Impact of plant extracts as molluscicides agent against *Biomphalaria alexandrina* snails and *Schistosoma mansoni*

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Abstract—In the present study, the efficacy of 6 different species of medicinal plants belonging to 5 different families against *Biomphalaria alexandrina* snails and *Schistosoma mansoni* stages were examined. During screening test of water suspension and methanol extract, results revealed that *Oreopanax reticulatum* (Family: Araliaceae) had the strongest molluscicidal activity against *Biomphalaria alexandrina* snails followed by *Azadirachta Indica A Juss* (Maliaceae) and *Dizygotheca kerchoveana* (Family: Araliaceae). Methanol extracts revealed more molluscicidal potency as compared to water suspension. Also, the effect of methanol extracts of *Oreopanax reticulatum, Azadirachta Indica A Juss* (Meliaceae) and *Dizygotheca kerchoveana* on the survival rate and cercarial production of *B. alexandrina* infected with *Schistosoma mansoni*, as well as on free living stages of *S. mansoni* (miracidia, cercariae and worm) were studied. The results show that the sublethal concentrations of methanol extract of these plants caused a considerable reduction in the survival rate of the snails and in the infectivity of *S.mansoni* miracidia to the snail. Reduction in the number of cercariae per snail during the patent period and in the period of cercarial shedding was also, observed. The prepatent period for snails exposed to sublethal concentrations of the tested plants during their exposure to miracidia has been shortened. The mortality rate of miracidia and cercariae and adult worms were elevated gradually by increasing the exposure period to methanol extract of these plants.

Key word — Plant extracts, molluscicidal, *Schistosoma mansoni*, *Biomphalaria alexandrina*

1 INTRODUCTION

Schistosomiasis is one of the most dangenous trematode diseases infecting human beings in tropical and subtropical areas of the world where it affects more than 900 million people, 600 million of them are probably at risk (1). The problem of schistosomiasis in Egypt became more complicated in recent years due to the introduction of *Biomphalaria glabrata*, which has been reported to hybridize with *B. alexandrina*. Both the introduced and the hybrid snails, also pose a threat with respect to *S. mansoni* transmission (2). Transmission of schistosomiasis is dependent on the specificity between host and parasite. *Bulinus, Biomphalaria* and *Oncomelania* spp. are the intermediate hosts for *S. heamatobium, S. mansoni* and *S. japonicum*, respectively.

The use of molluscicides in the control of snails had a significant effect in reducing both incidence and prevalence of schistosomiasis brought about by devastating reduction of the intermediate snail host population. Economic and ecological considerations increasingly the use of molluscicides that are selectively active, biodegradable, inexpensive and readily available in the affected areas. The imported synthetic molluscicides are high in their cost and toxicity for human, fish and domestic animals (3). In view of these disadvantages, increasing attention is currently given to plant molluscicides hoping that they may prove safe, cheap, easily available and simply applicable agents.

The use of plants with molluscicidal properties appears to be simple and nonexpensive alternative to chemical molluscicides (4). More than 1000 plant species have been screened for molluscicidal activity (5). In Egypt, screening of local plants for molluscicidal activity has received increasing attention (6,7, 8,9,10 ,11& 12).

The present work was planned to study the effect of sublethal concentrations of the most promising plant extracts on the mortality rate of *B. alexandrina* and their rates of infection with *S. mansoni* miracidia, in addition to cercarial production of infected snails, as well as on the free living stages (miracidia and cercariae) and adult worms of the parasite.

2 MATERIALS AND METHODS

2.1 Snails

The snails used in the present work were *B. alexandrina*, They were collected from irrigation canals not previously treated with any molluscicide nearby Abu–Rawash area, Giza province, Egypt. They were kept and examined for *S.mansoni* infection. Collected snails from field (noninfected snails) were kept in glass or plastic containers (50X30X20 cm) with transparent covers as 100 snails per each aquarium for 3 weeks before use to accommodate the laboratory conditions.

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2.2 Miracidia, cercaria and albino mice

S. mansoni ova, miracidia and cercaria were obtained from Schistosome Biological Supply Centre, Theodor Bilharz Research Institute (SBSC/TBRI), Cairo, Egypt.

Male inbred swiss CD1albino mice(Mus musculus), six- to eight-week-old, and maintained at standard laboratory care. Animal handling have been carried out according to the internationally valid guidelines and ethical conditions.

