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Assessment of the Antischistosomal Activity of Some Plant Extracts Against *Schistosoma mansoni* Infection

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Abstract: A total of 5 methanolic extracts of 5 Egyptian plant species representing 10 families were assayed *in vitro* for antischistosomal activity against adult *Schistosoma mansoni (S. mansoni)* worms using a well established cultured medium. Worms were treated with the extracts for 1-24 hours incubation periods and activity of extracts was assessed in terms of viability, motility and death of worms. Extracts of five plants; *Dryopteris filix*mas, *Tanacetum vulgare*, *Juglans nigra*, *Syzygium aromaticum* and *Allium sativum* exhibited a very strong potency at minimum effective concentrations of 50 µg/ml after 24 hours. Phytochemical screening of these 5 extracts was carried out to detect their major chemical constituents which may be responsible for activity. The methanol extract of *Allium sativum* proved to be the most potent one; so further *in vivo* study was carried out to evaluate its antischistosomal efficacy when administered 3 and 7 weeks postinfection, besides the infected praziquantel (PZQ) treated (Positive control) and infected untreated (negative control) groups. Our results reported a 27.6% and 21.7% reduction in worm burden in the groups which received *Allium sativum* 3 and 7 weeks postinfection, respectively. This reduction in worm burden was accompained by an equivalent reduction in the number of ova/g tissue (liver and intestine) and oogram pattern when compared to infected untreated control group. However, this reduction was not equevalent to the reduction produced by PZQ. In conclusion, *Allium sativum* was found to be effective *in vitro,* but less effective than PZQ *in vivo*. It is recommended to use a cocktial of plant extracts either alone or combined with PZQ to increase the efficacy of complete elimination of worms and ova.

Key words: Antischistosomal activity · *In vitro* · *In vivo* · Treatment

INTRODUCTION

Schistosomiasis is a chronic and debilitating disease that remains one of the most prevalent parasitic infections in the humid tropics, with an estimated 650 million people at risk of infection and 200 million actually infected in 74 countries [1]. It is encouraging that significant progress in the control of schistosomiasis has been achieved over the last several years in Brazil, China and Egypt. However, because of environmental changes linked to water resources development and rapidly increasing sizes and movements of population, the disease has spread to previously non-endemic or low endemic areas [2, 3].

Praziquantel (PZQ) remains the only antibilharzial drug effective against the four main schistosomes pathogenic to man [1, 4]. Although it has been reported that PZQ has minimal side effects [5]control of schistosomiasis using PZQ at a population level faces some problems. Resistance to PZQ has been recently induced in schistosomes by laboratory selection [6]. Reduced cure rates and failure of treatment after PZQ have been reported in Senegalese, Kenyan and Egyptian patients [7-9].

Therefore, investigators have been searching for alternative drugs by screening botanical and chemical compounds for their potential activity as antischistosomal agents. Few studies have addressed the use of medicinal plant species for their antischistosomal and anthelmintic activity. Some of them were evaluated using *in vitro* bioassay while the others were evaluated through *in vivo* techniques [10-12].

So it was thoughtful to screen some of the Egyptian flora as antischistosomal agents. This study aimed at screening of methanol extracts of some Egyptian plants belonging to different families against *Schistosoma mansoni* adult worm through *in vitro* bioassay tests. The most promising plant extract would be then assessed *in vivo* for its schistosomicidal potency.

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MATERIALS AND METHODS

Plants: Five Egyptian plants were collected from variable localities in Egypt such as: El-Orman botanical garden, Cairo-Ismailia road, Cairo-Suez road and El-Khanater El-Khairia road …..etc. Some of these plants are wild, while the others are cultivated species. Identification and classification of these plants were accomplished with the aid of plant taxonomy specialists.

***S. mansoni* Worms and Cercariae:** *S. mansoni* worms and cercariae were obtained from the Schistosome Biological Supply Center (SBSC) at Theodor Bilharz Research Institute (TBRI).

