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LABORATORY EVALUATION OF THE MOLLUSCICIDAL, MIRACIDICIDAL AND CERCARICIDAL PROPERTIES OF TWO EGYPTIAN PLANTS

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اثبت التقييم الاولي للمعلق المائي للبودرة الجافة لخمسين نباتا مصريا ضد قواقع بيومفلاريا الكسندرينا (العائل الوسيط للبلهارسيا المعوية في مصر) ان ثمار نبات سابيندس سابوناريا (عائلة سابيندسي) واوراق وسيقان نبات باديليا اسياتيكا (عائلة لوجانيسي) ذات قدرة ابادية عالية حيث كانت الجرعة المميتة هي ٩٠ و١٨٠ جزء في المليون للنباتين علي التوالي بعد فترة تعريض ٢٤ ساعة. وأيضا تم اختبار كفاءة مستخلصات الاثير البترولي والبنزين والكلوروفورم وخلات الاثيل والاسيتون والميثانول لكل من النباتين ضد نفس القواقع أظهرت النتائج ان اكثر من مستخلص لثمار السابيندس سابوناريا لهم قدرة ابادية ضد القواقع بينما مستخلص الميثانول فقط لنبات باديليا له قدرة ابادية. أظهرت الأعمار المختلفة من القواقع حساسية متدرجة تجاه المستخلص الميثانولي للنباتين ومن جهة أخرى اظهر الفحص الفيتوكيميائي الاولي لكلا النباتين ان الصابونين هو المادة الفعالة الرئيسية في النباتين ولهذا فقد تم تحضير الصابونين الخام لكلا النباتين وتمت تجربتهم وكانت الجرعة القاتلة لهما هي ١٩ و١١ جزء في المليون. وأيضا تمت تجربة المستخلص الميثانولي لكلا النباتين ضد السركاريا والميراسيديا للبلهارسيا المعوية ظهر ان تركيز ٩٠ جزء في المليون من مستخلص نبات باديليا فعال بدرجة عالية ضد الأطوار الحرة للطفيل بعد ساعة واحدة بينما تركيز ٤٥ جزء في المليون من مستخلص نبات سابيندس غير فعال ضد هذه الاطوار. وأيضا لم يؤثر كلا المستخلصين علي معدل فقس بيض البلهارسيا المعوية. ويجري الان فصل كروماتوجرافي للمكونات الكيميائية لكلا النباتين باستخدام طرق الفصل الكروماتوجرافية المختلفة.

Screening the aqueous suspensions of the dry powders of 50 Egyptian plants against Biomphalaria alexandrina snails, the intermediate host of Schistosoma mansoni in Egypt, revealed that the fruits of Sapindus saponaria Burm. (Family Sapindaceae) as well as the leaves and stems of Buddleia asiatica Lour. (Family Loganiaceae) have high molluscicidal activities (LC_{90}= 90 and 180 ppm for the two plants respectively) after 24 hours exposure times. Also, the petroleum ether, benzene, chloroform, ethyl acetate, acetone and methanol extracts of each plant were separately tested against the same snail species. Results showed that methanol, acetone, ethyl acetate and chloroform extracts of S. saponaria have activities whereas only the methanol extract of B. asiatica was active. Different ages of snails showed variable susceptibility towards the methanol extracts of both plants. Phytochemical investigation of the two plants was carried out and revealed that saponins are the major constituents in both plants so it may be responsible for the molluscicidal effectiveness of the two plants. To confirm this conclusion, the crude saponins of each of the two plants were prepared and they recorded very strong potency against B. alexandrina at 19 and 11 ppm. Also, the larvicidal potencies of the methanolic extract of each plant was tested against S. mansoni cercariae and miracidia. B. asiatica extract was lethal to both larvae at 90 ppm while 45 ppm of S. saponaria was not larvicidal at this concentration. However none of the methanol extracts of the two plants inhibited the hatchability of S. mansoni ova. Now, the two plants will be submitted to different chromatographic techniques to separate their active ingredients.