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2.3 The plants

The plants used in this study were *Oreopanax reticulatum*, *Dizygotheca kerchoveana* (Araliacaea) and *Ricinus communis* (Euphorbiaceae) were obtained from Orman garden, Egypt. *Azadirachta Indica A Juss* (Maliaceae) were obtained from El kanater –Egypt. *Matricaria recutita* (Asteraceae) and *Eclibta alba* (compositae) were obtained from Sinai- Egypt) These plants were kindly identified by specialists at the Botany Department, Faculty of Science, Cairo University. The whole overground parts of these plants were left to dry in air and then in an oven at 50°C and powdered by a mixer.

2.4. Preparation of Plant extracts

2.4.1. Water suspension.

To investigate the potency of water suspension for the whole over ground parts of each plant, weighed amounts of powdered material were added to 1000 ml of dechlorinated tap water to make the desirable series of weight / volume concentrations

2.4.2. Methanol extracts

Groups of each plant powder were exhaustively extracted with methanol (70%) by soaking at room temperature (25 ±1-3°C) for one week. The solvent was distilled off under vacuum and the crude extract residues were assayed as aqueous solutions.

The efficacy of the tested plant extract and water suspension against adult snails was determined according to the standard procedure recommended by WHO (13). 1965). LC_{10}, LC_{25}, LC_{50} and LC_{90} were previously determined in the laboratory according to the method of Litchfield & Wilconxin (14). LC_0 was determined as 1/10 LC_{50} (13).

2.4.3. Efficacy of tested plants on the infection rate of *B. alexandrina* with *S. mansoni* miracidiai

The effect of sublethal concentrations of methanol extract of *O. reticulatum, A. Indica* and *D. kerchoveana* on the infection rate of *B. alexandrina* with *S. mansoni* miracidia and cercarial production were examined by exposing group for each plant of 50 noninfected snails individually to a dose of 10 miracidia/snail and maintained in each concentration of the tested plant (LC_0, LC_{10}, LC_{25}) for 24 hours at room temperature (24 ± 2°C) and ceiling illumination. Control group of 50 snails was exposed to miracidia only without anytreatment and maintained under the same conditions. Examination of snails for cercarial shedding was carried out twice weekly.After 20 days of miracidial exposure, surviving snails were individually examined for cercarial shedding in multidishes,artificial light for 3 hr (stimulant period) and 2ml of dechlorinated tap water/snail. The product cercariae/snail were transferred to small pertridishes by poateur pipette, fixed in Boun´soln. and counted under astercomicrscope. This examin was repeated every 3 days until end of shedding.

2.4.4. Efficacy of tested plants on

2.4.4.1. Miracidia

S. mansoni ova were exposed to desk lamp for hatching at 25-27°C. One ml of dechlorinated tap water containing 25 freshly hatched miracidia was mixed with 1 ml of double concentration(this means, we tested 500 ppm we use 1000 ppm actually because addition of the same volume of water contanting the tested organisam which dilute concentrations to half) of each plant extract; three replicates from each concentration were used. Under the same conditions, 2 ml of dechlorinated tap water containing 25 nontreated freshly hatched miracidia was used as control. During exposure period mobility and mortality of miracidia were recorded at intervals of 1/4, 1/2, 3/4, 1, 1.5, 2, 2.5, 3 hr using stereomicroscopic.

2.4.4.2. Cercariae

One ml of each concentration was added in a test tube to the same volume of dechlorinated tap water containing 50 freshly shedded cercariae and mixed well (double concentration). The cercariae were observed for their mobility and mortality at different intervals. Two ml of dechlorinated tap water containing 50 freshly shedded cercariae was observed as control.

2, 5 Statistical analysis

Analysis of data was carried out applying student's t-test (15).Spiegel, 1981).

3. RESULTS

During the screening of the efficacy of the 6 tested plant spp.extracts, the results obtained (Tables1&2) revealed that the most potent water suspension extract were that of *O. reticulatum, A. Indica* and *D. kerchoveana* which have the highest molluscicidal *O. reticulatum*, *A. Indica* and *D. kerchoveana* activity (with LC_{50}) of 90, 100 and 110ppm, respectively. While that of *M. recutita, E. alba* and *R. communis* have showed the lowest molluscicidal activity with LC_{50} of 300, 350 and 6300 ppm, respectively. Therefore, *O. reticulatum, A. Indica* and *D. kerchoveana* have been selected for the present study. Methanol extract showed more toxicological activity against the tested snail as compared with the water suspension..

