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Research paper

Molluscicidal Activity of Some Sudanese Medicinal Plants Against Vector Snails of *Schistosoma mansoni*

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Abstract

Plants remain an abundant resource of phytochemicals useful for reserving human health and improving the environment where he lives. Molluscicides of plant origin are suggested to be a suitable, cheap, and environmentally friendly alternative for expensive and environmentally destructive synthetic molluscicides used for control and elimination of harmful snails such as those work as intermediate host of schistosomiasis disease. This study was carried out to investigate five Sudanese plants for their molluscicidal activity against the snail *Biomphlaria pfeifferi* the intermediate host of the parasite *Schistosoma mansoni*, the causative agent of intestinal schistosomiasis disease in Sudan. In an exploratory experiment, snails were exposed to serial concentrations of 1000, 500, and 250 ppm of 80% ethanolic extracts of the plants for 24 hours. Accordingly, the plants were further investigated by use of concentrations; 100, 50, and 25 ppm. Lc50 and Lc90 were calculated. Plants were subjected to preliminary phytochemical screening. All plants showed Lc90 less than 250 ppm. Leaves of *Combretum glutinosum* Perr. ex DC. showed activity by Lc50, and Lc90 of 117.57, and 220.84 ppm, respectively, fruits of *Solanum dubium* L. of 153.02, and 226.62 ppm, and remaining three plants male inflorescences of *Hyphaene thebaica* (L.) Mart., aerial parts of *Indigofera oblongifolia* Forssk, and aerial parts of *Rhynchosia minima* (L.) DC. showed similar potency by Lc50, and Lc90 of 158.11, and 228.11 ppm. The findings of the study suggest further phytochemical investigation so as to isolate and identify the active ingredients and also to be investigated for other different bioactivities.

Keywords: medicinal plants, molluscicidal activity, schistosomiasis, *Schistosoma mansoni*, *Biomphlaria pfeifferi*.

الفعالية الحيوية لبعض النباتات الطبية السودانية ضد القواقع العائلة لطفيل شيستوسوما مانسوناي

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المستخلص

تظل النباتات مصدر وفير للمركبات الكيميائية ذات الفائدة في المحافظة على صحة الإنسان وتحسين بيئته التي يعيش فيها. تُطرح مبيدات القواقع من أصل نباتي كونها زهيدة الثمن وصديقة للبيئة كبديل للمبيدات الصناعية غالية الثمن والمدمرة للبيئة أثناء استخدامها لإبادة أو السيطرة على القواقع الضارة كتلك التي تعمل كعائل وسيط للطفيل المسبب لمرض البلهارسيا. أُجريت هذه الدراسة بغرض إختبار فعالية خمسة نباتات سودانية ضد القواقع بيومفلاريا فيفيري (*Biomphlaria pfeifferi*) العائل الوسيط لطفيل شيستوسوما مانسوناي المسبب لمرض البلهارسيا المعوية في السودان. في تجربة إستكشافية تعرضت القواقع لتراكيز متسلسلة من مستخلصات الإيثانول 80% وهي 1000، 500، و250 ملجرام/مل وذلك لمدة 24 ساعة. من ثم أختبرت الفاعلية بتراكيز أقل وهي 100، 50، و25 ملجرام/مل لمدة 24 ساعة. كذلك أُخضعت النباتات قيد الدراسة لمسح كيميائي أولي. التركيز القاتل ل 90% من القواقع كان أقل من 250 ملجرام/مل لجميع النباتات. التراكيز القاتلة ل 50% و90% من القواقع التي أظهرها مستخلص أوراق النبات *Combretum glutinosum* Perr. ex DC. كانت 117.57 و220.84 ملجرام/مل علي التوالي، لثمار النبات *Solanum dubium* L. كانت 153.02 و226.62 ملجرام/مل، أما الثلاثة مستخلصات المتبقية وهي للأعضاء الذكرية لنبات *Hyphaene thebaica* (L.) Mart، الأجزاء الهوائية من نبات *Indigofera oblongifolia* Forssk.، والأجزاء الهوائية من نبات *Rhynchosia minima* (L.) DC. فقد كانت درجة الفعالية متطابقة بتراكيز 158.11 و228.11 ملجرام/مل علي التوالي. نتائج هذه الدراسة تقترح إختبارات كيميائية أكثر عمقا وذلك بغرض استخلاص وتعريف المركبات الفعالة، كما تقترح الدراسة إختبار هذه النباتات في فعاليات حيوية أخري.

