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Original Research Article

Molluscicidal Activity of Aqueous Methanol and Hexane Fractions of *Tetrapleura tetraptera* Stem Bark against *Biomphalaria pfeifferi*

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ABSRTACT

Snail infections and parasitic diseases associated with snails such as schistosomiasis remain a problem in some endemic communities in Nigeria. Consequently, effective molluscicides are needed to control these parasites. However, frequent use of commercially available molluscicides may cause unnecessary environmental pollution. This necessitates the advocacy for natural alternatives to commercially available synthetic molluscicides. This study aimed to investigate the molluscicidal activity of aqueous methanol and hexane fractions of *Tetrapleura tetraptera* stembark against *Biomphalaria pfeifferi* snails. A total of two hundred and eighty (280) mature snails from Giri River, Gwagwalada Area Council of the Federal Capital Territory (FCT), Abuja, Nigeria, were subjected to immersion bioassay with methanol and hexane fractions of *Tetrapleura tetraptera* stembark. Dechlorinated water was used as negative control while $CuSO_4.5H_2O$ (aq) was employed as positive control. Treatment concentration ranged from 0.39 mg/L to 12.5 mg/L and snail death was measured at 24, 48, and 72 hours post-exposure. The results revealed that the aqueous methanol fraction of *Tetrapleura tetraptera* stembark have high molluscicidal activity with LC_{90} of 2.184 ± 0.61 mg/L and LC_{50} of 0.647± 0.61 mg/: after 24 hours exposure time; while the hexane extract showed no significant toxicity towards the test organism. This observation may be attributed to the presence of tannins glycosides or carbohydrates prominent in the aqueous methanol fraction and were not present in the hexane fraction. Findings from this study provide considerable basis for further exploiting the aqueous methanol fraction of *Tetrapleura tetraptera* stembark and local indigenous plants for as potential molluscicidal agents.

Keywords: Molluscicides, Mortality, *Tetrapleura tetraptera*, Dose-response, Phyto-constituent

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Introduction

Schistosomiasis is one of several neglected tropical diseases often caused by exposure to schistosome-infected water.[1] The major causative agents of schistosomiasis in humans include: *Schistosoma haematobium, Schistosoma intercalatum, Schistosoma japonicum, Schistosoma mansoni* and *Schistosoma mekongi.*[2,3] Schistosomiasis has a long history of prevalence in subtropical and tropical regions but has recently emerged in Corsica a developed region in southern France.[4] In Africa *S. mansoni* (often transmitted by the *Biomphalaria* snails) is a major cause of schistosomiasis.[5] The intermediate snail hosts for *S. mansoni* include *Biomphalaria alexandrina, Biomphalaria glabrata, Biomphalaria choanomphala, Biomphalaria pfeifferi* and *Biomphalaria sudanica.* A recent study carried out by Dawaki *et al*. indicated the prevalence of this disease in Nigeria.[6,7] The disease is endemic to several communities in Nigeria, including Abuja.

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The disease causes economic and health burden to patients and communities where it is endemic, and Nigeria is the most schistosomiasis-endemic African nation.[8] The country has a total prevalence of 95 per cent and about 24 million people are at risk of schistosomiasis.[9]

In the Federal Capital Territory (FCT), where Gwako is located, the prevalence of urinary schistosomiasis was reported at 31.30%.[10] In general, urinary schistosomiasis is a persistent health problem among children in schistosomiasis-endemic communities. Poverty is an important factor that promotes the spread of schistosomiasis in Nigeria. Furthermore, the lack of safe drinking water, poor environmental sanitation, and inadequate toilet facilities exacerbates the situation.[11] In endemic communities such as Gwako, a large proportion of the population uses unsafe stream, ponds, and other stagnant seasonal water bodies for domestic and agricultural purposes.[12] The unrestricted disposal of human waste in water sources through open defecation also contributes to the spread of diseases and vectors.[11]