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Table (1) Molluscicidal activity of some plants as water suspension of the dry powder against *Biomphalaria alexandrina* snails after 24 hours of exposure under laboratory conditions.

Plant species	LC_{50} ppm	Confidence limit	LC_{90} ppm	LC_{25} ppm	LC_{10} ppm	LC_{0} ppm	Slope
O. reticulatum	90	64.3-126	180	45	20	9	2.1
A. Indica A Juss	100	76.9-130	190	60	40	10	1.9
D. kerchoveana	110	73.3-165	280	69	60	11	2.24
M. recutita	300	157.9- 570	1400	200	100	30	3.25
E. alba	350	145.5-840	2000	215	110	35	4.36
R. communis	6300	2520-15750	17800	3800	2300	630	5.15

Table (2) Molluscicidal activity of Methanol extract of some plants, against *Biomphalaria alexandrina* snails after 24 hours of exposure under laboratory conditions.

Plant species	LC_{50}	Confidence limit	LC_{90}	LC_{25}	LC_{10}	LC_{0}	Slope
A. indica	40	25-41	70	20	13	3	2
O. reticulatum	35	29- 42	80	30	16	3.5	2.51
D. kerchoveana	40	33.3-48	98	35	16	4	2.125

Table (3): Effect of sublethal concentrations of 70% methanol extract of *O. reticulatum* plant on the infectivity of *Biomphalaria alexandrina* snails.

Concentration Ppm	Survival at 1st shedding		Infection rate of snails		Prepatent period of cercaria Range (days)		Cercarial production Number of cercaria		Shedding Duration of cercaria (days)	
	No. survival	%	No.	%	Range	mean±S.D	Range	mean ± S.D	Range	mean±S.D
LC_{0}	14	70%	11	79%	22-36	29.1±0.47**	52-118	109±10.6***	3-9	6±0.42***
LC_{10}	12	60%	9	75%	20-32	27.2±0.74**	48-110	90±9.1***	3-6	4.7±0.12***
LC_{25}	9	45%	5	55.5%%	20-29	21 ±0.31***	27-62	52±8.5***	3-4	2,8±0.11***
Control	18	90%	16	88.8%	25-60	42.5±0.39	6-47	200±35	4-28	17±0.33

Table (4): Effect of sublethal concentrations of 70% methanol extract of *A. indica* plant plant on the infectivity of *Biomphalaria alexandrina* snails.

Concentration Ppm	Survival at 1st shedding		Infection of snails		Prepatent period range		Cercarial production		Shedding Duration	
	No. survival	%	No.	%	Range	Main±SD	Range	Main±S.D	Range	Main±S.D
LC_{0}	16	80%	13	81.3%	23-42	32.5±0.31**	68-132	128±19.7***	4- 8	6.3±0.12***
LC_{10}	14	70%	11	78.6%	26-38	30±0.314**	58-120	101±14.1***	3-7	5.1±0.128***
LC_{25}	11	55%	7	63.6%	27-34	29.2±0.44***	30-78	65±9.2***	3-5	3.8±0.166***
Control	18	90%	16	88.8%	25-60	42.5±0.39	125-243	200±35	4-28	17±0.33

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The LC_{50} values were 30 ppm, 40 ppm and 40 ppm for the methanol extract of *O.reticulatum, A. Indica, D. kerchoveana* plants, respectively.

From tables (3, 4 and 5), it is clear that, the snails exposure to LC_{25} of the three tested extracts were more significantly effective (P<0.01). The survival rate of *B.alexandrina* snails exposed to LC_{25} of *O. reticulatum, A. Indica* and *D. kerchoveana* extract was showed 45, 55, and 60% at first shedding, respectively, compared to 90% for the control group.

The result in tables (3, 4 and 5) revealed that the exposure of *B. alexandrina* to *S. mansoni* miracidia at sublethal concentrations had a positive effect on infection rates. The infection rate of *B. alexandrina* snails to *S. mansoni* miracidia exposed to LC_{25} of *O. reticulatum*, *A. Indica* and *D. kerchoveana* extract was 55.5%, 63.6% and 75%, respectively. These rates were significantly lower (P<0.01) than that of the control group (88.8%). The results showed that, infection rate significantly decreased (P<0.01) with the increase of concentrations of tested plant extracts. The infection rate showed percentages of 79%, 75%and 55.5% for the doses LC_0, LC_{10} and LC_{25} of the methanol extract of *O. reticulatum* respectively as shown in table (3). The rate was lower than that of the control (88.8%). A similar finding was observed for snails exposed to sublethal concentration of *A. Indica and D. kerchoveana* (tables 4 and 5).