INTRODUCTION

Schistosomiasis (Bilharzia) is one of the most widespread parasitic infections to man in tropical and subtropical areas including Egypt.[1] Schistosomiasis control usually takes place through two main means, chemotherapy and snails control.[2] Snail control is achieved mainly by using molluscicides. The numerous disadvantages of using synthetic molluscicidal

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agents stimulated endemic area to find a safe alternative mean, which is plant molluscicides.[3]

Medicinal plants regained their real position in the last two decades as a vital remedy for many diseases. This could be due to the fact that most of the synthetic drugs possess serious side effects, while using medicinal plants is more safe. Therefore, most countries all over the world paid a great attention to the production, manufacture and use of medicinal plants.[4,5]

One approach for using plant molluscicides in controlling snails intermediate host of schistosomiasis, is treating both snails and infectious larvae (cercariae and miracidia). This complementary methods is suggested by many researchers as the snails and the larvae shares the same aquatic habitats.[6-10]

In a continuing search for new plants for the control of the vectors of schistosomiasis, this work aims to evaluate some local Egyptian plants for their molluscicidal properties in the hope that these plants can be practically applied in field. Phytochemical screening of the active plants was carried out. Also, the promising plants were preliminary tested against *S. mansoni* cercariae and miracidia.

MATERIAL AND METHODS

Plant material

Samples of 50 plant species growing in different localities in Egypt were collected. They were kindly identified and classified by Mrs. Traes Labib, general manager and head of specialist for plant Taxonomy in El-Orman Botanical Garden, Giza, Egypt and with the help of standard references.[11,12] Voucher specimens of the plants were kept at department of Medicinal Chemistry. These plants were dried, powdered by electric mill.

Organisms

Wild *Biomphalaria alexandrina* snails (4-6 mm), the intermediate host of *Schistosoma mansoni*, were collected from canals in Giza Governorate, Egypt. The snails were maintained in aquaria filled with dechlorinated water under laboratory conditions (Temp. 25±2° and pH 7-7.7). Snails were examined for trematode infection and infected ones were excluded.

Cercariae, eggs and miracidia of *Schistosoma mansoni* were freshly obtained from experimentally infected *B. alexandrina* snails and homogenized liver of laboratory infected mice respectively.[13]

Experimental procedures

Preparation of biological agents

The biological agents examined in this study are the water suspensions, the extracts and the crude saponins and were prepared as follows: The fine dry powder of each plant was used as a water suspension in the experimental tests on the bases of weight/volume using preliminary serial concentrations up to 400 ppm.

The dry powder (150 g) of each active plant; *Sapindus saponaria* and *Buddleia asiatica;* was directly extracted by methanol, acetone, ethyl acetate, chloroform, benzene and petroleum ether (60-80°). Each extract was evaporated to complete dryness and kept for biological tests.

The crude saponins of the two plants were prepared from the methanol extract by defatting with petroleum ether then successively extracted with chloroform, ethyl acetate and finally partitioned between water and n-butanol. The butanolic layer was evaporated to dryness to yield the crude saponin.

Molluscicidal assay

Different dilutions (ppm) were prepared from the dry powder of each plant as aqueous suspensions using dechlorinated water. Also, gradual concentrations of each extract and crude saponin of the active plants were separately prepared on the bases of weight/volume. The exposure period was 24 followed by 24 hours recovery period and standard procedures were followed through this study.[14] Statistical analysis of the data were carried out according to Litchfield and Wilcoxon method.[15].

In comprehensive screening tests, wild four age-stages of *B. alexandrina* snails were used. Juvenile (2-3 mm), premature (4-6 mm), adult (8-11 mm) and old snails (13-15 mm) were exposed to *S. saponaria* and *B. asiatica* methanol extracts. Also *S. mansoni* -infected snails (4-6 mm)were exposed to the methanol extracts of each plant.

