water molecules and addition of missing hydrogen atoms. The metal ionisation was corrected to certify formal charge and force field treatment using Autodock tools. All co-crystallised ligands were cut from the protein complexes and used in validating the molecular docking protocol the through calculation of root mean square deviation (RMSD) using Biovia Discovery Studio. The proteins were optimised and refined for docking analysis using the Pyrx v 2008-2012 (Sargis Dallakyan, The Scripps Research Institute).

Ligand Preparation

The phytochemicals of *C. maxima* were retrieved from published literature (Kulczynski & Gramza-Michałowska, 2019), and their crystal structures were downloaded from the CHEMBL database (https://www.ebi.ac.uk/chembl/). The ligands were prepared using the Open Babel module of the Pyrx tool by using the force field uff.

Molecular Docking

Molecular docking simulations were done using Autodock Vina integrated with the Pyrx software. *C. maxima* phytochemicals were docked into the active sites of *SmPNP* (Pereira *et al.*, 2010) and *Sh*28GST (Acemoglu *et al.*, 2003) proteins. The grid was generated using the receptor grid generation module of the Pryx tool (coordinates are shown in Table 1). The grid box was adjusted to cover the catalytic site residues for *Sm*PNP and *Sh*28GST proteins.

The best 10 ligands according to the binding energy ΔG binding and RMSD values in each trial were chosen as novel inhibitors. The 17 compounds were docked against the catalytic site of

the proteins using the binding pocket of the co-crystallised ligands which had been removed before docking. Visualisation of the protein-ligand-complex was performed using Biovia Discovery Studio 2021.

Toxicity Analysis

The SMILE structures of ten compounds with the lowest binding energy were retrieved from CHEMBL. Using Lipinski's rule of 5, the prediction of absorption, distribution, metabolism, elimination, and toxicity (ADMET) analysis was done using the pkCSM server (http://biosig.unimelb.edu.au/pkcsm/) (Douglas E. V Pires, Tom L. Blundell, 2015). The following parameters were considered: human intestinal absorption (%), blood-brain barrier permeability (log BB), metabolic interactions with cytochromes CYP2D6 and CYP3A4, total clearance (log mL/min/kg), AMES toxicity; human ERG I inhibition, oral rat acute toxicity (LD50) in mol/kg and oral rat chronic toxicity lowest adverse effect levels (LOAEL) in log mg/kg body weight /day. ADMET properties of praziquantel were also predicted for comparative studies.

Results and Discussion

Molecular Docking

Virtual screening of a library of compounds from *C. maxima* was done using molecular docking against the targeted proteins *Sm*PNP and *Sh*28kDaGST. Each of the generated docked complexes was observed centred on minimum binding energy values (Kcal/mol). Pharmacokinetic profiling of the phytocompounds was further done to predict their drug-likeness properties. The interactions of the ligands within the binding pockets of *Sm*PNP and *Sh*28kDaGST are shown in Table 3.

The amino acid residues involved in the interactions and each of their position in their ligand-binding site were identified. Hydrophobic, pi-pi stacking, hydrogen bonding, and many other interactions between the protein and the ligands were demonstrated through molecular docking. The primary amino acids that were observed and shown to be involved in the *Sm*PNP-ligand interaction are CYS 33, ARG 86, HIS 88, TYR 90, ALA 118, ALA 119, PRO 200, TYR 202, GLU 203, VAL 219, MET 221, THR 244, ASN 245, PRO 257, and HIS 259. For the *Sh2*8kDa-GST-ligand interaction, the primary amino acids were PHE 11, ARG 16, TRP 41, LEU 53, GLU 70 and SER 71.

Among the ten compounds docked against proteins, Momordicoside I aglycone and Balsaminoside B were predicted to have the lowest binding energy values when bound to

*Sm*PNP and *Sh*28kDaGST, respectively. Momordicoside I aglycone is a triterpenoid saponin found in the Cucurbitaceae family and previous studies have shown the compounds' antidiabetic properties and anti-obesity properties through the reduction of fat accumulation(Fan *et al.*, 2019; Lin *et al.*, 2019; Tan *et al.*, 2008)[,]. Following the docking of Momordicoside I aglycone against *Sm*PNP, it displayed pi sigma bonding with amino acid residue HIS 88 with a binding affinity of -7.9kcal/mol. Balsaminoside B is a triterpene that has been shown to have antimalarial[35] and anticancer activity(Muronga *et al.*, 2021). The docking results from Balsaminoside B docked against *Sh*28dKaGST displayed a binding affinity of -7.69kcal/mol. Hydrogen bond interactions with the amino acid residues TRP 41, LEU 53, SER 77 and carbon-hydrogen bonds with ARG 16 were observed. Our results are in agreement with studies that have shown the *in vitro* anti-schistosomal activity of triterpenes in plants such as *Argemone mexicana, Momordica balsamina, Actinopyga echinites* and *Holothuria polii* (Neves *et al.*, 2015; Serala *et al.*, 2021).

Balsaminol E showed affinity on both *Sm*PNP and *Sh*28kDaGST with binding energies of -7.6 and -7.5 kcal/mol, respectively. For *Sm*PNP, the amino acid residue interactions observed with Balsaminol E were hydrogen bonds with ARG 86 and ASN 244, alkyl and pi alkyl bonds with TYR 202, VAL 219 and PRO 257. For *Sh*28dKaGST the interactions observed with Balsaminol E were hydrogen bonds with TRP 41, pi sigma bonds with PHE11, pi alkyl and alkyl bonds with LEU 53 and carbon-hydrogen bonds with ARG16.

CHEMBL468165 binds to *Sm*PNP with a binding affinity of -7.0 kcal/mol by hydrogen bond interactions with PRO 200 and pi sigma bond interactions with TYR 90 and HIS 259. Charantadiol A interacts with *Sm*PNP through hydrogen bonds with amino acid residues at CYS 33, VAL 219; alkyl and pi alkyl bonds at TYR 202 and MET 221. 3beta,25-diol had a binding affinity of -6.6 kcal/mol exhibiting pi sigma bonds with *Sm*PNP amino acid residues at HIS 259 and pi alkyl and alkyl bonds at TYR 202 and MET 221.

Neither visible interactions nor Lipinski violations were observed between Balsaminol C and *Sh*28kDaGST. Balsaminol E and Balsaminolside C, both triterpenoids, had the same binding affinities of -7.5 kcal/mol with *Sh*28kDaGST amino acid residues exhibiting pi sigma and hydrogen bonds. CHEMBL249658 binds to *Sh*28kDaGST with a binding affinity of -7.1 kcal/mol showing pi-sigma bond interaction with PHE 11 and TRP 41. Docking results showed that compounds generally exhibited good docking energy values with the highest binding energy values of -6.6kcal/mol for *Sm*PNP and -7.1kcal/mol for *Sh*kDaGST. Table 1 shows the

Pyrx grid box coordinates; Table 2 shows the drug-like properties of the ten best ligands and Table 3 shows the interactions between the ligands and the proteins in 3-dimensional images.

Table 1 shows the Pyrx grid box coordinates

Protein	**Centre X coordinates**	**Centre Y coordinates**	**Centre Z coordinates**	
*Sm*PNP		-6.4363	1.5792	30.1628
*Sh*28kDaGST	15.6356		0.7630	26.34

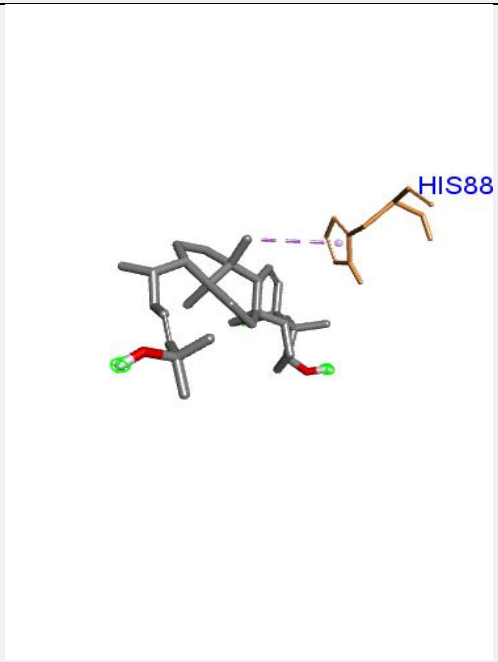
Table 2 shows the drug-likeness properties and binding affinities of the ten best ligands.

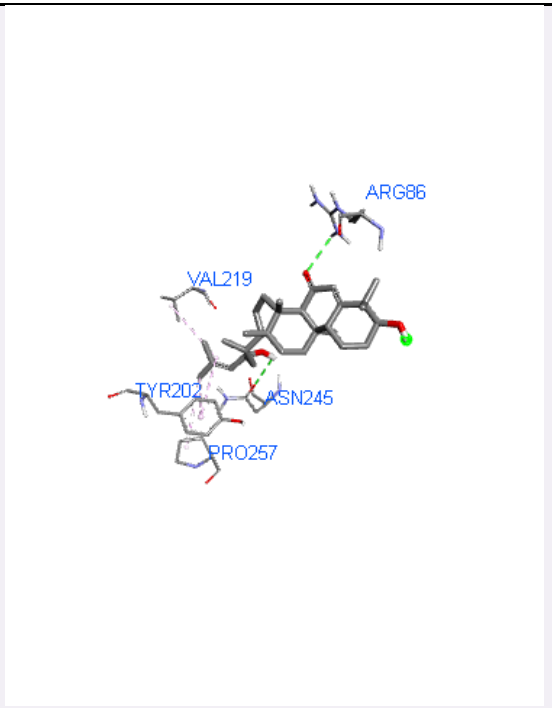
Protein-ligand-complex	**Molecular weight**	**LogP**	**Rotatable bonds**	**Acceptors**	**Donors**	**Surface area**	**Lipinski violations**	**Binding affinity in kcal/mol**
*Sm*PNP CHEMBL3264665 Momordicoside I aglycone	456.711	6.2946	4	3	2	201.616	1	-7.9
*Sm*PNP CHEMBL1254849 Balsaminol E	456.711	6.4848	4	3	2	201.670	1	-7.6
*Sm*PNP CHEMBL 468165	440.712	7.2259	4	2	2	7.2259	1	-7.0
*Sm*PNP	454.695	6.4182	4	3	2	201.616	1	-6.7

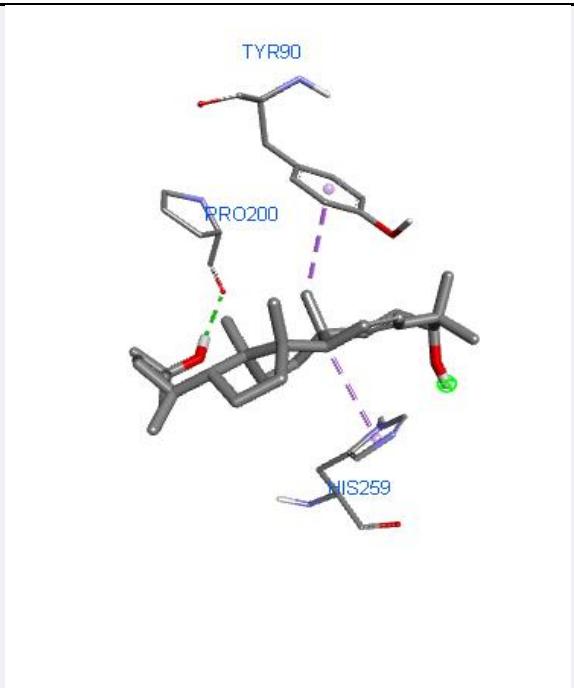
Charantadiol A CHEMBL3264664								
*Sm*PNP CHEMBL3264663 3beta,25-diol	486.737	6.2671	5	4	2	213.095	1	-6.6
*Sh*28kDaGST CHEMBL1928850 Balsaminoside B	620.868	4.1008	7	8	6	264.096	2	-7.6
*Sh*28kDaGST CHEMBL1254849 Balsaminol E	456.711	6.4848	4	3	2	201.670	1	-7.5
Sh28kDaGST CHEMBL1928851 Balsaminoside C	620.868	4.1008	7	8	6		2	-7.5
Sh 28dkaGST CHEMBL1254762	470.694		5	4	2	205.832	0	-7.2