The present results in Tables (3,4&5) indicated that, exposure of *B. alexandrina* continuously to the tested plants extract during miracidia exposure have shortened significantly (P<0.05), their prepatent period compared to their corresponding control. The prepatent periods of snail groups exposed to LC_{25} of *O. reticulatum, A. Indica, D. kerchoveana* plants were 21 ±0.31, 29.2±0.44 and 31.5±0.44 days, respectively, in comparison to 42.5±0.39 in the control group.

production throughout its entire life span compared to the control (P<0.001). The total number of cercariae exposed to LC_{25} of *O. reticulatum, D. kerchoveana* and *A. Indica* was 52±8.5, 65±9.2 and 74±11.1respectively, compared to the control group 200±35

Data given in tables (6, 7 and 8) indicated that, sublethal concentrations of the three plant extracts had a miracidial effect after one hour of exposure. However, at 500 ppm, *O. reticulatum* exhibit a harmful effect (100%) after 30 min, while the same concentration of *A. Indica* and *D. kerchoveana* gives 100% mortality after 45 min compared to 1% in the control group. As the concentration decreases, the harmful effect decreases. The most effective plant extract as miracicidal is of *O. reticulatum* and *A. Indica*. The recorded LC_{50} value for 3 hours for *O. reticulatum, A. Indica* and *D. kerchoveana* extract on *S. haematobium* cercariae were 40, 42 and 52 ppm respectively (Table 9).

The exposure of cercariae to the plant extracts (Tables10,11 and12) at they started to die after 45 minutes of exposure to 62.5 ppm of *O. reticulatum*, *A. Indica* and *D. kerchoveana* where 20%,15 % and 12% mortalities took place, respectively. The mortality rate was increased gradually by increasing the exposure period. It became 65, % 60 and 50% for 62.5 ppm of *O. reticulatum, A. Indica* and *D. kerchoveana* after 3 hours of exposure, respectively compared with 15% for control group.The high concentration (500 ppm) of *O. reticulatum* kills cercariae after 1.5 hr, while *A. Indica* gives 100% mortality after 2 hr and *D. kerchoveana* does the same act after 3 hr with comparison of control group (15%). The recorded LC50 value for *O. reticulatum, A. Indica* and *D. kerchoveana* on *S. mansoni* cercariae were 45, 35 and 60 for 3 ours respectively (Table 13).

Data given in tables (14, 15, and 16) revealed that, treated

Table (5): Effect of sublethal concentrations of methanol extract of D. kerchoveana plant on the infectivity of B. alexandrina snails.

Conc ppm	Survival at 1st shedding		Infection of snails		Prepatent period of cercaria (days)		Cercarial production		Shedding Duration of cercaria (days)	
	No.	%	No.	%	Range	Main±S.D	Range	Main±S.D	Range	Main±S.D
LC0	17	85%	15	88.2%	23-44	33.5±0.32**	77-153	130±22.3***	3-11	7.2±0.207***
LC10	15	75%	13	86.6%	27-39	33±0.315**	62-131	110±17.1***	4-7	5.9±0.175***
LC25	12	60%	9	75%	28-35	31.5±0.44**	34-89	74±11.1***	3-5	4.1±0.235***
Control	18	90%	16	88.8%	25-60	42.5±0.39	125-243	200±35	4-28	17±0.33

- p<0.05, ** p<0.01, *** p<0.001.

The results in tables (4, 6 and 8) indicated that, duration of cercarial shedding was significantly decreased in all groups of *B. alexandrina* tested with sublethal concentrations of the three tested plant extracts in comparison with the control group. The shortest period of cercarial shedding was observed for snails treated with LC_{25} of *O. reticulatum, A. Indica* and *D. kerchoveana* being 2,8±0.11, 3.8±0.166 and 4.1±0.235 days compared to 17±0.33 days for the control group (P<0.001).
Tables (3, 4 and 5) revealed that, treatment of snails with three tested plants during their exposure to *S. mansoni* miracidia resulted in highly significant reduction of total cercarial

worms died after 1.5 hr of exposure to 500 ppm of *D. kerchoveana* . While *A. Indica* gives the same effects after 2 hr. *O. reticulatum* has the lowest schistosomicidal effect on the adult worm at 500 ppm (95%) in comparison with control (10%). Mortality was gradually increased by the increase in the period of exposure and concentrations. In miracidia, cercaria and worms experiments, most of treated and control snails died after 24 hours. The recorded *LC_{50} value for 3 hours for O. reticulatum, A. Indica* and D. kerchoveana extract on *S. mansoni* cercariae were 70, 30 and 45 ppm respectively (Table 17).