Animals: Male CD1 Swiss albino mice (Weight 20 g ± 2 g) bred and maintained at the SBSC, TBRI, Egypt, were used in this study. Animal experiments have been carried out according to the internationally valid guidelines and ethical conditions [13].

Preparation of Plant Materials: Voucher specimens of each plant was kept at the department. Different parts of the collected plants were separated if necessary. Proper methods for drying the plants (In shade and well-aerated places) were followed to avoid fermentation and micro-organisms attack. An electrical mill was used to finely powder the plants.

Extraction of Plants: Known weights of each plant material was successively extracted with aqueous methanol at room temperature by percolation and the extract was filtered then dried using rotatory evaporator. The same method was also followed for preparing different extracts from the most potent plants. The crude extract of each plant was weighed and kept for biological tests [14,15]. The desired weight of each extract was dissolved in dimethyl sulfoxide (DMSO) (Sigma).

***In vitro* Bioassay Screening:** This test was assayed according to the method described by Jiwahinda *et al.* [16]. *S. mansoni* worms were washed several times in sterile RPMI-1640 media (Cutilab) (pH 7.5, with HEPES 20 mM and supplemented with penicillin (100 U/ml), sterptomycin (100 mg/ml) and 10% fetal calf serum (Gibco).

In 35 mm diameter (35 X 10 mm) polystyrene petri dish, ten adult *Schistosoma mansoni* worms were cultured in 10 ml sterile RPMI-1640 media with descending concentrations of plant extracts (400µg/ml, 40 µg/ml and 4 µg/ml) at 37 °C for 24 hrs. In parallel, the adult worms were cultured in RPMI-1640 media containing 10% DMSO (Served as solvent control) or 1 nmol PZQ (Served as standard drug control). The efficacy of different concentrations of plant extracts, motility and viability of worms, was observed (Determined or evaluated) using a stereomicroscope at different time intervals starting from 1 hour, 3 hours and 24 hours of incubation [10].

Phytochemical Screening: The methanol extracts of the five active plants were subjected to phytochemical investigations to detect their chemical constituents. Tests for sterols and/or triterpenes, carbohydrates and/or glycosides, flavonoids, tannins, alkaloides and/or nitrogenous bases and saponins were carried out following standard methods [14, 17-19].

Toxicity: Adult normal CD1 Swiss albino mice weighting 20 ± 2 gm were used to study the acute toxicity of *Allium sativum*. A pilot trial was conducted using a limited number of animals to determine the range limits of acute lethal dose. According to this data 36 normal mice were randomly divided into groups each comprised 4-6 mice. These groups received gradual increasing oral dose of *Allium sativum* (*A. sativum)* starting from 2 g/kg to 10 g/kg and the later dose didn't show any mortality [20].

***In Vitro* Study**

Infection of Animals and Experimental Design: Forty mice were infected with 80 *S. mansoni* cercariae/mouse using tail immersion method of Oliver and Stirewalt [21] and were divided into 4 groups (10 mice each). *Group I:* Infected untreated mice. *Group II:* 3 weeks post infection, mice were orally treated with 300 mg/mouse of *A. sativum* extract for 2 consecutive days. *Group III:* 7 weeks postinfection, mice were orally treated with 300 mg/kg *A. sativum* extract for 2 successive days. *Group IV:* Mice were orally treated with praziquantel (500 mg/kg/2 days) at 7 weeks post infection. Eight weeks post infection animals were sacrificed and subjected for parasitological assessment.

Assessment of Parasitological Criteria: Worm burden: Hepatic and portomesenteric vessels were perfused to recover worms for subsequent counting [22].

Tissue Egg Load: The number of ova/gm hepatic or intestinal tissue was counted after digestion overnight in 5% KOH [23].

Percentage egg developmental stages "Oogram pattern"

The percentages of eggs at the developmental stages were examined in three samples/mouse and the mean of each stage/animal was obtained [24].