Plant molluscicides are of economic importance, particularly in developing countries.[13] There is a continuous search for new plant species with ideal molluscicidal properities.[14,15] Different plants have been reported as molluscicides and in Egypt screening of local plants for molluscicidal activity has received increasing attention.[16 - 19]

Tetrapleura tetraptera is a tropical deciduous tree in the Mimosaceae family. It has distinctive four-wing fruit characteristic consisting of a woody shell, meat pulp and small brown-black seeds. The fruits have various applications in Nigerian folk medicine, and are widely used to treat a number of human diseases such as hypertension, arthritis, diabetes mellitus and epilepsy.[20]

The Igbo tribe of eastern Nigeria refers to the plant as ‘oshosho’ or ‘osakirisa’, the Yorubas call it ‘aidan’, Efik calls it ‘edeminang’, Ibibio

calls it 'Uyayak', Hausa calls it 'dawo', and also known as 'taub'. The potential use of these fruits with some of their corresponding phytochemicals was identified as toxic molluscicide, antimicrobial antiseizure and insecticide.[21] The insect-resistant characteristics of fruits have been attributed to their distinct fragrance due to their high content of essential oils.[22] The dried powdered fruits have been formulated into soap to increase antimicrobial activity and improve soap foam and hardness.[20] An infusion of the fruits is usually used to bathe to relieve fever.[23] The pulp is known to contain sugars, tannins, saponins and amino acids.[24] The pods are used as a popular seasoning spice in South eastern Nigeria and the dried fruits are used in flavouring soups particularly the traditional pepper soup a delicacy consumed by mothers from the first day of delivery to prevent post-partum contractions and as a lactation aid, and for gastrointestinal disorders especially stomach ulceration.[25 - 29] This study evaluated the molluscicidal effects of *Tetrapleura tetraptera* methanol and n-hexane stembark extracts against *Biophalaria pfeifferi* snails.

Materials and Methods

Plant collection and identification

Tetrapleura tetraptera stembark was collected from Owo, Ondo State, Nigeria in February 2024. Taxonomical identification was done at the National Institute of Pharmaceutical Research and Development (NIPRD), Idu Abuja. A herbarium specimen was deposited at the Institute, with voucher number NIPRD/H/7406.

Extraction of T. tetraptera stembark

The stembark of *Tetrapleura tetraptera* was cut into pieces and washed with distilled water to remove dirt. The sample was air-dried and pulverized using a hammer mill. Coarsely powdered stembark was then kept in airtight containers until required for further work. Crude methanol extract was obtained by exhaustive extraction of 5 kg of the powdered stembark by maceration. The crude methanol extract was partitioned with n-hexane to obtain the n-hexane fraction according to the method described by Afolayan *et al*., with slight modification.[30,31] Briefly, the coarsely powdered stembark was macerated in methanol in a stoppered glass container for 48 h with frequent agitation until all soluble matter was dissolved. Thereafter, the mixture was filtered through cotton wool to obtain crude methanol extract. The extract was mixed with n-hexane (1:1) and 10 mL of water was added. The mixture was allowed to separate into two layers which were collected in different beakers and labeled stembark methanol-water fraction (SBMe/H_2O) and stembark hexane fraction (SBHX) accordingly. The fractions were concentrated to dryness at room temperature in a fume hood.

Phytochemical analysis

Qualitative determination of major phytochemical constituents of the hexane and methanol fractions of *Tetrapleura tetraptera* was carried out according to methods described by Yadav.[32] Basically, the fractions were tested for the presence or absence of alkaloids, steroids, flavonoids, tannins, saponins, triterpennoids, glycosides, carbohydrates, phenols, and resins.