Phytochemical tests

The methanolic extracts of the fruits of *S. saponaria* and leaves & stems of *B. asiatica* were subjected to phytochemical tests to detect the presence of different components.[16-18]

Cercaricidal and miracidicidal assay

In this test LC_{90} (45 and 90 ppm) of *S. saponaria* and *B. asiatica* were prepared and about 200 freshly obtained cercariae or miracidia were mixed with these extracts. The observation periods were ½, 1, 1½, 2 after which the percent of mortality was recorded using stero-microscope.[7,19]

Effect on hatchability of *S. mansoni* eggs

The molluscicidal concentrations; 45 ppm of *S. saponaria* and 90 ppm of *B. asiatica* methanol extracts were supplied with *S. mansoni* eggs. The hatched miracidia were counted using stereo microscope. The hatchability was calculated as the ratio of hatched miracidia to that of the total No. of eggs.20

RESULTS AND DISCUSSION

The Preliminary molluscicidal screening of the aqueous suspensions of 50 local plants belonging to 29 families collected from different places (Table 1) proved that only two plants possess molluscicidal effects; *Sapindus saponaria* (Sapindaceae) and *Buddleia asiatica* (Loganiaceae) (100-200 ppm) whereas all other plants (48 species) did not show any molluscicidal potency against *B. alexandrina* snails up to 400 ppm.

Comprehensive screening carried out on the promising plants proved that different parts of *S. saponaria* showed variable attitudes towards snails. Only the fruits of *S. saponaria* exhibited strong molluscicidal activity (LC_{90}= 90 ppm), whereas the leaves and stems did not show any activity up to 400 ppm after 24 hours exposure. As to *B. asiatica* the leaves and stems exhibited moderate potency at 180 ppm (Table 2).

Molluscicidal screening of different extracts of the two plants (Table 3) proved that methanol, acetone, ethyl acetate and chloroform extracts of *S. saponaria* showed variable activities at LC_{90}= 45, 35, 37 and 100 ppm for the four extracts respectively whereas benzene and petroleum ether extracts of this plant did not show any effect up to 100 ppm. The methanolic extract of *B. asiatica* had high activity at LC_{90}= 90 ppm whereas other organic solvent extracts did not show any activity up to 100 ppm.

The crude saponins of both plants exhibited very remarkable potency with LC_{90} values 19 and 11 ppm for both plants respectively.

Table (4) indicate the comparative susceptibility of different ages of snails to the methanol extracts of the two plants. Results showed that juvenile snails are very sensitive to the action of both extracts as their LC_{90}= 29 and 63 ppm while the premature snails is the most tolerant stage. The infected snails proved to be slightly more susceptible than non-infected snails.

Preliminary phytochemical screening of the methanolic extracts of the two plants (Table 5) revealed that saponins are the principal components in these plants beside other natural product categories which are present with different proportions.

Testing the LC_{90} of the methanolic extracts of the two plants on cercariae and miracidia of *S. mansoni* showed that the methanolic extract of *B. asiatica* gave 100% mortality of cercariae after only 1/2 hr exposure time and 100% mortality of miracidia after 1 hr whereas *S. saponaria* methanol extracts did not show any activity against the two larvae within 2 hrs (Table 6). Also observing the hatchability of *S. mansoni* eggs exposed to *S. saponaria* and *B. asiatica* extracts showed that the ratio of hatched miracidia to the total number of eggs was 80% after an observation period of 1/4 hr which is identical with that of the control group.

Screening some of the rich Egyptian flora for molluscicidal and larvicidal activities against schistosomiasis; the most abundant disease in Egypt; is recommended.

From the obtained data it can be concluded that the active ingredients responsible for the molluscicidal potency of *S. saponaria* is found in the fruits and not in the leaves and stems. Many plant molluscicides showed that molluscicidal effects are restricted

Table 1: Preliminary molluscicidal screening of some Egyptian plants against *B. alexandrina* snails after 24 hours exposure.