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Table (6): Effect of sublethal concentrations of methanol extract *of O. reticulatum* on *Schistosoma mansoni* miracidia

Conc. (ppm)	% mortality of miracidia after the following intervals (hours)								
	1/4	1/2	3/4	1	1.5	2	2.5	3	24
62.5 ppm	20	25	40	50	70	75	85	100	100
125 ppm	40	55	65	70	85	100	100	100	100
250 ppm	60	70	80	90	100	100	100	100	100
500 ppm	80	100	100	100	100	100	100	100	100
Control	0	0	1	2	3	3	5	6	100

Table (7): Effect of sublethal concentrations of methanol extract of *A. indica* on *Schistosoma mansoni* miracidia

Conc. (ppm)	% mortality of miracidia after the following intervals (hours)								
	1/4	1/2	3/4	1	1.5	2	2.5	3	24
62.5 ppm	10	20	35	40	60	70	80	95	100
125 ppm	30	50	60	65	80	95	100	100	100
250 ppm	50	60	70	80	90	100	100	100	100
500 ppm	80	90	100	100	100	100	100	100	100
Control	0	0	1	2	3	3	5	6	

Table (8): Effect of sublethal concentrations of methanol extract of *D. kerchoveana* on *Schistosoma mansoni* miracidia

Conc. (ppm)	% mortality of miracidia after the following intervals (hours)								
	1/4	1/2	3/4	1	1.5	2	2.5	3	24
62.5 ppm	5	10	30	35	50	60	80	90	100
125 ppm	10	30	45	60	70	90	100	100	100
250 ppm	30	50	65	70	85	100	100	100	100
500 ppm	60	85	100	100	100	100	100	100	100
Control	0	0	1	2	3	3	5	6	100

Table (9): Miracidiacidal effect of 70% methanol extract of *O. reticulatum,A. indica* and *D.kerchoveana* after 3 hr of exposure in vitro.

Plant name	LC50 ppm	LC90 ppm	Slope
O. reticulatum	40	65	2.48
A. indica	42	70	2.33
D. kerchoveana	50	88	2.49

Table (10): Effect of sublethal concentrations of methanol extract of *O. reticulatum* on *Schistosoma mansoni* cercariae

Conc. (ppm)	% mortality of cercariae after the following intervals (hours)								
	1/4	1/2	3/4	1	1.5	2	2.5	3	24
62.5 ppm	6	12	20	30	40	55	60	65	100
125 ppm	10	20	25	35	50	60	75	80	100
250 ppm	20	30	35	55	70	75	85	90	100
500 ppm	50	70	85	90	100	100	100	100	100
Control	2	3	6	6	8	10	10	15	100

Table (11): Effect of sublethal concentrations of methanol extract of *A. indica* on *Schistosoma mansoni* cercariae

Conc. (ppm)	% mortality of cercariae after the following intervals (hours)								
	1/4	1/2	3/4	1	1.5	2	2.5	3	24
62.5 ppm	3	8	15	25	35	45	50	60	100
125 ppm	8	15	20	30	45	50	70	75	100
250 ppm	15	25	30	45	60	65	75	85	100
500 ppm	40	65	80	85	90	100	100	100	100
Control	2	3	6	6	8	10	10	15	100

Table (12): Effect of sublethal concentrations of methanol extract of *D. kerchoveana* on *Schistosoma mansoni* cercariae

Conc. (ppm)	% mortality of cercariae after the following intervals (hours)								
	1/4	1/2	3/4	1	1.5	2	2.5	3	24
62.5 ppm	2	6	12	20	30	40	45	50	100
125 ppm	6	10	15	20	35	40	50	60	100
250 ppm	10	20	25	40	50	60	70	80	100
500 ppm	20	40	50	60	70	85	95	100	100
Control	2	3	6	6	8	10	10	15	100

Table (13): Cercariacidal effect of 70% methanol extract of *O. reticulatum, A. indica* and *D. kerchoveana* after 3 hr of exposure in vitro.