Determination of molluscicidal activity

Sampling and preservation of snails

Adult snails (*Biomphalaria pfeifferi*) were collected using the handpick and sampling net technique. The sampling area was marked and demarcated with pegs and ropes during each sampling time or visit. Sampling was done once a week during the early hours of the day. Snails were searched for near the edges of slightly deep waters, rocky surface, buried sediments or plant materials and in discarded items including plastics and nylon bags. The sampling period lasted for 2-3 hours at each visit. Snails collected from the sample site were kept in separate labeled specimen plates/tanks. The snails were brought to the Biological Science laboratory, University of Abuja, identified and placed in a small plastic container filled with de-chlorinated water and fed with lettuce.[33,34] Snails were allowed to acclimatize for three days before treatment. During the period of acclimatization, the snails were subjected to 12-hour light and 12-hour dark cycle with a daily change of water (de-chlorinated water).[34,35] Dead snails were preserved in 70% alcohol for subsequent identification and classification.

Preparation of test solutions

Different concentrations (0.39 mg/L - 12.5 mg/L) of *T. tetraptera* stembark aqueous methanol (SB-Me/H_2O) and hexane (SB-HX) fractions were prepared by weighing appropriate amount of the dry fractions into a beaker and dissolving in small amount of water. The resulting solution was transferred quantitatively into a 1000 mL volumetric flask and made to the mark with distilled water. Six concentrations: 0.39, 0.78, 1.56, 3.125, 6.25 and 12.5 mg/L were prepared. Distilled water was used as the negative control and 1000 mg/L $CuSO_4.5H_2O_{(aq)}$ was used as the positive control.

Treatment of the snails with the test solutions

Intrinsic molluscicidal activity of the test samples was evaluated using immersion bioassay against a target snail species according to the method described by WHO, 2019.[36,37] Briefly, adult snails (*Biomphalaria pfeifferi*) were starved in the course of the study. The snails were pretreated with a wide range of concentrations of the test samples (SB-Me/H_2O and SB-HX), which was thereafter narrowed down to six concentration ranges. The test concentrations (working solution) consisted of three concentration ranges that kill <50% of the test organism and three concentration ranges that kill $50\% < X \leq 100\%$ of the test organism. Thus, the final working concentrations were obtained from the least effective concentration of the preliminary molluscicidal assay, these include; 12.5, 6.25, 3.125, 1.56, 0.78 and 0.39 mg/L. A group of ten snails were placed in a 500 mL beaker, the beaker was filled with each dilution at room temperature (to expose the organism to the test solution) and covered with wire gauze to prevent snails from escaping from the test solutions. At the end of the 24-hour exposure period, snails were removed from the test solution, rinsed, and transferred to containers containing dechlorinated water; after a 24-hour recovery period, mortality was recorded. This was done by noting the number of snails that remain completely within their shells and show no movement, which were suspected to be dead and transferred to a separate container and the number of snails that were alive after 24-hour recovery period were placed in freshwater with food, and monitored for a further 48 hours.[34,35] For confirmation of death, the soft tissue of the snail was stimulated with a needle to determine if there is a contractile response. After 48 hours, the number of dead snails was recorded. This process was performed in duplicate. Similar process was followed using equal number of snails using dechlorinated water as negative control and 1000 mg/L $CuSO_4.5H_2O$ (aq) as positive control.[36]

Statistical analysis

The relationship between dose and mortality was analyzed using log-probit and logit regression analysis.

Results and Discussion

Phytochemical constituent of T. tetraptera stembark fractions

The phytochemical screening of the fractions showed the presence of alkaloids, steroids, flavonoids, saponins, triterpenoids, phenols, and resins in the hexane fraction of *T. tetraptera* stembark, while tannins, glycosides, and carbohydrates were absent. The methanol fraction was found to contain all the phytochemicals tested except resins (Table 1). The result obtained in the study is similar to previous reports in literature.[38] Methanol fraction had higher number of secondary metabolites, and this may be due to the solubility of majority of the phytoconstituents in methanol-water solvent being more polar compared to hexane.[39]