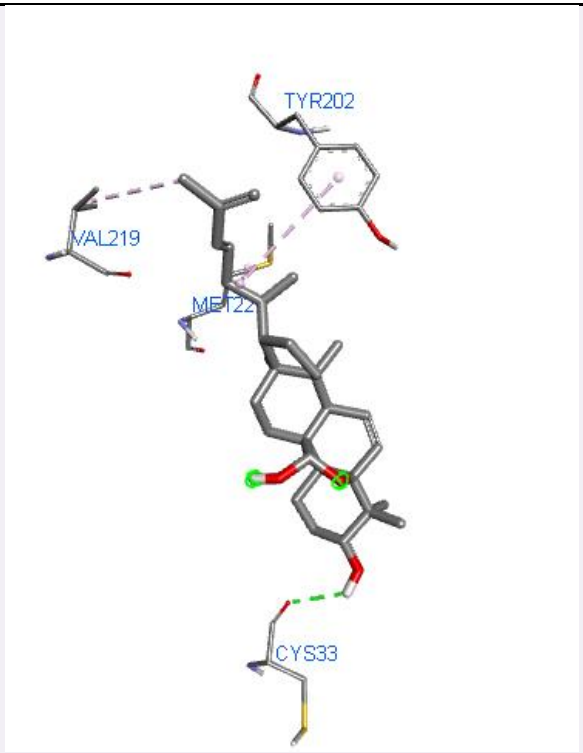
Balsaminol								
Sh 28dkaGST CHEMBL249658	468.722	7.0723	5	3	1	207.611	1	-7.1

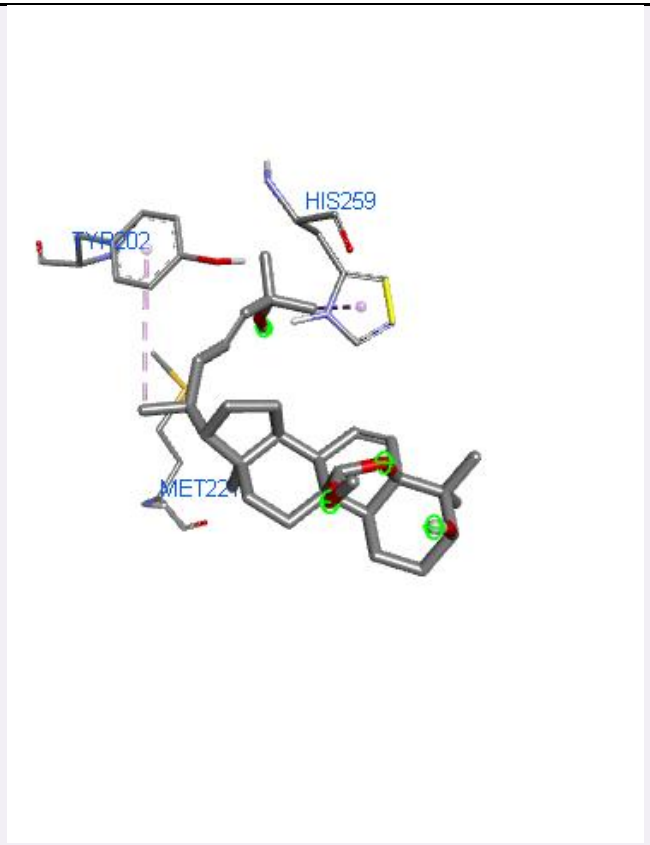
Table 3 shows the interaction between the docked ligands and the proteins in 3-dimensional images.

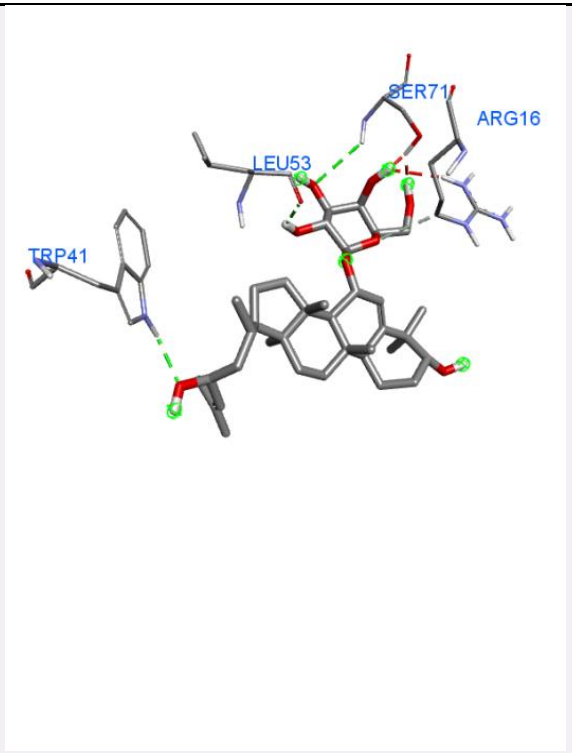
Protein-Ligand-complex		**3D interaction**
*Sm*PNP	Momordicoside I aglycone (CHEMBL3264665)	

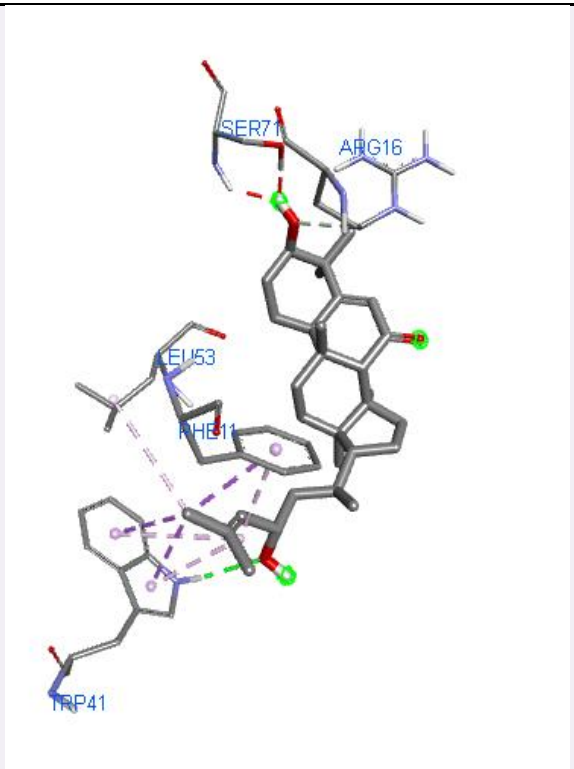
*Sm*PNP	Balsaminol E (CHEMBL1254849)		

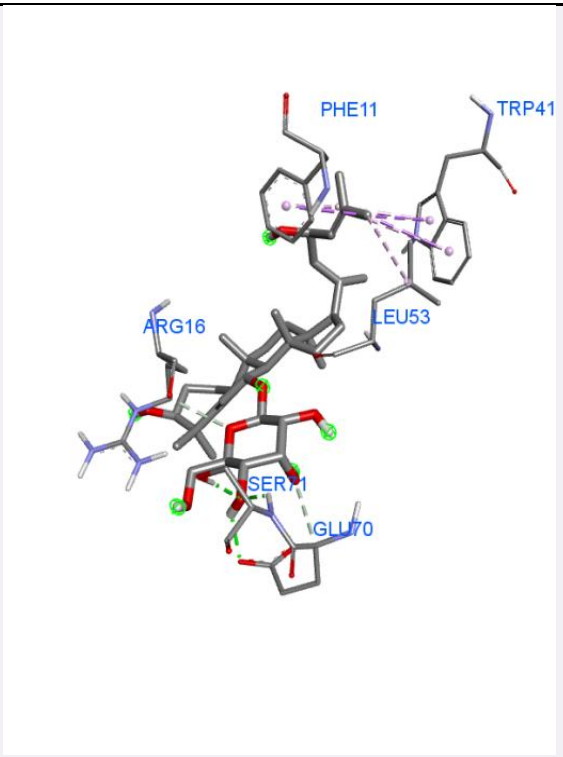
*Sm*PNP	CHEMBL468165		

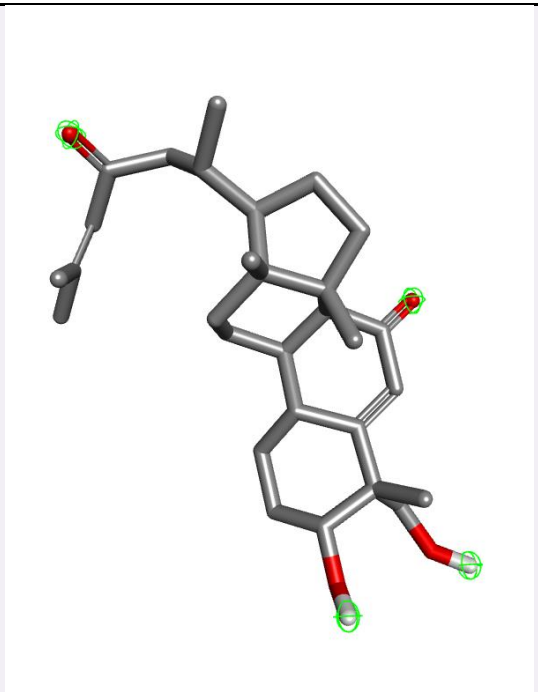
*Sm*PNP	Charantadiol A (CHEMBL3264664)		

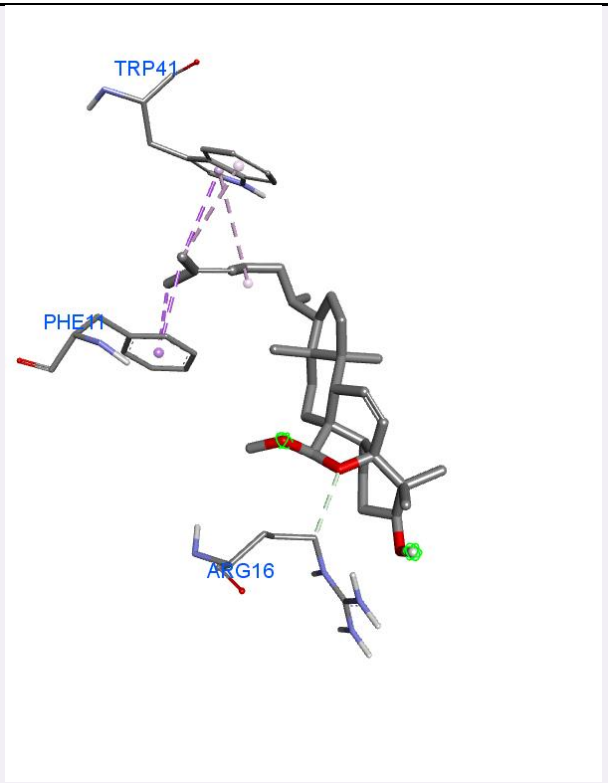
*Sm*PNP	3beta,25-diol (CHEMBL3264663)		

Sh 28kDaGST	Balsaminoside B (CHEMBL1928850)	

*Sh*28kDa GST	Balsaminol E (CHEMBL1254849)	

Sh28kDaGST	Balsaminoside C (CHEMBL1928851)	

Sh 28dkaGST	Balsaminol C (CHEMBL1254762)	

Sh 28dkaGST	CHEMBL249658	

Pharmacokinetic Studies

Tables 4 and 5 show the pharmacokinetics and drug-likeness parameters of each experimental compound. Momordicoside I aglycone's toxicity study confirms that it has an excellent intestinal absorption of 96,373% and acceptable blood-brain barrier permeability of log BB -0.117. The log BB value of Momordicoside I aglycone and all the compounds were less than the standard (log BB 0.3) which suggests that the compounds do not readily cross the blood-brain barrier. The compounds do not inhibit the cytochrome P3A4 and cytochrome 2D6 enzymes and can be easily excreted. The pharmacokinetic predictions suggest that none of the compounds was AMES toxic and none inhibited the potassium channels encoded by the human ether- a -go-go gene 1 (herG1). The pharmacokinetic properties of the favourable compounds were comparable to that of praziquantel with 70% of the compounds showing better intestinal absorption than praziquantel. The predicted intestinal absorption of all ten compounds was greater than the set standard of 30%. All of the compounds exhibited a total drug clearance prediction that was greater than zero. Also, all the compounds showed maximum tolerated values that were less than the standard (0.477mg/kg/day) and in the same range as praziquantel. The highest predicted rat LD50 value of the compounds was 4.256mol/kg exhibited by the compounds CHEMBL1928850 and CHEMBL19288.