Statisitical Analysis: Results were expressed as means ± standard deviation of the means (SD). Differences between groups were analyzed by using one way analysis of variance (ANOVA). The data were considered significant if p values were equal to or less than 0.05. Statistical analysis was performed with the aid of the SPSS computer program (Version 10 windows).

RESULTS

***In vitro* Bioassay Screening:** The 5 methanolic extracts extracted from 5 local plants, belonging to 10 families, were chosen to test their antischistosomal impactness on *S. mansoni* worms *in vitro*. Five plant extracts [*Dryopteris filix*mas (*D. filix* mas), *Tanacetum vulgare* (*T. vulgare*), *Juglans nigra (J. nigra)*, *Syzygium aromaticum (S. aromaticum)* and *Allium sativum (A. sativum*)] showed significant variation as antischistosomal activity as shown in Table 1. Culture of adult *S. mansoni* worms with 400 µg/ml of these 5 plant extracts led to immediate shrinking of worms, continuous muscle contraction and death which ranged from 75% to 100% of worms within the first and three hours after incubation were observed specially with *Allium sativum* plant extract (Fig. 1 A&D). The same results were recorded with 400 µg/ml after 24 hrs in all five extract, giving highest antischistosomal activity with *A. sativum* plant extract. At 40 µg/ml continuous muscle contraction and death which ranged from 50 % to 80% of worms within the three and 24 hours after incubation were observed specially with *A. sativum* plant extract (Fig. 1B). While at 4 µg/ml moderate antischistosomal activity was observed only with *A. sativum* plant extract after 24 hrs.On the other hand, 1 nmol of PZQ showed complete death of all worms (Fig. 1C).

Phytochemical Screening: The phytochemical screening tests of the five active methanolic extracts (Table 2) guided with review of literature of these plant species were carried out to detect the nature and amount of their chemical constituents, which may be responsible for the antischistosomal potency. *Dryopteris filix* mas, *Tanacetum vulgare*, *Juglans nigra*, *Syzygium aromaticum* and *Allium sativum* showed high content of triterpenoid saponin glycosides. (*Dryopteris filix* mas, *Tanacetum vulgare* showed also moderate amount of phenolic compounds. *Juglans nigra* showed high contents of phenolic compounds whereas *Syzygium aromaticum* showed major contents of diterpenoid compounds and moderate amounts of phenolic compounds.

Table 1: *In vitro* antischistosomal activity of different concentrations of methanolic extract of the applied plants at different time intervals of incubation.

	Antischistosomal activity of different concentrations of methanol extract								
	400 µg/ml			40 µg/ml			4 µg/ml		
Plant No.	*1 hr*	*3 hrs*	*24 hrs*	*1 hr*	*3 hrs*	*24 hrs*	*1 hr*	*3 hrs*	*24 hrs*
1	+++	+++	+++	-	+	++	-	-	++
2	-	+	+	-	-	-	-	-	-
3	-	+	+	-	-	-	-	-	-
4	-	+	++	-	-	-	-	-	-
5	-	++	++	-	-	++	-	-	+

+++ High antischistosomal activity 1- *Allium sativum* 2- *Syzygium aromaticum*
++ Moderate antischistosomal activity 3- *Juglans nigra* 4- *Tanacetum vulgare*
+ Low antischistosomal activity 5- *Dryopteris filix*mas

Table 2: Phytochemical investigation of the active plants showing the presence and ratio of chemical constituents.