Plant Species by Family	Part tested	Percent mortality of snails at concentrations (ppm)			
		400	300	200	100
Amaranthaceae					
Amaranthus viridis Desf.	Whole plant	-	-	-	-
Asteraceae					
Matricaria recutita L.	Whole plant	-	-	-	-
Senecio desfontainei Druce	Whole plant	-	-	-	-
Apocynaceae					
Catharanthus rosus L. G. Don	Leaves	-	-	-	-
Nerium oleander L.	Leaves	-	-	-	-
Bignoniaceae					
Kigelia pinnata DC.	Leaves	-	-	-	-
Chenopodiaceae					
Beta vulgaris L.	Leaves	-	-	-	-
Compositeae					
Solidaster luteus L.	Leaves & flowers	-	-	-	-
Aster squamatus tourn.	Leaves & flowers	-	-	-	-
Wedelia trilobara L. A. Hitchc	Leaves	-	-	-	-
Cruciferae					
Rorippa islandica Oeder	Leaves	-	-	-	-
Euphorbiaceae					
Ricinus communis L.	Leaves & fruits	-	-	-	-
Frankeniaceae					
Francoeuria crispa Cass.	Leaves	-	-	-	-
Labiatae					
Salvia splendes Jepson	Upper parts	-	-	-	-
Salvia farinacea L.	Upper parts	-	-	-	-
Mentha microphylla Koch	Upper parts	-	-	-	-
Leguminosae					
Enterolobium cyclocarbum Jacqu	Fruits	-	-	-	-
Cassia fistula L.	Leaves	-	-	-	-
Cassia glauca Mill.	Leaves	-	-	-	-
Loganiaceae					
Buddelia asiatica Lour	Leaves & stems	100	100	100	30
Malvaceae					
Hibiscus rosa L.	Leaves	-	-	-	-
Abutilon fruticosum Guill	Leaves	-	-	-	-

Table 1: Cont.

Plant Species by Family	Part tested	Percent mortality of snails at concentrations (ppm)			
		400	300	200	100
Meliaceae					
Melia azadirachta L.	Leaves & fruits	-	-	-	-
Moraceae					
Ficus benjamina Linn.	Leaves	-	-	-	-
Ficus carica Linn.	Leaves	-	-	-	-
Ficus hispida L.	Leaves	-	-	-	-
Ficus elastica Roxb.	Leaves	-	-	-	-
Myrtaceae					
Pisidium guajava L.	Leaves	-	-	-	-
Myristicaceae					
Myristica fragrans Houtt	Fruits	-	-	-	-
Punicaceae					
Punica granatum L.	Leaves	-	-	-	-
Polygonaceae					
Rumex vesicarius L.	Upper parts	-	-	-	-
Rosaceae					
Rubus sanctus Schreb.	Leaves & stems	-	-	-	-
Rutaceae					
Glycosmis pentaphylla Retz DC.	Leaves	-	-	-	-
Citrus aurantium L.		-	-	-	-
Salicaceae					
Salix tetrasperma Roxb.	Leaves & stems	-	-	-	-
Salix safsaf Forssk.	Leaves & stems	-	-	-	-
Sapindaceae					
Sapindus saponaria Burm.	Fruits	100	100	100	100
Koelrenteria pinculata Laxm.	Fruits & leaves	-	-	-	-
Harpullia pendula Planchon	Fruits & leaves	-	-	-	-
Scrophulariaceae					
Veronica anagalis L.	Leaves	-	-	-	-
Solanaceae					
Datura stromonium L.	Leaves, stems, fruits and roots	-	-	-	-
Datura tatula L.	Leaves, stems, fruits and roots	-	-	-	-
Hyoscyamus boveanus Dun	Whole plant	-	-	-	-

Table 1: Cont.