Table 4 shows the pharmacokinetic properties of the best 10 compounds

Compound	**Intestinal Absorption** **%**	**Blood-brain Barrier Permeability** **LogBB**	**CYP3A4** **inhibitor**	**CYP2D6** **inhibitor**	**Total Clearance** **log (mil/min/kg)**
CHEMBL3264665	96.373	-0.117	NO	NO	0.292
CHEMBL1254849	96.753	- 0.644	NO	NO	0.334
CHEMBL468165	95.149	-0.207	NO	NO	0.33
CHEMBL3264664	97.319	-0.001	NO	NO	0.401
CHEMBL3264663	97.757	-0.424	NO	NO	0.32
CHEMBL1928850	50.312	-1.118	NO	NO	0.492
CHEMBL1928851	50.312	-1.118	NO	NO	0.492
CHEMBL1254762	99.995	0.254	NO	NO	0.359

CHEMBL249658	98.037	0.107	NO	NO	0.423
Praziquantel	93.386	0.3	NO	NO	1.182

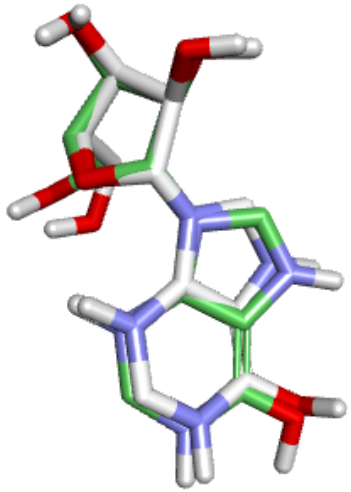
Table 5 shows the pharmacokinetic properties of the best 10 compounds

Compound	**AMES Toxicity**	**Max. Tolerated Dose (Human) Log(mg/Kg/day)**	**hERG I Inhibitor**	**Oral Rat Acute Toxicity (LD50) (mol/kg)**	**Oral Rat Chronic Toxicity (LOAEL) (mg/kg_bw/day)**
CHEMBL3264665	NO	-0.869	NO	3.398	1.796
CHEMBL1254849	NO	-0.734	NO	3.996	1.949
CHEMBL468165	NO	-0.976	NO	3.548	2.26
CHEMBL3264664	NO	-0.863	NO	3.085	1.818
CHEMBL3264663	NO	-0.441	NO	3.104	1.376

CHEMBL1928850	NO	-1.559	NO	4.256	2.923
CHEMBL1928851	NO	-1.559	NO	4.256	2.923
CHEMBL1254762	NO	-0.426	NO	3.45	1.693
CHEMBL249658	NO	0.072	NO	2.251	1.752
Praziquantel	NO	-0.554	NO	2.469	1.248

The calculated RMSD values and the superimposed co-crystallised and docked ligands are shown in Fig 1.

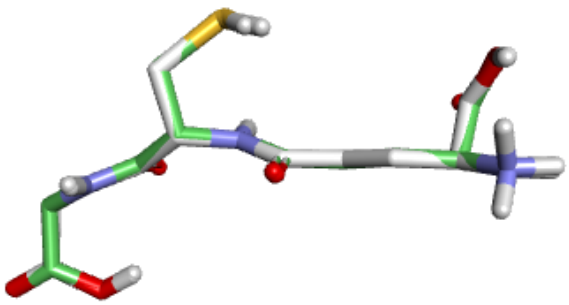
Fig 1 shows superimposed SmPNP co-crystallised, docked ligand and RMSD values used as validation of docking protocol



Heavy Atom RMSD to All

Name	Reference	RMSD (A)
NOS301_ghemical_E=280.89_out_model_0 1	NOS301_ghemical_E=280.89_out_model_0 1	0.0000
NOS301_out_model_0 2	NOS301_ghemical_E=280.89_out_model_0 1	0.4099
NOS301_out_model_0 2	NOS301_out_model_0 2	0.0000

Fig 2 shows superimposed 28kDaGST co-crystallised, docked ligand and RMSD values used as validation of docking protocol



Heavy Atom RMSD to All

Name	Reference	RMSD (A)
io7cont_ghemical_E=-10.12 1	io7cont_ghemical_E=-10.12 1	0.0000
control_ghemical_E=28.38 2	io7cont_ghemical_E=-10.12 1	0.0649
control_ghemical_E=28.38 2	control_ghemical_E=28.38 2	0.0000

Our results are in agreement with studies that have shown that Balsaminol F, a closely related tripetene to the promising compounds from this study, has anti- anthelmintic properties against *S. mansoni in vitro*(Augusta, 2015; Ramalhete *et al.*, 2012) with an LC50 value of 15 μM.

Balsaminoside B and Balsaminoside C had similar pharmacokinetic properties. The compounds had the lowest intestinal absorption of 50.312%. This can be attributed to the fact that they are both triterpenes with similar molecular formulae and their differentiation is difficult as also observed by Serala *et al., 2021*. The ligands Momordicoside I aglycone, Balsaminol E, CHEMBL468165, Charantadiol A, 3beta,25-diol, Balsaminol E and CHEMBL249658 violated the ROF with log P values greater than five. Although the log P-value which affects the compound's lipophilicity is a major determining factor in a compound's penetration across vital membranes and biological barriers, some researchers (Pathania & Singh, 2021) argue that these rules can be done away with at least during a virtual screening protocol, because it is essential to first look for a potent molecule and once potency is validated, improved kinetics can then be sought.

Hit-to-lead compound identification may take time, making it impossible for most current hit compounds to reach the market. However, various approaches are being brought forward in coming up with novel anti-schistosomal agents. An interesting iterative drug development process successfully identified derivatives of the OXA drug that are effective against all three species of the Schistosoma parasite (LoVerde *et al.*, 2021). Evaluation of 6-Gingerol and its modified analogues as therapeutic candidates against *S. mansoni* phosphofructokinase (Durojaye *et al.*, 2019; Ryan *et al.*, 2007) has also been done. Molecular docking methods showed oxadiazole-2-oxides derivatives furoxan exhibiting significant anti-schistosomal activity against *S. japonicum* (Li *et al.*, 2021). These *in silico* studies, including ours, lay a crucial foundation in the development of novel drugs against schistosomiasis.

Conclusion

Praziquantel has been the only drug used to treat schistosomiasis since 1970 making it vital to look for novel drugs to treat the disease. *C. maxima* seeds have been used in different parts of the world as traditional medicine for treatments of gastrointestinal parasites such as anthelmintic, urinary dysfunctions, hyperplasia of the prostate, dysuria, cardiovascular disease, enuresis and lowering blood glucose. This *in silico* study was aimed at exploring *Cucurbita maxima* compounds as potential therapeutics against schistosomiasis. We used computational modelling techniques to predict the inhibitory potential of *C. maxima* against *Sm*PNP a crucial protein in purine synthesis and *Sh*28kDaGST which is involved in parasite metabolic cycles. The binding of *C. maxima* compounds with *Sm*PNP and *Sh*28dKa, pharmacokinetic properties as established by docking studies, demonstrate that the *C. maxima* ligands are promising anti-schistosomal agents. Momordicoside I aglycone and Balsaminoside B exhibited the highest binding affinities with favourable drug-like properties. The ADMET properties of the compounds favour their consideration as drug candidates. We propose that *C. maxima* compounds be considered as potential therapeutics against schistosomiasis. We suggest that future research should involve molecular dynamic simulations to validate the structural stability of the selected ligands. Thereafter, *in vitro* and *in vivo* assays are required to get a more detailed analysis of the activity of the compounds within live organisms.

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Conflicts of Interest

No potential conflict of interest was reported by the authors.

Data Availability Statement

Data can be made available upon request.

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Chapter 4

In Vivo Evaluation of *Cucurbita maxima* seeds extracts and their synergistic combination with praziquantel against S*. mansoni* Infection in Murine Models.

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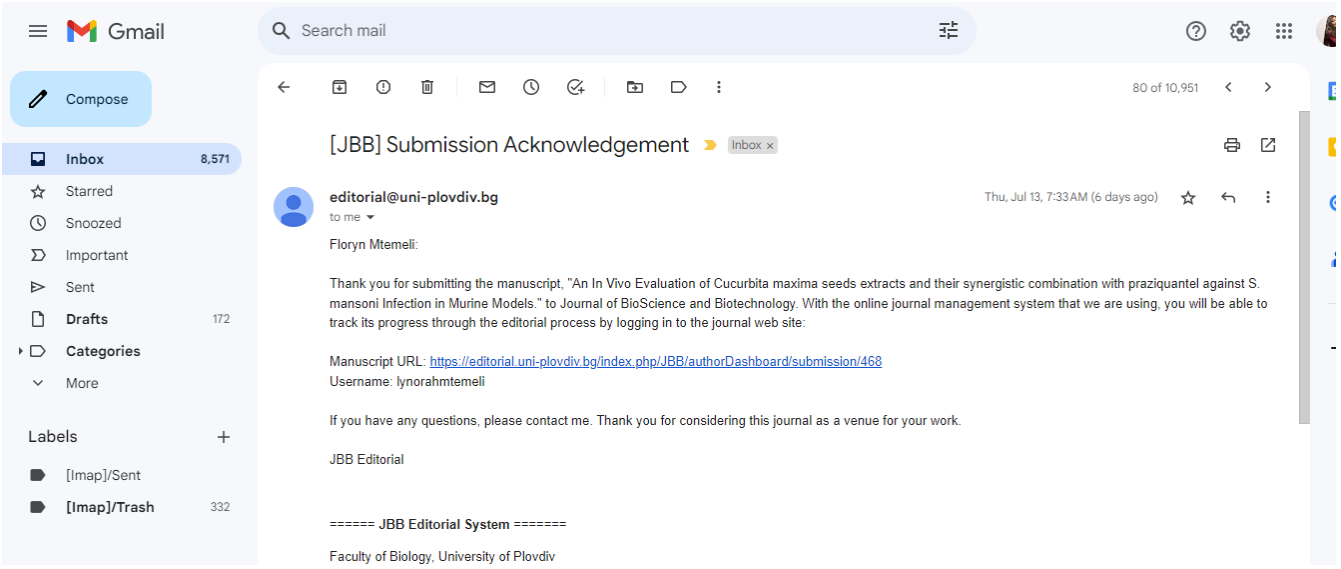

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Abstract

Over 200 million people worldwide are affected by schistosomiasis, a parasitic disease caused by infection with blood flukes of the genus *Schistosoma*. Presently, praziquantel (PZQ) is the only drug used to cure schistosomiasis. However, it is vital to look for alternative drugs and drug combinations, as reliance on PZQ may lead to drug resistance. The current study was aimed at evaluating the anti-schistosomal activity of *Cucurbita maxima* crude seeds extract alone and in combination with PZQ on *Schistosoma mansoni*-infected mice. The sampling of schistosomiasis-infected snails was done from the Mupfure River in the Shamva District of the Mashonaland Central province in Zimbabwe, particularly Matimure, Mwedziwandira, and Chikanza villages. Fifteen mice were used in each treatment bringing the total number of mice used in the study to 162. Five concentrations of the extracts were used to treat the infected mice (1 mg/mL; 0.1mg/mL;0. 01mg/mL; 0.001 mg/mL and 0.0001 gmL). The treatment was performed in a single dose of 400mg/kg in mice harbouring adult schistosomes at the 6-week post-infection mark. Our results indicate that there was a statically significant reduction in total egg count in the *C. maxima* extract, *C. maxima*+praziquantel and praziquantel treated group($p=0.00$). The combined treatment by *C. maxima* seeds extract and PZQ induced a significant reduction in the egg count in intestines, liver, and granuloma size ($p=0.00$; $p=0.00$ & $p=0.00$), respectively. There was no significant difference in the effect of the treatment on the parasites sampled from different sites ($p>0.05$). There was a significant difference in the concentration of the treatment administered ($p=0.00$) The synergistic effects of *C. maxima* extract in combination with praziquantel although minimal when compared to *C. maxima* extract alone were observed, and we propose that *C. maxima* extracts and their combination with praziquantel be considered as a candidate for the development of a new drug against schistosomiasis.