كلمات مفتاحية: نباتات طبية، فاعلية علي الرخويات، بلهارسيا

Introduction

The interest in molluscicides of plant origin historically goes back to 1930s when Archibald and Wagner advocated planting the desert palm, *Balanites aegyptiaca* and *B. maughamii*, along the water courses in Sudan and Southern Africa, respectively. The interest increased based on the philosophy of self-reliance, low cost, and saving of hard currency once the evolved plants are indigenous to the endemic areas of schistosomiasis (McCullough *et al*., 1980; Kloos and McCullough, 1982).

Since then numerous species of plant belonging to the different families have been investigated for their molluscicidal activity. Among these families some showed significant molluscicidal activity such as *Phytolaccaceae*, *Fabaceae*, *Caesalpiniaceae*, *Combretaceae*, *Rubiaceae*, *Olacaceae*, *Meliaceae*, *polygonaceae*, *Asteraceae*, *Euphorbiaceae*, and *Rhamnaceae* (Adewunmi, 1999; Kloos and McCullough, 1982).

The majority of the plants showed activity at the beginning of this trend are saponins containing and fish poisoning plants such as *Phytolacca dodecandra* (*Phytolaccaceae*), *Sapindus saponaria* (*Sapindaceae*), *Bobgunnia madagascariensis* (*Leguminosae*), *Diospyros zombensis* (Ebenaceae), and *Balanites aegyptiaca* (*Zygophylaceae*). However other sort of natural products belonging to phenolics and alkaloids classes were found active against mollusk (Adewunmi *et al*., 1989).

Many Sudanese plants have been investigated for their molluscicidal activity by a number of scientists; Amin *et al*. (1972), El-Kheir and El-Tohami (1979), El-Kheir and El-Tohami (1980), Ayoub (1982), Ahmed *et al*. (1984), Ayoub (1984), Ahmed *et al*. (1985), Ayoub (1985), Bashir *et al*. (1987), Ahmed *et al*. (2005), Osman *et al*. (2007a), EL-Kamali *et al*. (2010), Abdalla *et al*. (2011), and Ismail *et al*. (2016).

Schistosomiasis (clinically known as bilharzia) is one of the diseases considered by the World Health Organization (WHO) as neglected tropical diseases associated to poverty. It is remaining as one of the major public health problem affecting millions of people around the world. About 78 countries around the world suffering from the infection of the disease, 52 out of them are requiring preventive chemotherapy. With estimation of 250 million people infected annually, 91.4% out of them live in Africa. Up to 779 million people are at risk of infection (WHO, 2016; Toor *et al*., 2018). The global burden of schistosomiasis is estimated at 1.9 million disability-adjusted life years (DALYs) (French *et al*., 2018). This estimation is limited to the morbidity caused by schistosomiasis excluding mortality from other illnesses may associate to schistosomiasis like bladder cancer, cirrhosis, and colon cancer. Other estimation regarded these consequences of schistosomiasis raised (DALYs) to 24 – 29 million (Inobaya *et al.*, 2014).

During the last three decades there has been a serious increase in distribution of schistosomiasis in Sudan because of population movement, lack of governmental commitment, and failure of measures used to control the disease. It has been estimated that more than eight million people are at risk of infection with schistosomiasis (Cha *et al.*, 2019).

Schistosomiasis is a parasitic disease caused by digenetic nematode worms of the genus Schistosoma. The seven causative species for the disease in humans are *Schistosoma haematobium*, *Schistosoma japonicum*, *Schistosoma mansoni*, *Schistosoma intercalatum*, *Schistosoma mekongi*, *Schistosoma malayensis*, and *Schistosoma guineensis*. All species are causative for intestinal schistosomiasis except *S. haematobium* which is causative for urogenital schistosomiasis (McManus *et al*., 2018, WHO, 2013). However, *S. haematobium* and *S. mansoni* are the responsible species for the human schistosomiasis in Sudan (Elsammani *et al*., 2019).

The life cycle of the parasite involves a specific intermediate host snail for each species of schistosoma. Intermediate host snail releases the infective stage of the parasite cercaria in the fresh

water where it meets and infects the human definitive host comes in contact with contaminated water.

Breaking the life cycle by snail control is one of the effective strategies for control and elimination of schistosomiasis. Biological, environmental, and chemical controls are used for this purpose. Chemical control utilizes synthetic molluscicides such as nicolsamide or molluscicides of natural origin especially those extracted from plants.