Plant name	LC_{50} ppm	LC_{90} ppm	Slope
O. reticulatum	39	60	2.3
A. indica	35	90	2.4
D. kerchoveana	60	105	2.41

Table (14): Effect of sublethal concentrations of methanol extract of *O. reticulatum* on Adult worm of *Schistosoma mansoni*

Conc. (ppm)	% mortality of cercariae after the following intervals (hours)								
	1/4	1/2	3/4	1	1.5	2	2.5	3	24
62.5 ppm	7	10	18	20	30	45	50	80	100
125 ppm	10	15	20	25	40	50	60	95	100
250 ppm	25	30	40	45	50	80	90	100	100
500 ppm	40	45	70	80	100	100	100	100	100
Control	1	3	5	6	8	10	12	15	90

Table (15): Effect of sublethal concentrations of methanol extract of A. indica on Adult worm of Schistosoma mansoni

Conc. (ppm)	% mortality of cercariae after the following intervals (hours)								
	1/4	1/2	3/4	1	1.5	2	2.5	3	24
62.5 ppm	4	8	10	20	25	35	45	76	100
125 ppm	8	15	20	25	35	40	50	90	100
250 ppm	20	25	30	40	50	60	80	100	100
500 ppm	35	40	60	70	90	100	100	100	100
Control	1	3	5	6	8	10	12	15	90

ble (16): Effect of sublethal concentrations of methanol extract of *D. kerchoveana* on Adult worm of *Schistosoma mansoni*

Conc. (ppm)	% mortality of cercariae after the following intervals (hours)								
	1/4	1/2	3/4	1	1.5	2	2.5	3	24
62.5 ppm	3	7	10	15	20	30	35	60	100
125 ppm	6	10	15	20	30	40	45	70	100
250 ppm	15	20	25	35	45	50	75	90	100
500 ppm	30	40	50	60	85	95	100	100	100
Control	1	3	5	6	8	10	12	15	90

Table (17): Schistosomicidal effect of 70 methanol extract of *O. reticulatum*, *A. indica* and *D. kerchoveana* after 3 hr of exposure in vitro.

Plant name	LC_{50} ppm	LC_{90} ppm	Slope
O. rectituta	70	125	2.1
A. indica	30	79	1.8
D. kerchoveana	45	55	2.4

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4. DISCUSSION

Screening of the molluscicidal activity of six selected plants was worked out, namely *O. reticulatum, A. indica, Di. kerchoveana, E. alba, M. rectituta,* and *R. communis*. The results indicated that among these plants, *O. reticulatum,* had the strongest molluscicdal activity against *B. alexandrina* snails followed in descending order by *A. indica a*nd *D. kerchoveana*. The high molluscicidal activity of *O. reticulatum,, A. indica* and *D. kerchoveana* are apparently attributed to the high concentration of active constituent saponin and Flavonid. This is supported by Shoeb et al., (16), Mansour et al., (17), Bakry et al., 8 and 18, Abdel Kader (19). Based on the LC_{50} values after 24-hours exposure period, methanol extract of the tested plants administered more molluscicidal potency as compared with water suspension. This finding, however, may be attributed to several factors including plant specific differences in active gradients, type of the natural products, differences in their mode of action, method of penetration and the behavioral characteristic affect on the snails (7,10 & 20).

The infectivity of *S. mansoni* miracidia was greatly reduced by methanol extracts. The reduction rate increased with the increase of sublethal concentrations. This may be interpreted as the sublethal concentrations of methanol extracts of the three tested plants have weakened the ability of the miracidia to penetrate the tissues of the snails. This agreed with Bakry et al. (8), observation on *B. alexandrina* snails infected by *S. mansoni* miracidia and subjected to LC_{25} methanol extract of *Euphorbia lacteal*. El-Emam et al. (21), reported that 50 ppm of *Calendula micrantha* decreased the infection rate. Tantawy et al. (22), using *Solanium dubium* plant, Barky et al. (18) using *Agave franzosinii* , Mohamed et al. (23) using Abamectin, Sharaf El-Din et al.(24), using *Zygophyllum simplex* plant and Bakry et al. (20) using methanol extract of *O. reticulatum* and *Furcraea selloea* revealed similar conclusion. These results also were in accordance with many investigations that used various chemical and plant molluscicides (7, 25, 26, 27) . Thus, El-Ansary et al. (21) reported that *A. maritima* caused a remarkable decrease in cercarial shedding and cercarial production in *B. alexandrina* snails treated with this plant powder. Sharaf El-Din et al. (24), obtained similar reduction in cercarial shedding and cercarial production from *B. alexandrina* treated with sublethal concentrations of aqueous suspension of *Zygophyllum simplex*.