Keywords: Schistosomiasis, *Cucurbita maxima*, *in vivo*, anti-schistosomal, praziquantel

Introduction

The WHO ranks schistosomiasis as a neglected tropical disease that comes second after malaria in severity and the disease leads to 200 000 annual deaths *per* year (WHO, 2022). Two major species that cause morbidity in sub-Saharan Africa are *Schistosoma haematobium* and *Schistosoma mansoni* which account for approximately 90% of all infections (WHO, 2020). Infection by various species of the flatworm parasite, *Schistosoma*, cause morbidity and the parasites which live in the blood vasculature produce eggs leading to different pathologies (Adekiya *et al.*, 2022). *S. mansoni* causes intestinal schistosomiasis, which primarily affects the liver and the intestines, while *S. haematobium* is responsible for urinary schistosomiasis, which primarily affects the bladder and the kidneys (WHO, 2020). The most common symptoms of the disease include abdominal pain, diarrhoea, and traces of blood in the urine (haematuria) (WHO, 2022). In severe cases, the disease can lead to anaemia, malnutrition, and chronic kidney disease (Adekiya *et al.*, 2022). Long-term infection can also lead to the development of bladder cancer and liver fibrosis (WHO, 2020). The disease also has a significant economic impact, as it can lead to reduced productivity and increased healthcare costs (Wang *et al.*, 2012). The costs associated with the disease can have a significant impact on the economy of affected countries, particularly in terms of lost productivity and increased healthcare costs (Gemma *et al.*, 2019).

While schistosomiasis has been effectively controlled in many countries, the disease remains prevalent in sub-Saharan Africa (Faust *et al.*, 2020). Prevalence has been reported in 78 countries (WHO, 2020). Extensive intervention is vital in 52 of these countries where moderate to high transmission has been reported (Molehin *et al.*, 2022). The WHO strongly advocates for mass drug administration with PZQ through intermittent treatment given to at-risk populations (Webster *et al* . 2014). Since the 1970s chemotherapy solely relies on PZQ and this poses a serious threat to the medical sector as the *Schistosoma* parasite could develop resistance to the drug due to selective pressure (Ammar *et al.*, 2020). Furthermore, there is evidence of animal-to-human schistosome species hybridization, leading to new zoonotic infections increasing (Borlase *et al.*, 2021; Fall *et al.*, 2021). Attempts to eliminate schistosomiasis in endemic African countries by 2030 have been made by the WHO (WHO, 2022).

The development of anti-schistosomal drugs from natural sources has become vital in recent years (McManus, 2021). While various studies have been done in an attempt to come up with

new drugs that target the *Schistosoma* parasite, however, presently none of the drug candidates is close to reaching the market (Gouveia *et al.*, 2018).

The *Cucurbita maxima* plant, commonly known as pumpkin, has been used in traditional medicine to treat gastrointestinal parasites, hyperplasia, dysuria, and urinary and bladder infections (Roy & Datta, 2015). A study was done to assess the molluscicidal potential of *C. maxima* on Schistosoma vectors and the potency of the plant in this regard was observed. Approximately 130 compounds from *C. maxima* are available on natural product databases such as CHEMBL, Lotus Natural Compounds Database (https://lotus.naturalproducts.net/) and PubChem https://pubchem.ncbi.nlm.nih.gov/. An *In-silico* study that was aimed at identifying the compounds that target the *Schistosoma haematobium* 28kDa glutathione transferase and the *Sm*PNP proteins was done using molecular docking studies (Mtemeli *et al.*, 2022). Desirable drug-like properties for the ten compounds in the plant that displayed the lowest binding energies following pharmacokinetic studies were observed (Mtemeli *et al.*, 2022).

Cases of schistosomiasis in the Madziva community in Shamva district Mashonaland Central Province, Zimbabwe were reported in June 2021 (Marume *et al* 2021). The Local Mupfure clinic records roughly 50 cases of schistosomiasis in the district *per* month. According to the local clinic records, ten primary school-aged children from Mupfure, Chikanza and Mwedziwandira villages were infected with schistosomiasis in May 2022. In this study, the collection of snails from specific sites where villagers do their laundry and take baths in the river was done during the same period. To validate our findings (Mtemeli *et al.*, 2022), crude C. *maxima* seed extracts alone and in combination with praziquantel were assessed *in vivo.* The study was aimed at evaluating the activity of *C. maxima* extracts alone, *C. maxima* extracts in combination with praziquantel and lastly the activity of praziquantel alone. The study also aimed to compare the difference in the susceptibility of the parasites sampled from the three different villages to the treatments administered. The antischistosomal activity of *C. maxima* extracts alone and in combination with praziquantel was observed. The difference in activity of the extracts in combination with praziquantel was significantly higher than that of praziquantel alone. Our study lays a crucial foundation for the development of new drug combinations or praziquantel adjuvants.

Methodology

Plant Extract Preparation

Briefly, organic *C. maxima* plants were purchased from a local farmer in Chinhoyi. The identification of *C. maxima* was done using taxonomic morphological keys. The seeds were

sundried for 72 hours to a moisture content of 12% and crushed using a blender. Extraction using ethanol as a solvent was done as previously reported by Mtemeli *et al*.,2021 where ethanol extracts displayed better potency than water extracts. An estimated 300g of the seeds were blended into a fine powder. The refined pumpkin seeds were mixed with 900 ml of absolute ethanol and left in a dark cupboard for 7 days. Filtration was then done using 0.1mm Whatman filter paper. The filtrate was concentrated to dryness using a Buch Rotavapor (R205) set at 78°. A total yield of 6g was achieved. Distilled water was used to dissolve the extract, and it resulted in a 100mg/mL solution which was considered as the pure extract. The resulting concentrations of the *C. maxima* seeds extract serial dilutions were 1mg/mL; 0.1mg/mL; 0.01mg/mL; 0.001mg/mL and 0.0001mg/mL.

Bulb C Mice

Approximately 5-week-old male, Swiss albino, mice, weighing 20-25 grams were used in this study. Collection of infected *Biomphalaria pfeifferi* and *Bulinus globosus* snails was done from three different sites of the Mupfure River in Madziva, Shamva District of the Mashonaland Central province in Zimbabwe. Cercariae from the snails were shed by alternating the snails' exposure to direct sunlight and darkness for 45 minutes. Approximately *S. mansoni* 50 cercariae were used to infect each mouse according to the protocol (Tavares & Mourão, 2021). After randomizing the infected mice into groups of five in well-labelled cages, they were maintained with pellets and water *ad libitum*.

A group of infected mice treated only with distilled water was used as a negative control. At the 39-day post-infection mark, the treatment was performed in a single dose of 400mg/kg in mice harbouring adult schistosomes (Roquini *et al*., 2019). Praziquantel tablets were crushed and administered as a suspension to mice at a dose of 400 mg/kg intramuscularly. A combination of dissolved praziquantel and *C. maxima* extracts was administered as the combined treatment. At the 42-day post-infection mark, the mice were euthanised in a random manner using carbon dioxide and dissected to harvest the intestines and liver. The Kato Katz technique was used to analyse the egg burden in the faeces from the intestines. The animal studies were reported in compliance with ARRIVE guidelines (de Brito *et al*., 2020; Xavier *et al*., 2020). Observation, measurement of granuloma, and harvesting of eggs were performed as described in the protocol (Tavares & Mourão, 2021).

Guinea Pigs

A total of 16 guinea pigs weighing 0.3–0.60 kg were obtained from the animal house unit at the University of Zimbabwe. The guinea pigs were kept in a well-aerated room with free access

to a standard diet and water ad libitum. The 16 guinea pigs were divided into four main groups of four animals each: treated with *C. maxima* pure extract, treated with *C. maxima* pure extract plus praziquantel; treated with praziquantel alone and untreated. Immunosuppression was induced using 5 mg/kg prednisolone administered orally daily for five consecutive days before infection. Guinea pigs were infected by subcutaneous injection of *S. haematobium* cercariae. Screening of urine and eggs in faeces and urine was done 9 weeks post-infection. The guinea pigs were euthanised after 14 weeks. Unfortunately, the infection wasn't successful as no worms were recovered from the faeces and urine neither were they recovered from the hepatic and mesenteric vessels.

Table 1 outlines the experimental design employed in this study

Treatment administered	**Number of mice per treatment**
Negative control 1(uninfected untreated) (-ve control)	**9**
Negative control 2 (infected untreated) (-ve control2)	**9**
Positive control (infected PZQ treated) (+ve control)	**9**
Parasite from Site 1	**5**
Praziquantel treatment (replicated three times)	**5**
	5
Parasite from Site 2	**5**
Praziquantel treatment	**5**
	5

Parasite from Site 3 **Praziquantel treatment**	**5**
	5
	5
Parasite from Site 1 ***C. maxima* extract + praziquantel treatment**	**5**
	5
	5
Parasite from Site 2 ***C. maxima* extract + praziquantel treatment**	**5**
	5
	5
Parasite from Site 3 ***C. maxima* extract + praziquantel treatment**	**5**
	5
	5
Parasite from Site 1 ***C. maxima* extract treatment**	**5**
	5
	5
Parasite from Site 2 ***C. maxima* extract treatment**	**5**
	5
	5
Parasite from Site 3 ***C. maxima* extract treatment**	**5**
	5
	5

Pathological Studies

Using 41.7 mg of stool *per* slide, two Kato Katz slides were prepared from each stool sample. Individuals were defined as *Schistosoma*-infected if the *Schistosoma* eggs were detected by microscopy. A section of liver tissue from each group was promptly fixed in 10% formalin, dried, prepared for paraffin sectioning, and stained with hematoxylin and eosin. Five fields from various sections and different mice were used to calculate the mean granuloma diameter (in millimetres) for each group. Granulomas were counted in accordance with Abd El-Aal *et al.*, 2021. For each specimen, the number of granulomas per low-power field (10 10) was calculated (Hams *et al.*, 2013).

Statistical Analysis

The significance of the effects of the treatments was tested using a multifactor Analysis of Variance (ANOVA). The selected ANOVA model allowed us to test the significance of the treatment while accounting for egg count in the intestines, liver, and size of granuloma size. All of the possible interactions were included in the model to determine whether the effects of the treatment varied with concentration, site and type of treatment. Non -significant interactions were not considered. Data were considered statistically significant at P=0.05. All tests were done using IBM SPSS Statistics (Version 26) Tables 1 and 2.