This study is concerned with the investigation of the molluscicidal activity of some Sudanese medicinal plants against the fresh water snail *Biomphlaria pfeifferi* the intermediate host of the parasite *Schistosoma mansoni* the causative agent of intestinal schistosomiasis in Sudan.

Materials and methods

Plant material

Collection

The five Plants under study (Table 1) were collected from River Nile state except *Combretum glutinosum* Perr. ex DC. which was collected from South Kordofan state during the year 2018. They were brought to the herbarium of Medicinal and Aromatic Plants and Traditional Medicine Researches Institute (MAPTMRI), Khartoum, Sudan. They were authenticated and identified by the taxonomists in the herbarium.

Extraction of Plants

Amount of 50 grams of each plant was extracted by maceration in 500 ml of aqueous ethanol 80% in Erlenmeyer's flasks, sealed with aluminum foil, and kept for 72 hours with occasional stirring. The mixture was filtered through a filter paper and the filtrate was concentrated under reduced pressure using a rotatory evaporator. The concentrated material was kept in a petri dish of known weight and left on the lab bench for complete drying. The marc was returned to the Erlenmeyer's flask with the recovered solvent for more extraction. This process was repeated for three times to the exhaustion of the plant material. The yield percentage was calculated by weighing the extract. The dry extracts were covered and reserved in temperature of - 8^0C.

Preliminary Phytochemical Screening

Phytochemical screening was conducted according to Harborne (1984) and Sofowora (1993) with few modifications.

1. Detection of Steroids and Triterpenes

About 0.5 g of the extract washed three times with petroleum ether and dissolved in 10 ml of chloroform. To 5 ml of the solution, 0.5 ml acetic anhydride was added and then three drops of concentrated sulphuric acid at the bottom of the test tube. At the contact zone of the two liquids a gradual appearance of green, blue pink to purple color was taken as an evidence of the presence of sterols (green to blue) and/ or triterpenes (pink to purple) in the sample.

2. Detection of Alkaloids

About 0.5 g of the extract was heated with 5 ml of 2N HCl in water bath and stirred for about 10 minutes, cooled filtered and divided into two test tubes. To one test tube few drops of Mayer's reagent was added while to the other tube few drops of Valser's reagent was added. A slight turbidity or heavy precipitate in either of the tow test tubes was taken as presumptive evidence for the presence of alkaloids.

3. Detection of Flavonoids

About 0.5 g of the extract was washed three times with petroleum ether, dissolved in 30 ml of 80% ethanol. The filtrate was used for following tests:

A. To 3 ml of the filtrate in a test tube 1ml of 1% potassium hydroxide solution in methanol was added. Appearance of a yellow color indicated the presence of Flavonoids. Flavones or and chalcone.

B. To 2 ml of the filtrate 0.5 ml of 10 % lead acetate was added. Appearance of creamy turbidity was taken as an evidence of flavonoids presence.

4. Detection of Tannins

About 0.5 g of the extract washed three times with petroleum ether, dissolved in 10 ml hot saline solution and divided in two tests tubes. To one tube 2-3 drops of ferric chloride added and to the other one 2 – 3 drops of gelatin salts reagent added. The occurrence of a blackish blue colour in the first test tube and turbidity in the second one denotes the presence of tannins.

5. Detection of Saponins

A weight of 0.3 g of the extract was placed in a clean test tube. 10 ml of distilled water was added, the tube stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of foam, which persisted for least an hour, was taken as evidence for presence of saponins.

6. Detection of Cumarins

A weight of 0.2 g of the extract dissolved in 10 ml distilled water in test tube and filter paper attached to the test tube to be saturated with the vapor after a spot of 0.5N KOH put on it. Then the filter paper was inspected under UV light, the presence of cumarins was indicated if the spot adsorbed the UV light.

Molluscicidal activity assay

Collection of snails

The snails were collected during the periods from January- March and November of the year 2018 from eastern bank of the White Nile River starting from Jebal Awlia damp 40 kilometers south of Khartoum up to the center of the city at the conjunction of White Nile with Blue Nile.

The collection was done by using scooping technique with local made scoop (longitudinal stick of 150 cm length and in front a squared metal sieve 40X40 cm). The collected snails were placed in a plastic jar with wet cotton and immediately imported to indoor aquaria at the Department of Microbiology and Parasitology, MAPRI, NRC, Khartoum, Sudan.

There in the aquaria the authentication of the snail was done by the major parasitologist of the department (Dr. W.S. Koko). Only *Biomphlaria pfeifferi* species of middle age and size, and proofed to be none infected were kept to the molluscicidal studies after a week of collection day. In the meantime, snails were fed with lettuce and fresh water.