	Presence of chemical constituents				
Phytochemical test	*Allium sativum*	*Dryopteris filix*mas	*Tanacetum vulgare*	*Juglans nigra*	*Syzygium aromaticum*
Sterols and/or triterpenes	++	+	+	++	+
Carbohydrates and /or glycosides	++	+	++	+	+
Phenolic compounds	++	+	+	+	+
Saponins	+++	+	+	+	+
Alkaloides and/or nitrogenous bases	±	±	±	±	+

+++ High amounts ++ Moderate amounts + Low amounts
± Minor amounts

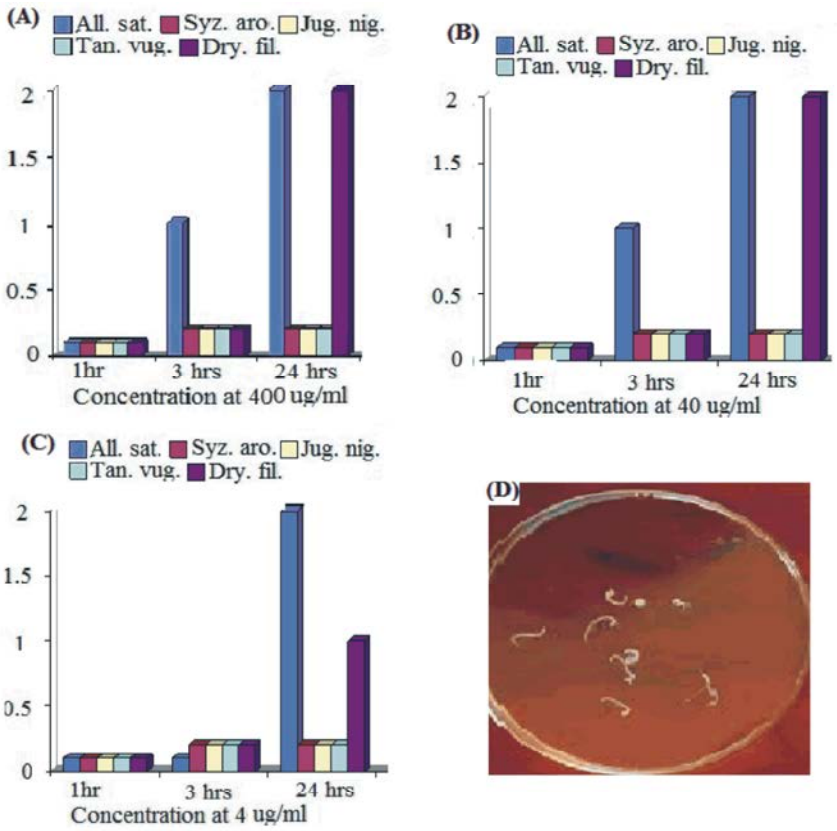

Fig. 1: *In vitro* antischistosomal activity of different concentrations of methanolic extract of the applied plants (A): 400 µg/ml, (B): 40 µg/ml and (C): 4 µg/ml at different time intervals of incubation. (D): Culture of adult *S. mansoni worms*.

Table 3: Effect of *Allium sativum* (3 & 7 weeks postinfection) and PZQ (500 mg/kg/2 days, 7 weeks postinfection) on worm load and tissue egg load.

Groups (n= 9)	Worm load	Tissue egg load in	
		Liver (X 10^3)	Intestine (X 10^3)
Group I Infected control	27.2 ± 3.7	23.6 ± 4.2	41.8 ± 6.2
Group II *Allium sativum* (3 wks PI)	19.7 ± 4.6[a] (27.6%)	17.8 ± 2.9[a] (24.6%)	29.6 ± 5.7[a] (29.2%)
Group III *Allium sativum* (7 wks PI)	21.3 ± 4.1[a] (21.7%)	20.7 ± 3.3 (12.3%)	34.2 ± 6.3 (18.2%)
Group IV PZQ (7 wks PI)	1.6 ± 0.6[a] (94.1%)	9.2 ± 2.4[a] (60.6%)	6.7 ± 1.3[a] (84%)

PI=postinfection

[a]p< 0.05 relative to control group.