Results

Overall, *C. maxima* crude extracts had a significant anti-schistosomal activity as shown by the eggs recovered from the intestines, liver, and granuloma (P<0.00005). Furthermore, *C. maxima* crude extracts in combination with praziquantel had slightly greater anti-schistosomal activity than the *C. maxima* crude extracts alone with an estimated mean difference of 0.311 (Table 2);1.333(Table 3) & 13.244(Table4) in the egg count from the intestines, liver, and size of granuloma respectively. Treatment with praziquantel had a satisfactory anti-schistosomal effect although significantly lower than *C. maxima*+ praziquantel with an estimated mean difference of 39.556;12.445&80.178 in the egg count from the intestines, liver, and size of granuloma respectivel.

Table 2 shows the mean difference in the egg count recovered from the intestines where treatment 1 was praziquantel alone; treatment 2 was a *C. maxima*+ Praziquantel and treatment 3 was *C. maxima* extracts alone.

Table 2

Dependent Variable: @#ofeggsinintestines

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	57.556	.743	56.079	59.032
2	18.000	.743	16.523	19.477
3	18.311	.743	16.835	19.788

Table 3 showing the mean difference in the egg count recovered from the liver where treatment 1 was praziquantel alone; treatment 2 was a combination of *C. maxima* + Praziquantel and treatment 3 was *C. maxima* extracts alone.

Table 3

Dependent Variable: @#ofeggs in liver

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	20.667	.977	18.725	22.608
2	8.222	.977	6.281	10.163
3	6.889	.977	4.948	8.830

Table 4 shows the mean difference in the size of granuloma where treatment 1 was praziquantel alone treatment 2 was a *C. maxima*+ Praziquantel and treatment 3 was *C. maxima* extracts alone

Table 4

Dependent Variable: granuloma size

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	139.689	2.057	135.603	143.775
2	59.511	2.057	55.425	63.597
3	39.267	2.057	35.181	43.352

Based on the tests between-subjects effects, there was no significant effect of the site from which that parasite was sampled i.e., Matimure, Mwedziwandira, and Chikanza ($P>0.05$) on egg count in the intestines(Table5) liver(Table6) and size of granuloma (Tables7).

Table 5 shows the tests between-subjects effects of the treatments on the number of eggs recovered from the intestines

Dependent Variable: @#of eggs in intestines

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	83454.400[a]	44	1896.691	76.297	.000
Intercept	132164.267	1	132164.267	5316.501	.000
Concentration	29068.919	4	7267.230	292.335	.000
Site	2692.978	2	1346.489	54.164	.000
Treatment	46572.978	2	23286.489	936.733	.000
concentration * site	257.837	8	32.230	1.296	.255
concentration * treatment	4146.726	8	518.341	20.851	.000
site * treatment	72.178	4	18.044	.726	.577

concentration * site * treatment	642.785	16	40.174	1.616	.080
Error	2237.333	90	24.859		
Total	217856.000	135			
Corrected Total	85691.733	134			

a. R Squared =.974 (Adjusted R Squared =.961)

Table 6 shows the tests between-subjects effects of the treatments on the number of eggs recovered from the liver

Table 6

Dependent Variable: @# of eggs in liver

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	12632.593[a]	44	287.104	6.683	.000
SIntercept	19200.741	1	19200.741	446.914	.000
Treatment	5197.037	2	2598.519	60.483	.000
Site	197.037	2	98.519	2.293	.107
Concentration	4062.222	4	1015.556	23.638	.000
treatment * site	291.852	4	72.963	1.698	.157
treatment * concentration	1040.000	8	130.000	3.026	.005
site * concentration	862.222	8	107.778	2.509	.017
treatment * site * concentration	982.222	16	61.389	1.429	.146
Error	3866.667	90	42.963		
Total	35700.000	135			
Corrected Total	16499.259	134			

a. R Squared =.766 (Adjusted R Squared =.651)

Table 7 shows the tests between-subjects effects of the treatments on the size of granuloma

Table 7

Dependent Variable: granuloma size

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	537816.400[a]	44	12223.100	64.222	.000
Intercept	852995.267	1	852995.267	4481.761	.000
Treatment	253844.044	2	126922.022	666.867	.000
Site	950.933	2	475.467	2.498	.088
Concentration	153008.548	4	38252.137	200.982	.000
treatment * site	42139.689	4	10534.922	55.352	.000
treatment * concentration	51849.363	8	6481.170	34.053	.000
site * concentration	10788.696	8	1348.587	7.086	.000
treatment * site * concentration	25235.126	16	1577.195	8.287	.000
Error	17129.333	90	190.326		
Total	1407941.000	135			
Corrected Total	554945.733	134			

a. R Squared =.969 (Adjusted R Squared =.954)

Based on Pairwise comparison, the concentrations, and treatment led to a significant difference in the egg count and granuloma size (P<0.00005). A concentration dependant decrease in egg count from the intestines even at the weakest concentration of 0.0001mg/ml was observed. The

same concentration dependant antischistosomal effect was observed at the following concentrations 0.001mg/mL (Fig2);0.01mg/mL(Fig3);0,1mg/mL(Fig4)and 1mg/mL(Fig5).The mean egg count in the liver also significantly differed according to the concentration of each treatment(P<0.00005).0.0001mg/mL(Fig6);0.001mg/mL(Fig7);0.01mg/mL(Fig8);0,1mg/mL(Fig9)and 1mg/mL(Fig10). The mean granuloma size also significantly differed according to the concentration of each treatment (P<0.00005) 0.0001mg/mL(Fig11);0.001mg/mL(Fig12);0.01mg/mL(Fig13);0,1mg/mL(Fig14)and1mg/mL (Fig15). The average egg count and size of granuloma in the negative and positive controls were uniform in all trials. The results also showed that there was a significant difference in the egg count from the intestines when comparing treatment with praziquantel alone and its combination with *C. maxima* extracts (P<0.00005). There was however no significant difference between the praziquantel treatment and *C. maxima* extract treatment in terms of the eggs recovered from the intestine (P=0.7 68). The eggs recovered from the liver were significantly lower when comparing treatment with praziquantel alone and its combination with *C maxima* extracts (P<0.00005). There was however no significant difference between the praziquantel treatment and *C. maxima* extracts treatment in terms of the eggs recovered from the liver (P=0337). There was a significant reduction in the size of granuloma after pairwise comparisons across all three different treatments with the greatest granuloma size reduction observed in the *C. maxima* + praziquantel treatment (P<0.00005)

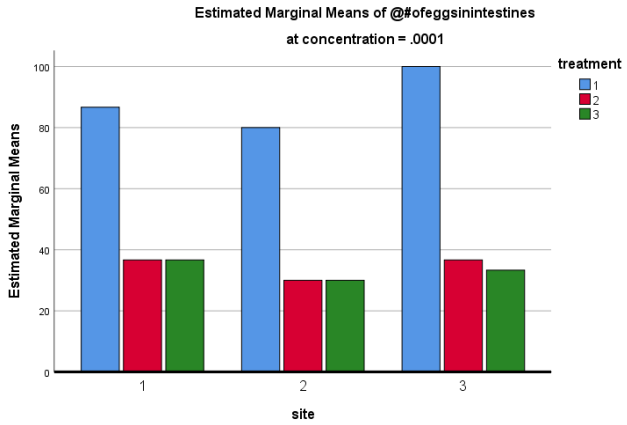

Fig 1: shows the estimated marginal means of the eggs recovered from the intestines after treatment with 0.0001 mg/ml. Treatment 1 is praziquantel alone, treatment 2 is a combination of *C. maxima* extracts and praziquantel and treatment 3 is *C. maxima* alone

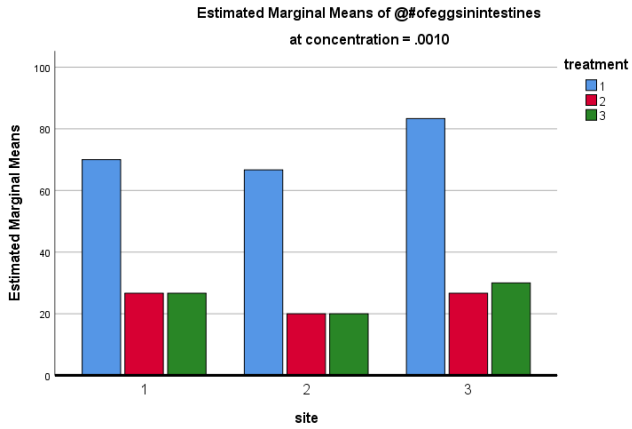

Fig 2: shows the estimated marginal means of the eggs recovered from the intestines after treatment with 0.001 mg/ml. Treatment 1 is praziquantel alone, treatment 2 is a combination of *C. maxima* extracts and praziquantel and treatment 3 is *C. maxima* alone

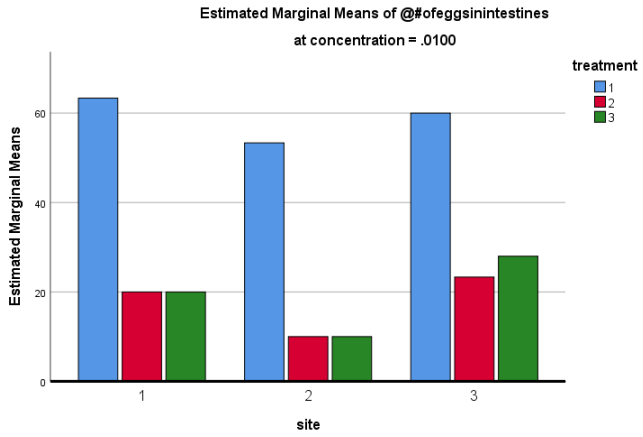

Fig 3: shows the estimated marginal means of the eggs recovered from the intestines after treatment with 0.01 mg/ml. Treatment 1 is praziquantel alone, treatment 2 is a combination of *C. maxima* extracts and praziquantel and treatment 3 is *C. maxima* alone

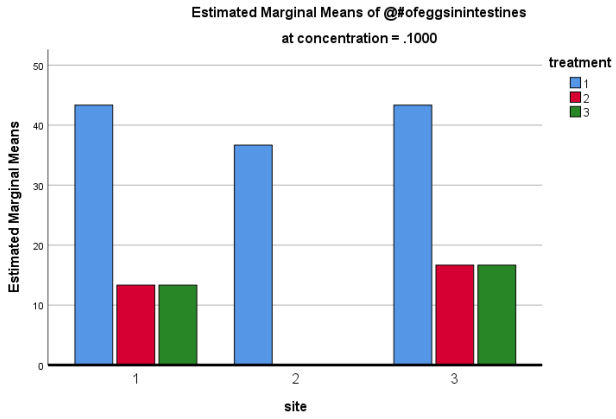

Fig 4 :shows the estimated marginal means of the eggs recovered from the intestines after treatment with 0.1 mg/ml. Treatment 1 is praziquantel alone, treatment 2 is a combination of *C. maxima* extracts and praziquantel and treatment 3 is *C. maxima* alone

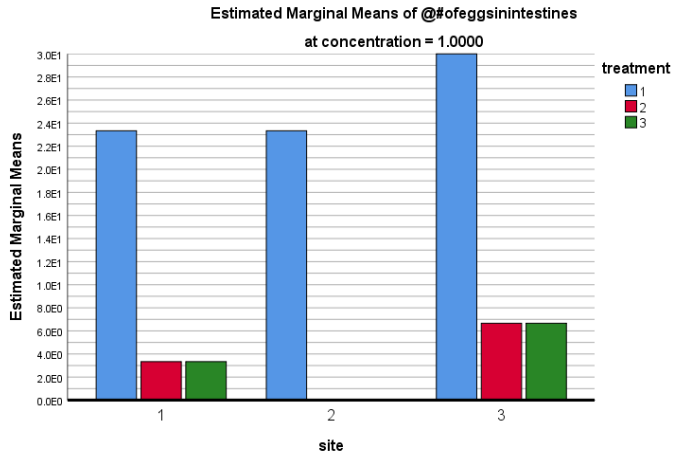

Fig 5: shows the estimated marginal means of the eggs recovered from the intestines after treatment with 1 mg/ml of each treatment.
Treatment 1 is praziquantel alone, treatment 2 is a combination of *C. maxima* extracts and praziquantel and treatment 3 is *C. maxima* alone