Molluscicidal activity test

Molluscicidal activity test was conducted as described by WHO, 1965. In an exploratory experiment, snails were exposed for 24 hours to serial concentrations; 250, 500 and 1000ppm in three replicates for each concentration. Then the snails were kept in de-chlorinated water for more than 24 hours for recovery and then the dead snails were counted. Plants those showed 100%

mortality in the lowest concentration (250 ppm), were tested for their potency using concentrations 100, 50, and 25 ppm.

Statistical Analysis

Microsoft Excel Programme 2010 was utilized to calculate LC_{50} and LC_{90} (lethal concentration for 50% and 90% of exposed snails) from the obtained results using linear regression equation.

Results and discussion

Table (1) shows the plants under study, family, vernacular name, used part of plant, yield percentage of the extracts and area of collection in Sudan; Table (2) represents the lethal concentrations of aqueous ethanolic (80%) extracts that killed 50% (Lc_{50}) and 90% (Lc_{90}) of snails *Biomphlaria pfeifferi*; and Table (3) represents the result of the preliminary phytochemical screening for the major groups of secondary metabolites in the plants.

Table (1): Plants under study

No.	Plant scientific Name	Family of plant	Local Name	Part of plant	Yield % (weight of extract/weight of sample)%	Area of Collection
1	*Combretum glutinosum* Perr. ex DC.	Combretaceae	Habeel Algabal	leaves	13.48	S. Kordofan
2	*Hyphaene thebaica* (L.) Mart.	Arecaceae	Doum	Male Inflorescences	8.22	River Nile
3	*Indigofera oblongifolia* Forssk.	Fabaceae	Dahseer	Aerial parts	12.08	River Nile
4	*Rhynchosia minima* (L.) DC.	Fabaceae	Adan Alfar	Aerial parts	14.33	River Nile
5	*Solanum dubium* L.	Solanaceae	Jobain	Fruits	17.16	River Nile

Table (2): Lethal concentrations of ethanolic (80%) extracts that killed 50% (Lc_{50}) and 90% (Lc_{90}) of snails *Biomphlaria pfeifferi*

No.	Plant	Part of plant	Lc_{50} ppm/ 24 hours	Lc_{90} ppm/ 24 hours
1.	*Combretum glutinosum* Perr. ex DC.	Leaves	117.57	220.84
2.	*Hyphaene thebaica* (L.) Mart.	Male Inflorescences	158.11	228.11
3.	*Indigofera oblongifolia* Forssk.	Aerial	158.11	228.11
4.	*Rhynchosia minima* (L.) DC.	Aerial	158.11	228.11
5.	*Solanum dubium* L.	Fruits	153.02	226.62

The preliminary phytochemical screening for the five plants showed presence of different groups of secondary metabolites that probable to cause the activity such as saponins, tannins, or alkaloids. However, on this level the molluscicidal activity could not be attributed to a specific group of secondary metabolites. Further phytochemical investigation is needed to specify the group or compound/s responsible for the activity.

Table (3): Preliminary phytochemical screening for the major groups of secondary metabolites in five Sudanese medicinal plants

Plant	Part of Plant	Major Groups of Secondary Metabolites							
		Steroids	Triterpenes	Saponins	Alkaloids	Flavonoids	Tannins	Cumarins	Anthraquinone glycosides
***Combretum glutinosum* Perr. Ex DC.**	Leaves	-	++	+++	-	+++	+++	++	-
***Hyphaene thebaica* (L.) Mart.**	Male inflorescences	-	-	+++	-	-	+	+	-
***Indigofera oblongifolia* Forssk.**	Aerial parts	+	+	++	-	-	+++	+	-
***Rhynchosia minima* (L.) DC.**	Aerial parts	-	-	+	-	+	+++	+	-
***Solanum dubium* L.**	Fruits	+	-	+	+	+	+	++	-

+++ = high, ++ = moderate, + = low, and - = absent

Leaves of *Combretum glutinosum* Perr. ex DC.showed activity by Lc_{50}, and Lc_{90} of (117.57, and 220.84 ppm). It showed high content of saponins, flavonoids, and tannins, medium content of triterpenes and cumarins, and lack of steroids, alkaloids, and anthraquinone glycoside.