Phytochemicals such as phenols, flavonoids, saponins, steroids, terpenoids, and alkaloids present in the plant fractions are generally classified as phenolic compounds. It is well documented that phenolic compounds are one of the largest ubiquitous groups of plant secondary metabolites with immense biological properties such as antileshmania,

antiapoptotic, antiaging, anticarcinogenic, antiinflammatory, antiatherosclerotic, cardioprotective, and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferative activities.[31,32,40] Antioxidant properties of medicinal plants which are rich in phenolic compounds had been expantiated in literature, and this property has been attributed to the presence of phytochemicals like flavonoids, tannins, etc.[32,38] Tannins have been reported to modulate protein synthesis by binding to proline-rich protein. Flavonoids which are hydroxylated phenolic compound produced in plants as a result of response to external stimuli were reported to display antimicrobial properties against a wide array of microorganisms *in vitro.* The antibacterial activity of flavonoids have been credited to their capacity to complex with soluble proteins and extracellular proteins in bacterial cell wall.[32] They also are effective antioxidants and show strong anticancer activities.[38,41] Saponins are celebrated for their inhibitory effect on inflammation and ability to precipitate and coagulate the red-blood cell.[32] Steroids well-charted for their relationship with compounds such as sex hormones and alkaloids are famous for their cytotoxicity, analgesic, antisplasmodic, and antibacterial properties.[32, 43-48] Based on the results obtained from this study, the phytochemicals in the methanol and hexane fractions of *T. tetraptera* stembark may be bioactive against molluscs, as this plant has been proven to be valuable repository of bioactive compounds of significant medicinal worth.

Table 1: Phytochemical constituents of *Tetrapleura tetraptera* stembark

Metabolite	SB HX	SB (ME/H_2O)
Alkaloids	+	+
Steroids	+	+
Flavonoids	+	+
Tannins	-	+
Saponins	+	+
Triterpenoids	+	+
Glycosides	-	+
Carbohydrates	-	+
Phenols	+	+
Resins	+	-

Key: SB (ME/H_2O): Stem Bark (aqueous methanol fraction); SB HX: Stem Bark (Hexane fraction). +; indicate presence of metabolite, -; indicate absence of metabolite.

Molluscicidal activity of aqueous methanol fraction of T. tetraptera stembark

The dose-response curve for the molluscicidal activity of the methanol fraction of *Tetrapleura tetraptera* stembark after 24-hour exposure is presented in Figure 1. The result showed that with increase in concentration of the fraction, the mortality increased gradually until a threshold was reached at 6.250 mg/L. This represents the maximum concentration needed to achieve 100% mortality after 24-hour exposure, which indicate that the molluscicidal effect of *T. tetraptera* was most active at this concentration. Similar result has been reported by Joseph and co-workers, on the molluscicidal activity of oil extract of *T. teraptera.*[49] The mean molluscicidal activity of aqueous methanol fraction of *T. tetraptera* stembark against adult *Biomphalaria pfeifferi* snail is presented in Table 2. The result shows that the methanol fraction exhibited varying degrees of mortality at different concentrations and time of exposure. A 100% snail mortality was recorded at 6.25 mg/L, and 12.5 mg/L for 24, 48 and 72 hours of exposure, respectively. Similarly, 100% mortality was recorded at 1.56 mg/L, and 3.126 mg/L for 48 hours, and 72 hours of exposure, respectively. At 72 hours of exposure 0.78 mg/L of the fraction also showed 100% mortality. The study revealed that fraction at concentration of 0.78 mg/L and above gave mortality >50%, and fraction concentration 0.39 mg/L resulted in mortality <50 % (Table 2).

Table 2: Molluscicidal activity of aqueous methanol fraction of *T. tetraptera* stembark

	Percentage mortality (%)				
Fraction conc. (mg/L)	24 h	48 h	72 h		P-value
0.390	35	70	80	$LC_{50} \pm SEM$	0.007
0.780	60	95	100	0.647 ± 0.61	
1.560	85	100	100		
3.126	75	100	100	$LC_{90} \pm SEM$	0.007
6.250	100	100	100	2.184	
12.500	100	100	100		
Positive control	100	100	100		
Negative control	0	0	0		

SEM: standard error of mean.