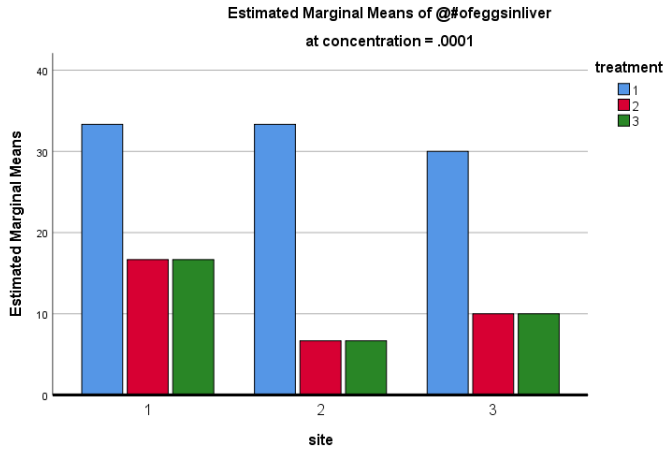

Fig 6: shows the estimated marginal means of the eggs recovered from the liver after treatment with 0.0001 mg/ml of each treatment.
Treatment 1 is praziquantel alone, treatment 2 is a combination of *C. maxima* extracts and praziquantel and treatment 3 is *C. maxima* alone

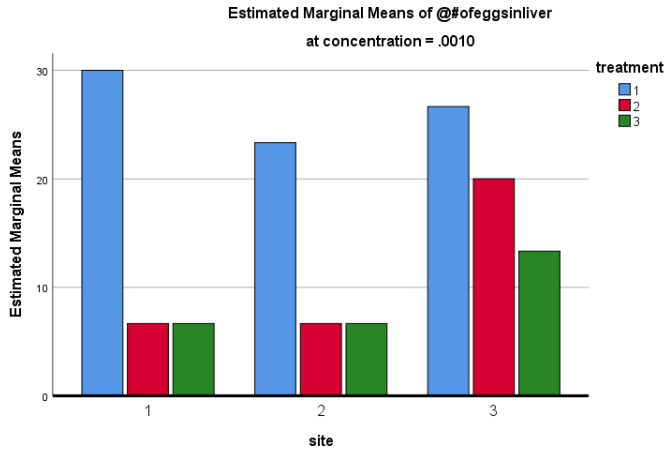

Fig 7: shows the estimated marginal means of the eggs recovered from the liver after treatment with 0.001 mg/ml of each treatment.
Treatment 1 is praziquantel alone, treatment 2 is combination of *C. maxima* extracts and praziquantel and treatment 3 is *C. maxima* alone

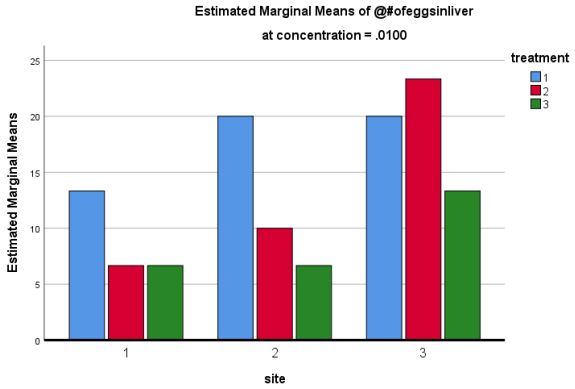

Fig 8: shows the estimated marginal means of the eggs recovered from the liver after treatment with 0.01 mg/ml of each treatment.
Treatment 1 is praziquantel alone, treatment 2 is combination of *C. maxima* extracts and praziquantel and treatment 3 is *C. maxima* alone

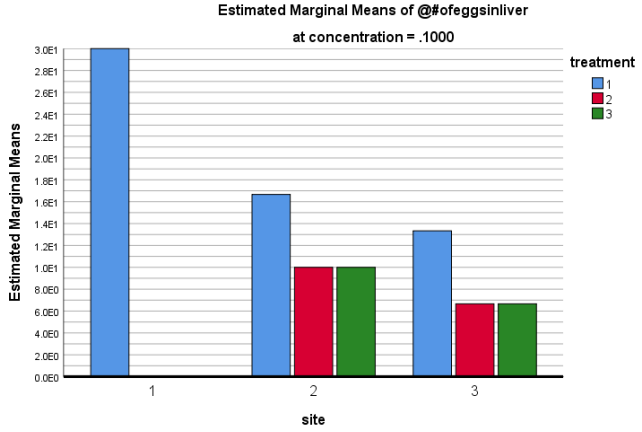

Fig 9: shows the estimated marginal means of the eggs recovered from the liver after treatment with 0.1 mg/ml of each treatment.
Treatment 1 is praziquantel alone, treatment 2 is combination of *C. maxima* extracts and praziquantel and treatment 3 is *C. maxima* alone

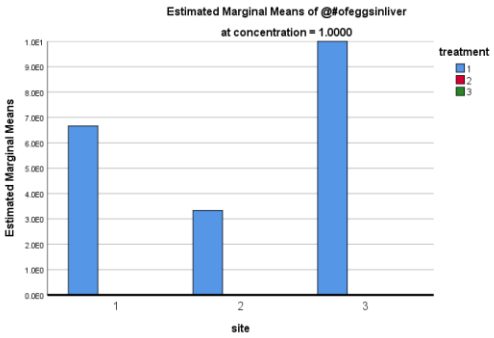

Fig 10: shows the estimated marginal means of the eggs recovered from the liver after treatment with 1 mg/ml of each treatment.
Treatment 1 is praziquantel alone, treatment 2 is combination of *C. maxima* extracts and praziquantel and treatment 3 is *C. maxima* alone

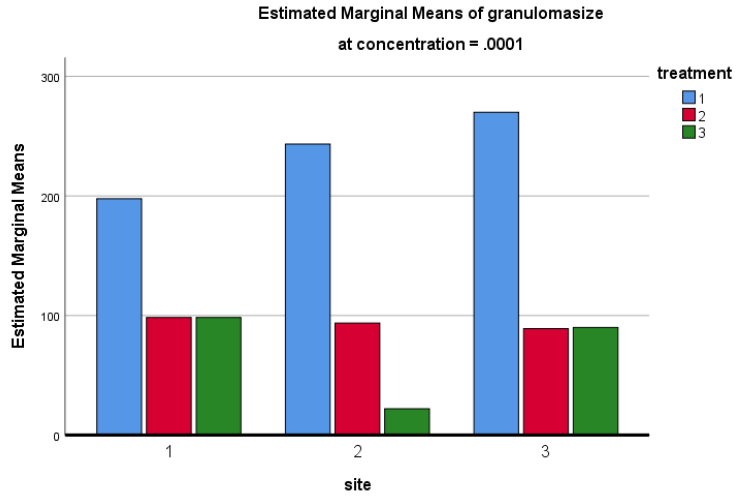

Fig 11: shows the estimated marginal means of the size of granuloma after treatment with 0.0001 mg/ml of each treatment.
Treatment 1 is praziquantel alone, treatment 2 is combination of *C. maxima* extracts and praziquantel and treatment 3 is *C. maxima* alone

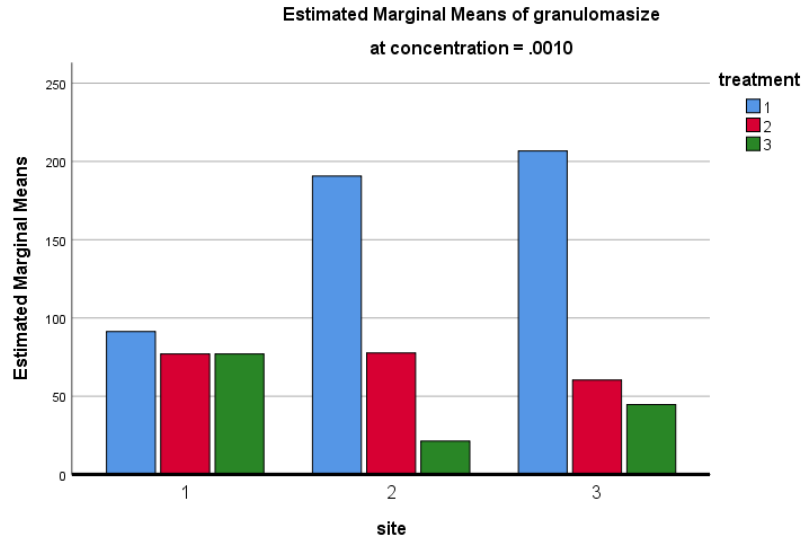

Fig 12: shows the estimated marginal means of the size of granuloma after treatment with 0.001 mg/ml of each treatment.

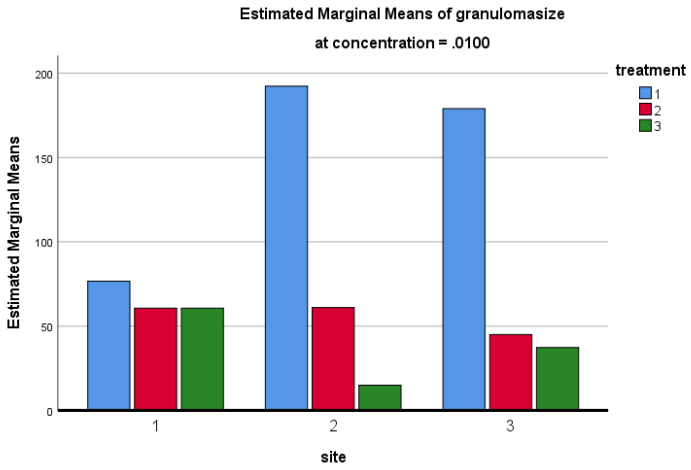

Fig 13: shows the estimated marginal means of the size of granuloma after treatment with 0.01 mg/ml of each treatment

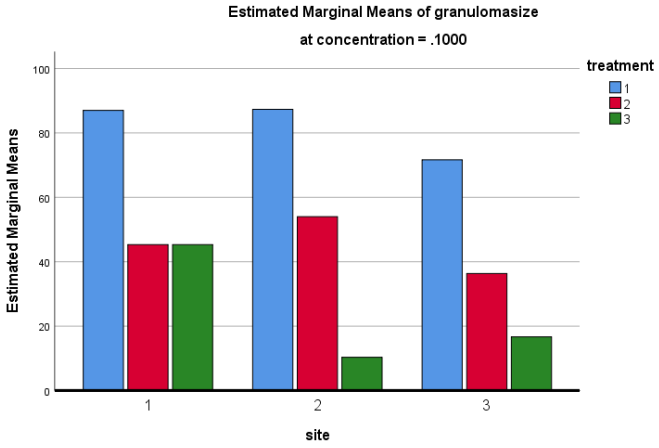

Fig 14: shows the estimated marginal means of the size of granuloma after treatment with 0.1 mg/ml of each treatment

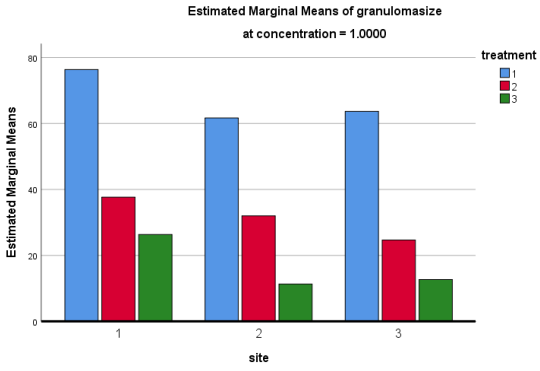

Fig 15 : shows the estimated marginal means of the size of granuloma after treatment with 1 mg/ml of each treatment

Discussion

The plant *C. maxima*, commonly known as pumpkin, is a plant that belongs to the family Cucurbitaceae. This is one of the first studies, to our knowledge to examine the anti-schistosomal activity of *C. maxima* on *S. mansoni* by assessing egg count in the intestines and liver and granuloma size. In recent years, there has been increasing attention on the probable use of *C. maxima* as an antihelminthic agent (Muchirah *et al*., 2018).