Combretum glutinosum was reported previously to have strong molluscicidal activity against both species snails *Biomphlaria pfeifferi* and *Bulinus truncatus* (Osman *et al*., 2007b). The difference in the values of findings in the two studies may due to environmental factors such as the area of collection of plants or snails affected the response of snails. In relation to this study *Combretum glutinosum* was found to have activity against miracidia and cercaria of *Shistosoma mansoni* (Lima *et al*., 2012; Albagouri *et al*., 2014). Also the plant was found to possess antibacterial, antifungal, antiviral, and antitussive activities (Lima *et al*., 2012).

Fruits of *Solanum dubium* L. showed activity by Lc_{50}, and Lc_{90} of (153.02, and 226.62 ppm). It showed moderate presence of cumarin, low of steroids, saponins, alkaloids, and flavonoids, and devoid of triterpenes and anthraquinone glycoside.

Solanum dubium L. belongs to the family *Solanaceae* which showed significant molluscicidal activity represented by many species such as Egyptian *S. nigrum, S. villosum, and S. sinaicum* which showed activity against *Biomphalaria alexandrina* the intrermediate host of *Schistosoma mansoni* in Egypt (El-Sherbini *et al.*, 2009). Also *Solanum nigrum* was found to have activity against the snail *Galba truncatula*, an intermediate host of fasciolasis (Hammami *et al*., 2011). Glycoalkaloid extracts of *Solanum sodomaeum* and *Solanum elaeagnifolium* were found of strong molluscicidal activity against *Bulinus truncatus* the intermediate host of *Schistosoma haematobium* in Morocco (Bekkouche *et al*., 2000). Also the glycoalkaloid compounds isolated from *Solanum asperum* were found of strong molluscicidal activity against *Biomphalaria glabrata* the intermediate host of *Schistosoma mansoni* in Brazil (Silva *et al*., 2008). This suggests the molluscicidal activity of this family including *Solanum dubium* L. may be attributed to the alkaloid type of compounds work alone or synergistically with other type of secondary metabolites like saponins.

The three remaining plants which are male inflorescences of *Hyphaene thebaica* (L.) Mart., aerial parts of *Indigofera oblongifolia* Forssk., and aerial parts of *Rhynchosia minima* (L.) DC. showed similar potency by Lc_{50}, and Lc_{90} of (158.11, and 228.11 ppm).

Male Inflorescences of *Hyphaene thebaica* (L.) Mart. in this investigation showed high content of saponins, low content of tannins and Cumarins, and devoid of steroids, triterpenes, alkaloids, flavonoids, and anthraquinone glycoside. This is not far from the statement of El-Beltagi *et al*. (2018) that the secondary metabolites such as tannins, saponins, steroids, glycosides, flavonoid, and terpenes were found at low and moderate concentrations in the fruits of the plant. The fruits of this plant showed several bioactivities including antimicrobial, anti-hypertension and hyperlipidemia, management of type-2 diabetes, and as antioxidant, anticancer, and anti-inflammatory activities (Faten, 2009; Mohamed *et al*., 2010; Oduje *et al*., 2016).

Indigofera oblongifolia Forssk. showed high content of tannins, moderate of saponins, low contents of steroids, triterpenoids, and cumarins, lack of alkaloids, flavonoids, and anthraquinone glycoside.

The genus *Indigofera* which belongs to the family *Fabaceae* is found to have several biological activities such as antimicrobial, antimlarial, antioxidant, anti-hepatitis, antidiabetic, and insecticidal. *Indigofera oblongifolia* Forssk. was found to possess antimicrobial, hepatoprotective, and lipoxygenase inhibitory activities (Ur Rahman *et al.*, 2018).

Rhynchosia minima (L.) DC. also belongs to the family *Fabaceae* showed high presence of tannins, moderate presence of saponins, flavonoids, and Cumarins, and devoid of steroids, triterpenes, alkaloids, and anthraquinone glycoside.

This plant was reported to possess some bioactivities. Essential oil obtained from the leaves of this plant was found to have significant antibacterial, antifungal, and antioxidant activities (Gundidza *et al*., 2009). The antioxidant property of the plant was reported also in addition to the anthelmintic activity by Mali *et al*., 2008 and Yellasubbaiah *et al*., 2015.

Sudan because of its great area, geographical location, and variety of climate and environment, is considered as a rich country of flora which represents a huge reservoir of biologically active phytochemicals. This study as it contributes a little in this issue it recommends organized investigation for the Sudanese medicinal plants. The findings of the study suggest further phytochemical investigation so as to isolate the active ingredients and test the chemical modifications for their molluscicidal activity. Also it suggests subjecting isolated compounds and modifications to different biological assays.

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