Lower concentrations (< 0.39 mg/L) did not give 100% mortality, which indicated that *Biomphalaria pfeifferi* snails were able to withstand the toxic effect of the aqueous methanol fraction at lower concentrations. Reduced mortality observed at lower concentrations may be due to the low concentration, and the short exposure time, while higher mortality observed at higher concentrations within a short time can probably be linked to the aqueous methanol fraction toxicity, and the concentration used. Using the percentage mortalities at various concentrations for 24 hours exposure, the LC_{50} and LC_{90} of the aqueous methanol fraction was calculated to be 0.647 ± 0.61 mg/L, and 2.184 ± 0.61 mg/L, respectively using the Log-porbit/logit regression analysis.[36] The high toxicity shown by aqueous methanol fraction of *T. tetraptera*

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stembark can be attributed to the presence of higher number of plant metabolites in the fraction. The molluscicidal activity of *T. tetraptera* had been reported by Oniya and colleagues, where a 100% mortality rate of adult snails at 1.2 mg/L, and 1.6 mg/L after 48 hours of exposure.[50]

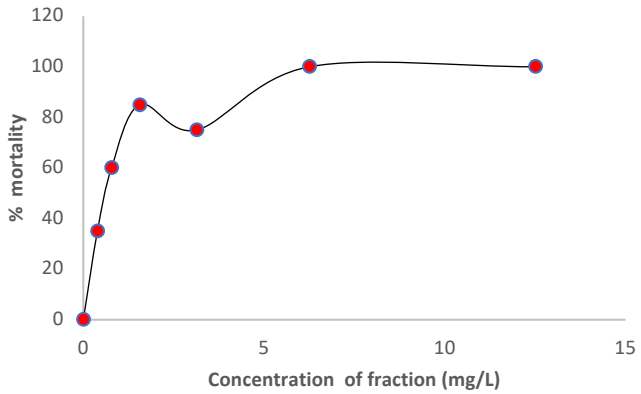

Figure 1: Dose-response curve for molluscicidal activity of aqueous methanol fraction of *T. tetraptera* stembark

Molluscicidal activity of hexane fraction of T. tetraptera stembark

The mean molluscicidal activity of hexane fraction of *T. tetraptera* stembark was expressed as adult *Biomphalaria pfeifferi* snail mortality and the results are presented in Table 3. The result shows that the hexane fraction did not exhibit any mortality at the concentrations tested, and time of exposure except for 10% mortality displayed at 1.56 mg/L. This observation indicated that *Biomphalaria pfeifferi* snails were able to withstand the toxicity of the hexane fraction at the concentrations tested. This may be due to limited number of plant metabolites present in the hexane fraction compared to the aqueous methanol fraction. Also, the absence of tannins and glycosides in this fraction may account for the observed activity as postulated in literature.[51]

Conclusion

It has been proven that botanical molluscicides are important as they are cheap, less toxic, and effective in the control of freshwater snail population, which play an essential role in the life cycle of the parasite responsible for schistosomiasis. The findings from the present study shows that the aqueous methanol fraction of *T. tetraptera* stembark could distrupt the schistosome life cycle due to its molluscicidal properties. In addition, the molluscicidal properties of the fraction increased with increase in concentration and exposure time. Indeed, the use of *T. tetraptera* plant as a molluscicides could be one of the natural means of controlling schistosomiasis and other trematode infections.

Table 3: Molluscicidal activity of hexane fraction of *T. tetraptera* stembark

	Percentage mortality (%)		
Fraction conc. (mg/L)	**24 h**	**48 h**	**72 h**
0.390	0	0	0
0.780	0	0	0
1.560	10	10	10
3.126	0	0	0
6.250	0	0	0
12.500	0	0	0
Positive control	100	100	100
Negative control	0	0	0

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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