Our results indicate that there was a statically significant decrease in total instestinal and liver egg count in the *C. maxima* extract and *C. maxima* extracts+praziquantel treated group, suggesting that the extracts and PZQ have, though minimal, some synergistic effects. The results we obtained are in agreement with (Mokbel *et al*., 2020) who assessed the anti-schistosomal action of curcumin-loaded nanoparticles *versus* praziquantel in the treatment of *S. mansoni in vivo.* Their studies showed a significant reduction in worm burden of 45.45% by curcumin in the 3rd week. Even greater worm reduction of 97.4% and 70.1% reduction in granuloma size in the 3rd week was observed when curcumin-loaded nanoparticles were combined with praziquantel. Curcumin, one of the bioactive components of *C. maxima*, has recently gained attention from chemists because of its vast potential biological uses as an anti-carcinogenic, antioxidant, anti-inflammatory, and more recently, as anti-carcinogenic, antioxidant, anti-inflammatory, and more recently, anti-schistosomal agent (Abd El-Hak Rashed *et al.*,2020 and Mokbel *et al.*, 2020)).

Our results, also concur with (Beshay *et al.*, 2018) who studied the anti-schistosomal activity of *C. pepo* seed oil on *S. mansoni in vitro*. In the study the seed oil-induced microsatellite instability and tegumental damage to *S. mansoni* immature and adult worms *in vitro.* The *in vivo* schistosomicidal, antifibrotic and antioxidant activity of *C. pepo L*. seed oil on *S. mansoni* study they observed that the seed oil was effective in treating fibrosis and oxidative stress. Fadladdin, 2021 also studied the anti-schistosomal Activity of *Origanum majorana*, *Ziziphus spina-christi*, and *Salvia fruticosa* plant extracts on *S. haematobium*-infected hamsters at concentrations 500, 250, 125, 62.5, and 31.25mg/ml, respectively, and the effectiveness of the extracts was observed in these studies after 12-24 hours. The anti-schistosomal activity of *Z. officinale*, *Piper nigrum*, and *Coriandrum sativum* extracts on *S. mansoni*-infected hamsters was also studied (Abou El-Nour & Fadladdin, 2021). They observed that concentrations of 500,

250, and 125 µg/ml of *Z. officinale* and *P. nigrum* caused 100% mortality of adult worms within 6 and 12 hrs of incubation, respectively.

A study El-Sayed *et al*. (2020), showed that *C. maxima* aqueous extract could inhibit the growth and reproduction of *S. mansoni* adult worms *in vitro*. The study also showed that the extract was able to reduce the expression of some genes involved in the parasite's survival. Another study observed that *C. maxima* fruit peel extract could inhibit the multiplication of *S. mansoni* miracidia, thus preventing its further development into adult worms El-Sayed *et al*. (2021).

The anthelminthic activity of *C. maxima* has been attributed to several compounds that have been isolated from the plant (Ndhlala *et al.* 2015). For example, compounds like cucurbitacin E, a triterpenoid compound, have been reported to have anthelmintic activity. Similarly, compounds like cucurbitacin B, also a triterpenoid compound, have been reported to have anthelmintic activity (Grzybek *et al*., 2016). These compounds have been shown to have a direct effect on the parasite by causing paralysis and death of the worms. Some studies have shown that these compounds may also have an indirect effect on the parasite by increasing the host's immune response (Ndhlala *et al.* 2018).

The anthelminthic activity of *C. maxima* has been reported to be mediated by several mechanisms of action. For example, Abdel Aziz *et al*., 2018 showed that cucurbitacin E, a compound isolated from *C. maxima*, caused paralysis and death of the *Ascaridia galli* worms by binding to the voltage-gated sodium channels in the worm's nerve cells, resulting in a disruption of the worm's nervous system. Similarly, another study showed that cucurbitacin B, another compound isolated from *C. maxima*, caused paralysis and death of the *Haemonchus contortus* worms by binding to the muscle-type nicotinic acetylcholine receptors in the worm's body wall muscles, resulting in a disruption of the worm's muscular system (Wetmore *et al*., 2019).

In a study where we observed the inhibition potential of compounds from *Cucurbita maxima* using molecular docking studies on *Sm PNP* and *Schistosoma haematobium* 28-kDa glutathione S-transferase (*Sh*28kDaGST), it was observed that Momordicoside I aglycone binds to *S. mansoni* purine nucleoside phosphorylase with the lowest binding affinity of -7.9 kcal/mol. Balsaminoside B also binds to *S. haematobium* 28kDa glutathione transferase with a binding affinity of −7.6 kcal/mol (Mtemeli *et al.,* 2022). It is recommended to isolate these compounds from *C. maxima* and assess them *in vitro* and *in vivo*. Combined with PZQ,

Momordicoside I aglycone and Balsaminoside B could display synergistic anti-schistosomal activity with greater potency and a wider spectrum of activity.

Conclusion

Schistosomiasis is one of the utmost significant tropical diseases that annually leads to millions of morbidities and mortalities. Various scholars have investigated various herbs and drugs that target the disease. Our study presented a trial for treating schistosomiasis in bulb C *mice* using *C. maxima* seed extracts alone and in combination with PZQ. *C. maxima* has been shown to have anti-schistosomal properties in bulb c mice with a significant decrease in egg count in the intestines, liver, and granuloma size. Our results show a statistically significant reduction in total egg count in the *C. maxima* extracts and *C. maxima* extract +praziquantel treated group. Our future work will involve the synthesis of the high-scoring ligands from our molecular docking studies and testing their efficacy *in vitro* and *in vivo.* We also recommend that the synergistic effects of the isolated compounds and PZQ be assessed *in vivo* and *in vitro.*

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Author Contribution

All the authors contributed to the study's conception and design. The primary investigation, formal analysis and data interpretation were carried out by Floryn Lynorah Mtemeli. The initial draft of the manuscript was written by Floryn Lynorah Mtemeli and all authors commented on the previous versions of the manuscript. Ryman Shoko obtained funding and supervised the study. All authors have read and agreed to submit the final version of the manuscript.

Conflicts of Interest

No potential conflict of interest was reported by the authors.

Ethical Clearance Statement

Ethical clearance for this study was obtained from the Graduate Studies of the Chinhoyi University of Technology.

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Chapter 5

General Discussion

Overview

In the discovery of drugs for schistosomiasis, a combination of computational methods and conventional laboratory methods has become increasingly important, offering a faster and more cost-effective approach than traditional experimental methods (Gallinger *et al.*, 2022). Over the past few years, computational tools for drug discovery have advanced significantly, including virtual screening, molecular docking, and molecular dynamics simulations. Virtual screening is a widely used computational technique that involves screening large compound databases to identify potential drug candidates (Mtemeli *et al.*, 2022). For schistosomiasis, several studies have used virtual screening to identify compounds that might inhibit key enzymes or proteins involved in the parasite's life cycle. For example, studies by Gallinger *et al.*, 2022; Li *et al.*, 2015; Tripathi & Chetri, 2020 used virtual screening to identify potential inhibitors of *S. mansoni* thioredoxin glutathione reductase, a key enzyme involved in parasite redox balance.

Molecular docking is another computational method used in drug discovery that involves the prediction of the binding affinity of a small molecule to a target protein (Moreira-Filho *et al.*, 2021). Following molecular docking simulations to predict the inhibitory potential of *C. maxima* against *S. mansoni* PNP, a critical protein in the synthesis of purines and *Schistosoma haematobium* 28kDaGST a protein involved in parasite metabolic cycles, we observed that the binding of *C. maxima* compounds with *Sm*PNP and *Sh*28dKa as well as the pharmacokinetic properties proved that the *C. maxima* ligands are promising anti-schistosomal agents. The compounds Momordicoside I aglycone and Balsaminoside B displayed the highest binding affinities and favourable drug-like properties. While further validation of these computational predictions is needed, these methods may accelerate the discovery of new drugs for treating schistosomiasis. The validation of these natural compounds as anti-schistosomal agents can be validated *in vitro* and *in vivo*.

Natural Products

For centuries, natural products have been used to treat schistosomiasis. For example, observations were made from a study by Gobaara, 2020, where the anti-schistosomal properties of the marine sponge extract from the Gulf of Aqaba, in Egypt were determined.

The extracts were administered orally at a dose of (7.85, 11.25 and 10, mg/kg body weight/mouse, respectively) to infected mice at 9 weeks post-infection. The extracts induced a noteworthy reduction, in the number of male and female *Schistosoma* worms.

Ethnobotanical surveys conducted during the past few decades in Africa have recorded the presence of plant species that possess anti-schistosomal activities. Examples of these plant species are *Abrus precatoriu, Afzelia quanzensis, Antidesma venosum, Boswellia carteri Birdw., Berkheya speciosa, Cassia abbreviata, Cissampelos murconata, Euclea divinorum Hiern., Euclea natalensis, Faurea saligna Harv, Macaranga kilimandscharica, Maytenus senegalensis, Mondia whitei Skeels, Ocimum americanum, Ocimum canum Sims, Ormocarpum trichocarpum, Pterocarpus angolensis, Protasparagus buchananii, Rhus gueinzii, Rumex lanceolatus, Rumex nepalensis, Sclerocarya birrea, Tephrosia macropoda Harv, Terminalia phanerophlebia* and, *Vernonia amygdalina, Ximenia americana. americana, Ximenia caffra and Cucurbita pepo* (Cock *et al.*, 2018).

Cucurbita Species

Cucurbita sp, commonly known as pumpkins, belongs to the family Cucurbitaceae. The seeds of these plants are rich in monounsaturated and polyunsaturated fatty acids. Their seed oil is constituted of a large quantity of linoleic acid, oleic acid, palmitic acid, ECN-44, ECN-46, tocopherols, ß-sitosterol, and delta-7-sterols (Batool *et al.*, 2022). In recent years, there has been increasing interest in the potential use of *Curcubita sp* as an anthelminthic agent. Some *in vitro* and *in vivo* studies have reported the plant's anthelminthic potency. For example, in one study, *Cucurbita pepo* seed oil-induced microsatellite instability and tegumental damage to *S. mansoni* immature and adult worms *in vitro* (Ammar *et al.*, 2020) and all tested stages of *S. mansoni* were susceptible to the seed oil, which was more effective than PZQ on juvenile worms and schistosomula. An evaluation of pumpkin seed oil on the embryonic and larval development of *Toxocara cati* eggs reported disruption of egg hatching in *T. cati* eggs. The results displayed that the eggs were only developed into two cells stage at rates of 61.9% and 43.2% at the first and fourth week of incubation, respectively. The rate of undeveloped eggs was 35.7% and 56.75% in the first and fourth weeks, respectively (Hussein & Shukur, 2020). *C. maxima* oil also paralyzed *Haemonchus contortus* worms by binding to nicotinic acetylcholine receptors in the worm's body wall muscles, disrupting their muscular system (Wetmore *et al*., 2019). *Cucurbita sp* has also been shown to have nematicidal potency. Grzybek *et al.*, 2016 conducted a study to evaluate the *in vitro* and *in vivo* anthelmintic efficiency of *C. pepo* on two model nematodes namely *Caenorhabditis*

elegans and *Heligmosoides bakeri*. Fatty acids, cucurbitine, amino acids, berberine and palatine were identified in the extracts. All of the *C. pepo* seed extracts displayed a nematicidal potential *in vitro,* against *H. bakeri* larvae.