Table 4: Effect of *Allium sativum* (3 & 7 weeks postinfection) and PZQ (500 mg/kg/2 days, 7 weeks postinfection) on oogram pattern

Groups (n= 9)	Oogram pattern		
	Immature	Mature	Dead
Group I Infected control	65.3 ± 5.4	31.1 ± 3.4	3.6 ± 0.4
Group II *Allium sativum* (3 wks PI)	56.9 ± 4.8[a]	37.7 ± 4.3[a]	6.4 ± 1.5
Group III *Allium sativum* (7 wks PI)	41.5 ± 5.2[a]	43.9 ± 5.6[a]	13.6 ± 3.9[a]
Group IV PZQ (7 wks PI)	2.3 ± 0.4[a]	1.9 ± 0.2[a]	95.8 ± 4.9[a]

PI=postinfection

[a]p< 0.05 relative to control group.

Assessment of Parasitological Criteria: Concerning *in vivo* study, there was a significant decrease in worm load in groups treated with *Allium sativum* either 3 or 7 weeks postinfection when compared with infected control group (p< 0.05) with a percent reduction 27.6% and 21.7%, respectively. Tissue egg load showed a significant decrease in both hepatic and intestinal egg load in both *Allium sativum* groups when compared with control group (p< 0.05) with a percent reduction 24.6%; 29.2% and 12.3%; 18.2%, respectively (Table 3, Fig. 2).

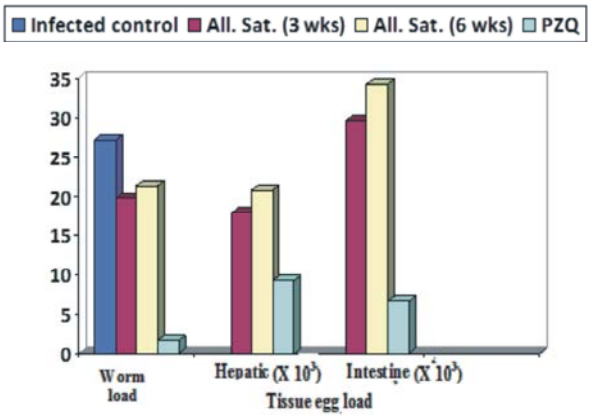

Fig. 2: Effect of Allium sativum (3 & 7 weeks postinfection) and PZQ (500 mg/kg/2 days, 7 weeks postinfection) on worm load & tissue egg load.

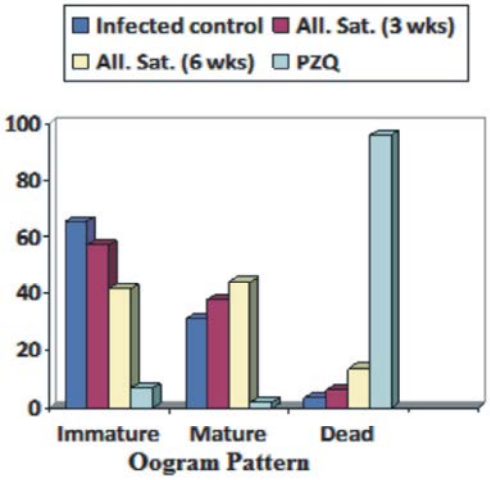

Fig. 3: Effect of Allium sativum (3 & 7 weeks postinfection) and PZQ (500 mg/kg/2 days, 7 weeks postinfection) on oogram pattern.

Also, the oogram pattern showed a significant difference ($p< 0.05$) when compared to the infected control group (Table 4, Fig. 3).

On the other hand, PZQ was found to reduce significantly the worm burden by 94.1% and tissue egg load (Liver and intestine) (60.6% and 84%, respectively) with nearly complete absence of immature ova and increase in dead ova.

DISCUSSION

Plants provided a rich source of biologically active compunds which played a significant role in control of many diseases such as schistosomiasis, malaria, filariasis and fascioliasis etc…. [25, 26].

In vitro results revealed that the applied plant extracts (*Dryopteris filix* mas, *Tanacetum vulgare*, *Juglans nigra*, *Syzygium aromaticum* and *Allium sativum*) showed high antischistosomal activity and *Allium sativum* showed the highest percentage of mortality (100%) after 1 hour.