Plant Species by Family	Part tested	Percent mortality of snails at concentrations (ppm)			
		400	300	200	100
Typhaceae					
Typha domingensis Pers.	leaves	-	-	-	-
Verbenaceae					
Verbena bipinnatifida L.	leaves	-	-	-	-
Duranta repens Jacq.	leaves	-	-	-	-
Clerodendron splendens G. Don.	leaves	-	-	-	-
Zygophyllaceae					
Tribulus bimucronatum Viv.	Whole plant	-	-	-	-
Umbelliferae					
Anethum graveolens L.	Leaves & stems	-	-	-	-
Crithmun maritimum L.	Leaves & stems	-	-	-	-

Table 2: Effect of the aqueous suspensions of the dry powder of *Sapindus saponaria* and *Buddleia asiatica* against *Biomphalaria alexandrina* snails after 24 hours exposure.

Plant species	Part tested	LC_{50}	LC_{90}	S
Sapindus saponaria	Fruits	60 (53.74-68.72)	90	1.24
	Leaves	-ve up to 400 ppm		
	Stems	-ve up to 400 ppm		
Buddleia asiatica.	Leaves & stems	150 (139.41-168.31)	180	1.22

Table 3: Comparative susceptibility of different kinds of *B. alexandrina* snails exposed to *S. saponaria* and *B. asiatica* methanol extracts.

Kind/Age of snails	*Sapindus saponaria*			*Buddleia asiatica*		
	LC_{50} (ppm)	LC_{90} (ppm)	S	LC_{50} (ppm)	LC_{90} (ppm)	S
Wild-uninfected Juvenile (2-3 mm)	18 (12.5-24.6)	29	1.41	42 (38. 7-47. 5)	63	1.28
Premature (4-6 mm)	32 (27.9-36.58)	45	1.27	60 (50. 21-70. 42)	90	1.21
Adult (8-11 mm)	30 (25.8-36.7)	42	1.32	57 (51. 8-62. 4)	86	1.44
Old (13-15 mm)	24 (19.5-28.6)	35	1.39	52 (46. 7-59. 4)	81	1.36
Infected (4-6 mm)	29 (24.5-34.8)	41	1.28	56 (51. 32-64. 53)	85	1.45

Table 4: Effect of some extracts of *Sapindus saponaria* fruits and the leaves and stems of *Buddleia asiatica* against *B. alexandrina* snails after 24 hours exposure.

Extract	*Sapindus saponaria*			*Buddleia asiatica*		
	LC_{50} (ppm)	LC_{90} (ppm)	S	LC_{50} (ppm)	LC_{90} (ppm)	S
Methanol	32 (27.97-36.48)	45	1. 27	60 (50.21-70.42)	90	1. 21
Acetone	26 (22.53-30.58)	35	1. 25	-ve up to 100 ppm		
Ethyl acetate	27 (23.78-32.41)	37	1. 27	-ve up to 100 ppm		
Chloroform	63 (52.31-73.51)	100	1. 31	-ve up to 100 ppm		
Benzene	-ve up to 100 ppm			-ve up to 100 ppm		
Pet. Ether	-ve up to 100 ppm			-ve up to 100 ppm		
Crude saponins	16.5 (13.72-17.56)	19	1.45	8.3 (6.33-9.81)	11	1.48

Table 5: Preliminary phytochemical screening of the methanol extracts of *Sapindus saponaria* and *Buddleia asiatica.*

Constituents	*S. saponaria*	*B. asiatica.*
- Sterols and / or triterpenes	++	++
- Flavonoids	±	+
- Tannins	±	+
- Carbohydrates and / or glycosides	++	++
- Saponins	+++	+++
- Alkaloids and / or nitrogenous bases	±	±

+++ Large amount of constituents. ++ Moderate amount of constituents.
+ Small amount of constituents. ± Rare amount of constituents.