Examples of key secondary metabolites assumed to be responsible for anthelmintic activity in *Cucurbita sp* seeds are a triterpenic compound named cucurbitacin B, a non-proteic amino acid named cucurbitin, saponins, and sterols. The non-proteic amino acid cucurbitin, which is only present in the seeds, has been focused on as the active principle responsible for the anthelmintic activity, notably schistosomicidal action (Grzybek *et al.*, 2016b). The non-proteic amino acid cucurbitin (3-amino-pyrrolidine-3-carboxylic acid) is assumed to be the active principle. There are few side effects reported in dogs and humans with cucurbitin (Kaushik *et al.*, 2015). We assessed the potency of *C. maxima* seed extracts on *S. mansoniin vivo.* Our results were in agreement with previous studies that have exhibited the anthelmintic potential of *Cucurbita sp* (Ammar *et al.*, 2020). Our study also assessed the potency of *C. maxima* seed extracts in combination with PZQ and the synergistic effects of this combination was observed.

The Synergism of Plant Extracts in Targeting *Schistosoma*.

Several studies have investigated the synergistic effect of phytochemicals in treating schistosomiasis. For example, a study by Oliveira *et al.* (2013) found that a combination of two phytochemicals, cubebin and amentoflavone, had a synergistic effect in inhibiting the growth and viability of *S. mansoni* adult worms. An additional study by Abdel-Lateff *et al.* (2015) reported that ursolic acid and luteolin combined had a synergistic effect on S. *mansoni* adult worms. Also, Campelo *et al.* (2017) demonstrated that epiisopiloturine combined with piplartine and praziquantel had anti-schistosomal activity indicating synergistic activity in reducing the worm burden, liver egg count, and oogram pattern of *S. mansoni*--infected mice. Observations made also involved surface alterations in the tegument of adult schistosomes and decreased with isolated compounds in three different lines of mammalian cells using laser confocal microscopy.

The use of natural products as adjuvants to PZQ therapy is an attractive strategy to improve treatment outcomes and reduce the risk of PZQ resistance (Campelo *et al.*, 2017). The synergistic effect of natural products with PZQ can be attributed to several mechanisms. A few natural products have been reported to increase PZQ's efficacy by enhancing its uptake by schistosomes. For example, the flavonoid quercetin, found in many fruits and vegetables,

was shown to increase the uptake of PZQ by *S. mansoni* adult worms *in vitro*, enhancing the drug's anti-schistosomal activity (Gouveia *et al.*, 2018).

Studies have shown that natural products can reverse PZQ resistance in schistosomes, restoring the drug's efficacy (Greenberg, 2013). For example, the sesquiterpene lactone helenalin, isolated from the plant *Inula helenium*, was shown to reverse PZQ resistance in *S. mansoni* worms *in vitro* and *in vivo* by inhibiting the expression of drug transporter genes and increasing the permeability of the worm membrane (Ferreira *et al.*, 2013; Pinto-Almeida *et al.*, 2021). Moreover, natural products enhance the host's immune response to schistosomes, thus augmenting PZQ's anti-schistosomal properties. For example, the polysaccharide fraction from the fungus *Ganoderma lucidum* was shown to enhance the host immune response against *S. mansoni* in mice, leading to a significant reduction in worm burden and egg output when combined with PZQ (Huseein *et al.*, 2022).

There is evidence that some natural products can complement PZQ, thereby increasing the overall anti-schistosomal activity (Gouveia *et al.*, 2020). For example, the compound pristimerin, isolated from the plant *Maytenus guianensis*, was shown to inhibit the activity of an essential schistosome enzyme, farnesyl diphosphate synthase, which is involved in the synthesis of isoprenoids (Gouveia *et al.*, 2019).

Another study investigated the synergistic effect of the methanolic extract of *C. pepo* seeds and PZQ (Beshay *et al.*, 2018). The combination treatment was shown to significantly enhance the efficacy of PZQ against adult *S. mansoni* worms *in vitro*, with a synergistic index of 1.87. The combination treatment was also shown to significantly decrease the worm burden and egg count in infected mice *in vivo*, compared to treatment with PZQ alone. In this study, our results indicate a statistically significant reduction in granuloma size in the *C. maxima* extract +praziquantel treated group, with a 13.24% margin in comparison with the *C. maxima* extracts treated group. The mechanism of the synergistic effect of *Cucurbita sp* with PZQ has not been fully elucidated. However, we hypothesise that the bioactive compounds in *C. maxima* seeds may enhance the penetration of PZQ into the worms, leading to increased efficacy. Additionally, the phytochemicals in the *C. maxima* seeds may also possess immunomodulatory properties that can enhance the host's immune response against the parasite, thereby contributing to the efficacy of the combination treatment. Natural products, generally considered safe, with minimal side effects, are suitable for use in resource-limited settings. Furthermore, the use of *C. maxima* can reduce the risk of drug resistance, as the

Schistosoma parasites are less likely to develop resistance to a combination of drugs with different mechanisms of action.

Chapter 6

Conclusion and Recommendations

Drug discovery for schistosomiasis has been a significant area of research in recent years. Among the interventions recommended by the WHO are regular mass drug administration, improved access to safe water, improved sanitation, and the development of new drugs. In addition, health education and community mobilisation are also important components of efforts to control the disease. The identification of new compounds with anti-schistosomal activity has been accomplished in several ways, including identifying new compounds from natural products, repurposing existing drugs, and using computational methods such as virtual screening and molecular docking. However, it is imperative to note that most of this research has been conducted in *vitro* or *in vivo* animal models and more research is needed to confirm their efficacy and safety in human trials.

To combat the high prevalence of schistosomiasis and improve the quality of life of affected populations, new adjuvants to PZQ therapy are necessary. The use of combinations of phytochemicals could provide a more effective and sustainable treatment for schistosomiasis. Our thesis's main aim was to determine the anti-schistosomal activity of *C. maxima* on *S. mansoni* and *S. haematobium in silico* and *S.mansoni in vivo*. We also explored the synergistic activity of *C. maxima* seed extracts combined with PZQ. Our results demonstrated that *C. maxima* has significant anti-schistosomal activity and does have a slight synergistic anti-schistosomal effect with PZQ.

Further work

Further research is needed to explore the mechanisms of action and safety of the combination. Further studies are required to explore the potential of *C. maxima* seed extracts as anti-schistosomal agents and to elucidate the underlying mechanisms of their synergistic effect with PZQ and optimise the dosing and formulation of the extracts alone and the combination treatment. Although there is a need to ascertain the safety of this treatment and its combination with praziquantel in humans. The use of *C. maxima* seed extracts and their combination with PZQ has the potential to provide a safe, effective, and sustainable alternative for the treatment of schistosomiasis in resource-limited settings. We recommend

further anti-schistosomal studies that involve assessing the maxima seed anti-schistosomal activity of the compounds Momordicoside I aglycone, Balsaminoside B from the *C. maxima* plant. We also recommend selectivity tests of the two compounds on human GST and human PNP. Further work also involves assessing the combination of each of these compounds with PZQ as treatment and a prophylactic *in vitro* and *in vivo*

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7.0 Appendix

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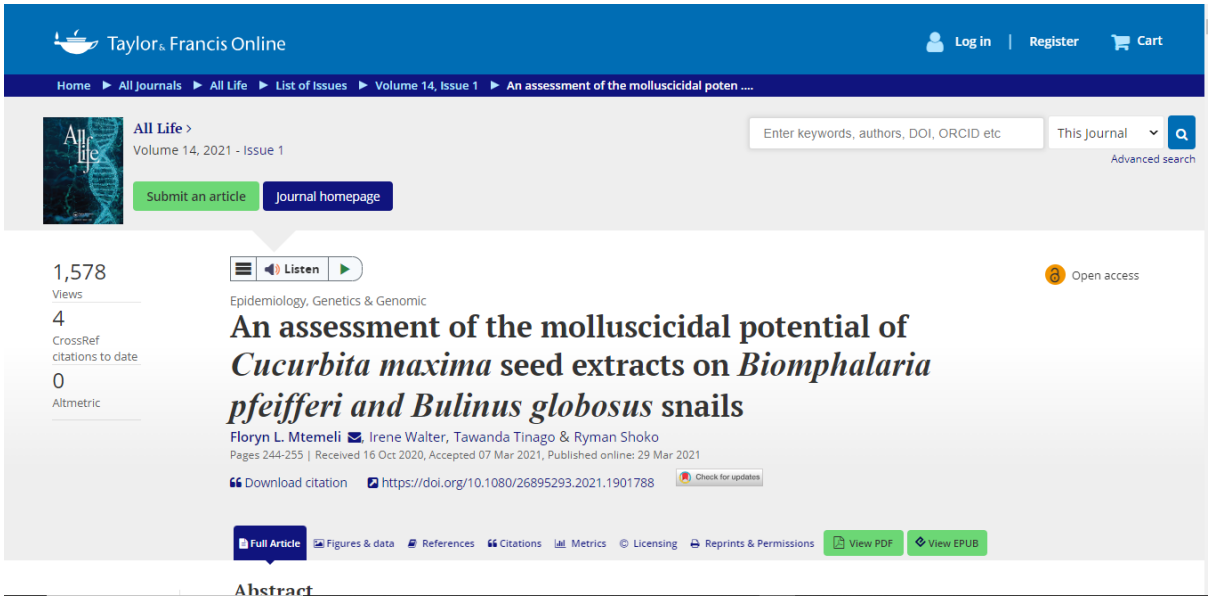

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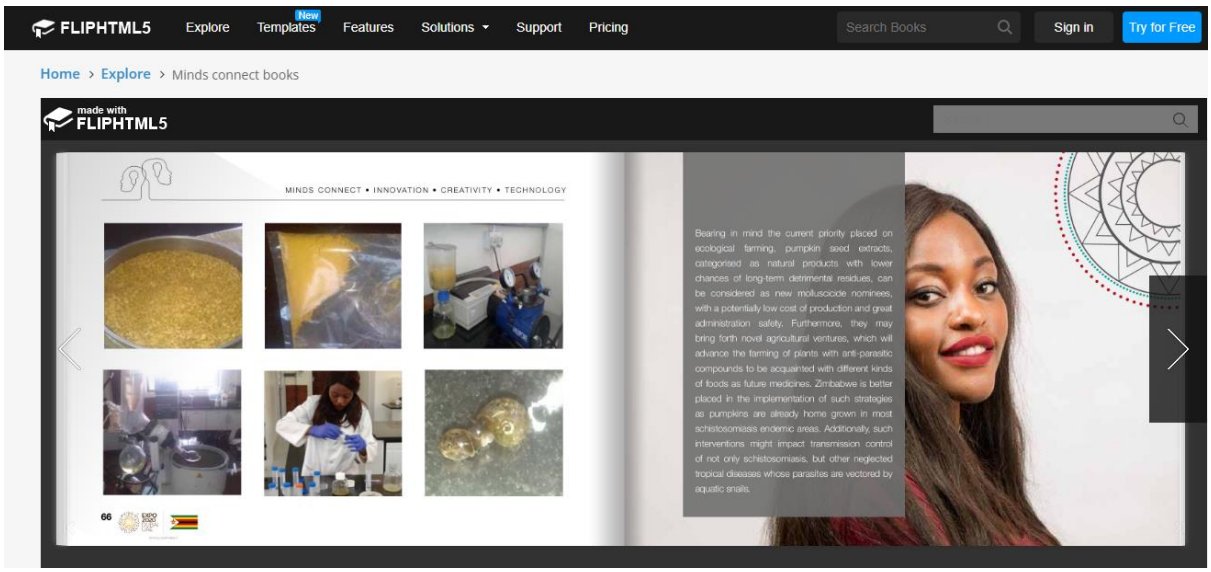

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