It had been long recognized that the biological activity appeared to be found in certain plants more than others. Also, plants of the same family might possess different degrees of activities against the target organisms. The variation in activity of different extracts might be due to the different nature and amount of active components released with various solvents used in the extraction processes [27].

From phytochemical screening tests, results in the present study showed that the methanolic extracts of *Dryopteris filix* mas, *Tanacetum vulgare*, *Juglans nigra,* had high contents of triterpenoidal saponin glycosides and these results had been previously reported [28-30].

Also results in the present study were in agreement with those of Kumar *et al*. [31] who reported that natural products of triterpenoidal glycosides isolated from the bark of *Schina araentea* (Theaceae) were found to be effective as an oral anthelmenthic agent. Also the triterpenoidal glycoside saponins from *Hedera Helix* (Araliaceae) killed the liver flukes *Fasciola hepatica* at concentration around 5 µg/ml *in vitro* [32]. For *Polygonum sp.* the basic components responsible for its biological activity were related to its high contents of phenolic compounds; flavonoides and anthraquinones [33]. The *Euphorbea peplus* had been used as a traditional medicine for the treatment of skin conditions including warts and cancer [34]. The activity of this genus and related species was found to be due to its high contents of diterpenoid compounds concentrated in its milky secretions [35].

Carvalho *et al.* [36]studied the effect of different concentrations of *Euphorbia* (Family) species on different life cycle stages of *S. mansoni* and reported that it had high toxic effect against them. Abdel-Hamid [37] studied the effect of different concentrations of latex extract of *Euphorbia* (Family) *in vitro* and reported that it had mollluscidal and schistosomicidal effect.

Concerning *in vivo* study, *Allium sativum* reduced worm burden by 27.6% and 21.7% when administered 3 or 7 weeks postinfection, respectively, accompanied by a parallel reduction in hepatic and intestinal tissue egg load and in the oogram pattern when compared with the control group; but this reduction was not comparable with that produced by PZQ.

In fact, the efficacy of *Allium sativum as* an antischistosomal agent was not studied before but some authers studied their effecacy as anti-inflammatory and antioxidant agents and found that low concentration of *Buddleja* species had anti-inflammatory and antioxidant properities due to its content of flavonoids, triterpenoids, diterpenoids and caffeic acid derivatives [38, 39].

The difference between *in vitro* and *in vivo* results might be due to several factors including both host factors and the nature of the parasite itself. Host secretions might change the activity of the functional groups of the plant extracts. On the other hand, parasites contained a variety of somatic antigens, some of which were stage specific and transitory in nature and others persisted throughout the life cycle of the parasite and might continually stimulate a diversity of immunological responses. Host responses to parasite antigens were complicated by the fact that many epitopes shared antigenic moieties not only with other infectious agents but also with antigens of the host [40]. Antibodies did not normally bind to the surface of schistosomes. The surface of schistosomes acquired erythrocytes and other host antigens, which were thought to prevent host antibodies from binding to the tegument of the worms [41]. These factors together might lead to such difference in the *in vitro* and *in vivo* results. PZQ treatment *in vitro* and *in vivo* exposed antigens on the tegument of mature and developing schistosomes [42] appeared to interact with the lipid constituents of the tegumental membrane and caused membrane destabilization including contraction of muscles, increased permeability of the surface membrane to cations such as calcium, vacuolation of the tegument, depolarization of the worm tegumental resting potential and derangement of glucose metabolism [43].

The phytochemical screening tests gave a preliminary idea about the natural product classes which might be responsible for activity, but it is recommended to separate, isolate and identify the pure chemical constituents of each plant. This process also must take place according to bioassay-guided fractionation, separation and isolation.

In conclusion, *Allium sativum* was found to be effective *in vitro* but it was less effective than PZQ *in vivo*. It is recommended to use a cocktial of plant extracts either alone or combined with PZQ to increase the efficacy of complete elimination of worms and ova.

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