Regarding the prepatent period, the present results showed that the prepatent period for snails exposed to sublethal concentrations of the tested plants during their exposure to miracidia has been shorted. This was in accordance with Mahmoud (25) using sublethal concentrations of Abamectin, Tantawy et al. (22), using S. dubium and Bakry et al. (18) using *A. franzosinii*.

The present results showed that duration of cercarial shedding was shorter for tested snails than their corresponding control. This shortening in cercarial shedding period may be due to rapture of snail's tissues through miracideae penetration that caused an increase in the harmful effect of these treatments. Such stress could disturb the physiological activities of treated snails and result in shorter life span and shedding period in comparison with their control groups (25). Similar findings have been reported by Mahmoud (25), Mohamed (23) and Barky et al. (18). The authors found that, the period of cercarial shedding in snails treated with experimental molluscicides during their exposure to miracidia are significantly shorter than that in control snails. It is also agreed with Badawy (28) who found that, exposure of *B. alexandrina* to 50 ppm from F. cretica during exposure to *S. mansoni* miracidia decreased the duration of cercarial shedding.

The present data claimed that the mean number of cercariae produced by each infected *B. alexandrina* previously tested with plants throughout its life span was significantly less than that revealed by Badawy (28) on *B. alexandrina* snails treated with *F. cretica* at the early prepatent period. El-Emam et al. (21), found that, 0.050g/L from the dry powder of *C. micrantha* for 24hr highly reduced the cercarial production from infected *B. alexandrina* treated at early and late prepatent period. Adewunmi et al. (29) recorded that the active compound aridanin isolated from T. tetraptera at 0.25 m/mg reduced *S. mansoni* cercarial shedding. The same result was observed by Ahmed and Ramzy (30), Tantawy (22), Bakry et al. (18), Mostafa and Tantawy (31), Sharf El-Din et al. (24) and El-Ansary et al. (32). The authors used the plants *S. nigrum, S. dubium, A. arvensis, A. franzosinii, Z. simplex and P. harmula,* respectively against *S. mansoni* cercarial production inside the soft tissues of *B. alexandrina*.

From the previous results, it was clear that, *B. alexandrina* snails exposed to sublethal concentration of *O. reticulatum, A. indica, D. kerchoveana* plants were less susceptible to the infection with *S. mansoni* miracidia. Also, the cercarial production decreased during the entire life of the infected snails treated with the extracts than those infected and untreated. These results declared that, such sublethal concentrations when used in the field could suppress the development of *S. mansoni* inside snails and shorten the duration of cercarial shedding. It also could induce decrease in the number of cercariae produced by infected snails and thus, can interrupt parasite transmission.

Concerning the effect of sublethal concentrations of methanol extract of the tested plants against *S. mansoni* miracidia ,cercariae and adult worm. The present results showed that mortality rates of *S. mansoni* miracidia , cercariae and adult worm were increased by increasing sublethal concentrations of the tested plants as well as by the period of exposure. Therefore, when these plants are used in snail control, they will kill the free-living larval stages of *Schistosoma.*

Previous studies on the plants Tetra pleura (33), Zingber offionale (29), F. cretica and A. filifera (28), *A. arvensis, A. lophantha* and *B. murciata* (34), *A. arvensis and C. mircantha* (31), *A. franzoinii* (18) and *E. peplus* (35) against *S. mansoni* miracidia and cercariae revealed similar conclusion. It was concluded from the present study that methanol extract of the three plants have toxic effects against *B. alexandrina* at various sublethal concentrations after 24 hours as well the free living larval stages (miracidia and cercariae) and adult worm of *S. mansoni*. Also, *O. reticulatum* was the most effective on the snail activity compared to the other two plant extracts namely *A.*

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indica and *D. kerchoveana*.

In conclusion, In the present study, *O. reticulatum*, *D. kerchoveana* and , *A. indica* extracts are the most poomising natural products. As mol, *A. indica*, molluscicidal either as water suspension or methanol extract. They have a miracicidal and cercacicidal but *D. kerchoveana* and *A. indica* extracts have the most powerful effect as schistosomicidal.

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