Table 6: Effect of 45 ppm of *Sapindus saponaria* and 90 ppm of *Buddleia asiatica* methanol extracts on *Schistosoma mansoni* cercariae and miracidia after different intervals of time.

Biological test		*S. saponaria*	*B. asiatica*	Control
% mortality of cercariae after:	½ hr	0. 0	100	0
	1 hr	0. 0	100	0
	1,1/2 hrs	0. 0	100	0
	2 hrs	0. 0	100	0
% mortality of miracidia after:	½ hr	0. 0	80	0
	1 hr	0. 0	100	0
	1,1/2 hrs	0. 0	100	0
	2 hrs	0. 0	100	0

to only one part of the plant and not the other parts.[21,22] *Sapindus saponaria* fruit was reported to possess molluscicidal effect against snails in Zanzibar.[1] The LC_{90} value of *B. asiatica* is similar to the molluscicidal effect of other *Buddleia* sp. such as *B. madgascariensis*.[23]

Different extracts of *S. saponaria* fruits were recently reported to possess various medical activities such as, antivenom and antiulcer[24,25] so it proved the safety of this plant to mammalian animals.

The lethal concentrations of the methanol extract of both plants are less than the threshold of 100 mg/liter set for a potent molluscicide by the World Health Organization[1] and as it gave a good yield, so the methanol extracts were chosen for further comprehensive investigation.

In water canals usually different stages of snails and both infected and non-infected snails are encountered. So it was necessary to test the effect of the two plants on different stages and types of *B. alexandrina* snails. In this work premature snails (4-6 mm) proved to be more tolerant to the action of the methanol extracts of the two plants than mature snails. These data are in good agreement with Ragab *et al*.[26] and El-Khayat[27], however the authors did not give an explanation to these results. It can be concluded that snails maturity and the process of oviposition consumes a lot of nourishing constituents and makes the mature snails became relatively more vulnerable to the toxic action of molluscicides. It is reasonable that the sensitivity of juvenile and old snails to the toxic action of the plants can be attributed to the fact that juvenile snails are delicate while old ones are senile. Also infected snails proved to be more sensitive to the effect of the extracts than non-infected snails as invading of snails with miracidia causes various metabolic disorders.[28]

Phytochemical tests of both plants revealed that they are rich in saponins and this conclusion is in good agreement with Ribeiro *et al*.[29] as they isolated 3 saponin compounds from *S. saponaria* pericarp. Many classes of natural compounds are reported sometimes to be responsible for the molluscicidal properties of the active plants.[30] However the most abundant molluscicidally active compounds seems to be saponins[4,31] and it is in good accordance with the present work.

Also, the responsibility of saponins about the molluscicidal potency would explain the mode of action of these plants on snails as it was noticed that dead snails diffuse their blood in the surrounding media. Both Lemmich *et al*.[32] and Sindambiwe *et al*.[33] correlated between the haemolytic activity and the high molluscicidal properties of saponins.

The cercaricidal and miracidicidal tests of the plants proved that miracidia are slightly less susceptible to the action of *B. asiatica* than cercariae. This finding is in full agreement with previous results.[34] The very quick action of *B. asiatica* methanol extract on both larvae (1/2-1 hr) proves that the larvae is much more sensitive than snails to this extract. This finding is in good agreement with Mendes *et al*.[6]. The absence of larvicidal effect of *S. saponaria* is in good accordance with Mansour *et al*.[35] as they reported that some molluscicidal plants gave negative results in testing their cercaricidal activities.

Finally it is worthy to mention that Mansour *et al*.[35] stated that the cercaricidal and miracidicidal activities of plants having molluscicidal effect add further advantages to such plants. As these plants also possess strong effects on different stages of snails so it may have a future as a potent molluscicides. Moreover, continuous biological studies and a bioactivity-guided fractionation to isolate and identify their active components will be carried out Knowing that only few compounds have been identified so far from *S. saponaria*.[29]

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