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Molluscicidal potency of *Ficus exasperata* (Vahl) against juvenile and adult *Biomphalaria pfeifferi*

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This study was undertaken to assess the molluscicidal potency of ethanolic and aqueous extracts of roots, leaves, bark, and seeds of *Ficus exasperata* (Vahl) against juvenile and adult *Biomphalaria pfeifferi* that are intermediate hosts of schistosome species causing intestinal schistosomiasis. Snails were collected and reared in the laboratory. Plants were identified, authenticated, and phytochemically screened. Cold water and organic solvent (ethanol) extraction of different parts of the plant were carried out. The walls of the digestive tract of the snails were histopathologically assessed. *F. exasperata* (Vahl) contains alkaloids, cardenolides, anthraquinones, saponins, tannins, and flavonoids which vary in different parts. Both ethanolic and aqueous extracts were potent for snails after 24 h exposure, but ethanolic extracts exhibited significant toxic effects when compared to aqueous extracts. Bark ethanolic extract showed the highest molluscicidal potency with lethal concentration LC_{50} and LC_{90} values of 0.36 and 0.62 ppm, respectively, for juveniles. This was followed by leaf ethanolic extract with LC_{50} and LC_{90} values of 0.39 and 0.69 ppm, respectively, for adults. The juvenile and adult snails were both susceptible to the leaf aqueous extract with LC_{50} and LC_{90} values of 0.45 and 1.50 ppm, respectively, for juveniles, and 0.60 and 1.05 ppm, respectively, for adults. Seed ethanolic and aqueous extracts showed the least molluscicidal potency. Investigation of the histopathological changes following exposure to the different plant parts revealed that the extracts caused visible degeneration of the epithelial and glandular cells of the digestive tract. Eruption of mitochondria and endoplasmic reticulum was evident, the cell nuclei were atrophied and calcium cells were equally affected. It is concluded that *F. exasperata* seems to be a promising molluscicidal candidate and thus needs further studies.

Įvertintas *Ficus exasperata* (Vahl) šaknų, lapų, žievės ir sėklų vandeninių ir etanolio ekstraktų moliuskocidinis poveikis jaunoms ir suaugusioms *Biomphalaria pfeifferi* sraigėms – žarnyno šistosomozę sukeliančių šistosomų rūšių tarpiniams šeimininkams. Sraigės buvo renkamos ir auginamos laboratorijoje. Atlikta tiriamų augalų fitocheminė analizė, pagaminti įvairių augalo dalių vandeniniai ir organinio tirpiklio (etanolio) ekstraktai. Sraigių virškinamojo trakto sienelės ištirtos histopatologiškai. Nustatyta, kad *F. exasperata* turi alkaloidų, kardenolidų, antrakvinonų, saponinų, taninų ir flavonoidų. Etanolio ir vandeninių ekstraktų poveikis sraigėms išliko ir po 24 valandų. Etanolio ekstraktų poveikis buvo stipresnis. Stipriausią moliuskocidinį poveikį sraigių jaunikliams turėjo žievės etanolio ekstraktas ($LC_{50} = 0{,}36$ ppm, $LC_{90} = 0{,}62$ ppm). Lapų etanolio ekstraktas stipriausiai veikė sraigių suaugėlius ($LC_{50} = 0{,}39$ ppm, $LC_{90} = 0{,}69$ ppm). Lapų vandeniniam ekstraktui buvo jautrūs sraigių jaunikliai ($LC_{50} = 0{,}45$ ppm, $LC_{90} = 1{,}50$ ppm) ir suaugusios sraigės ($LC_{50} = 0{,}60$ ppm, $LC_{90} = 1{,}05$ ppm). Silpniausią moliuskocidinį poveikį turėjo sėklų vandeninis ir etanolio ekstraktai. Įvairių augalo dalių ekstraktų poveikis sukėlė *B. pfeifferi* virškinamojo trakto epitelinių ir liaukinių ląstelių degeneraciją. Buvo stebimas akivaizdus mitochondrijų ir endoplazminio tinklelio irimas, ląstelės branduolio atrofija ir kalcio ląstelių pažeidimas. Daroma išvada, kad *F. exasperata* gali būti laikomas perspektyviu moliuskocidu, kurio poveikį reikia ištirti išsamiau.

Keywords: molluscicidal potency; schistosomiasis; *Ficus exasperata*; phytochemical screening; histopathology

Introduction

Schistosomiasis is one of the neglected tropical diseases of poverty, discrimination, and stigma that causes massive but hidden and silent suffering and frequently kills, but not in numbers comparable to the death caused by HIV/AIDS, tuberculosis or malaria (Otunbanjo 2007; WHO 2010). It is also considered as a major health problem in many developing areas, including Africa, South America, the Caribbean, the Middle East, and Asia (Chitsulo et al. 2000) and has been ranked second to malaria in tropical Africa. Approximately 60% prevalence of this disease has been found in children living in vast rural areas which are ill provided with social amenities like good water; they therefore resort to infected streams and lakes (Clark, Appleton, and Drewes 1997). The World Health Organization (WHO 1993) estimated that over 200 million people are infected with schistosomiasis in 73 countries while about 5% of the world's

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population (500–600 million) are at risk of being infected. The Global Burden of Disease reported that schistosomiasis caused the loss of more than 1.7 million disability-adjusted life years (DALYs) worldwide per year primarily as a result of organ damage, haemorrhage, and cancer resulting from infection, of which 82% (1.4 million DALYs) were lost in Sub-Saharan Africa alone in 2001, and Nigeria is still a seriously endemic of this disease since 16 million children are infected (Carter Centre 2011).

Since the transmission of schistosomiasis requires a specific snail vector as an obligate intermediate host, control of vector snails is therefore relevant to the control of this parasitic disease (Taylor 1986). To achieve a successful control of this disease, an integrated approach should be adopted, which includes chemotherapy, ecological, and biological methods and, most importantly, control of snail hosts using molluscicides. Recently, molluscicide still remains the most effective measure in the control of schistosomiasis (Webbe 1987; Madsen 1990).

According to Kloos and McCollough (1982), desirable characteristics for the selection of a candidate molluscicidal plant includes its high toxicity to the target organism but low or no toxicity to nontarget organisms at molluscicidal concentrations. It should be a perennial rather than an annual growth, must have retention of molluscicidal potency under physical and chemical influences and should have an ethnobotanical value among others. Niclosamide (Bayluscide) is the only synthetic molluscicide recommended by WHO for large-scale use for control of snail populations. However, it is very expensive, so there is a need to develop a better, cheaper, and more cost-effective molluscicide. This suitable alternative led to the exploration of natural products of plants with strong molluscicidal properties (Kloos and McCullough 1987; WHO 2006).

F. exasperata (Vahl) belongs to the family Moraceae. It is a terrestrial Afro-tropical shrub or deciduous tree that grows up to about 20 m in height and prefers evergreen and secondary forest habitats (Berg 1989; Berg and Wiebes 1992). It is locally known as a sand paper tree/plant usually characterized by very rough leaves. Native to tropical equatorial Africa, the southern Arabian Peninsula and southern India, the forest sandpaper fig, or *Ficus exasperata*, grows wild. In Nigeria, it spreads widely in all eco-regions (Lawal et al. 2012). Ethnobotanically, it is a medicinal plant used traditionally and otherwise for the treatment of different diseases like diarrhea, dysentery, flatulence, malaria, infantile convulsions, tonsillitis, bacterial and fungal infections and worm infestation (Ogueke, Ogbulie, and Njoku 2006). Moreover, phytomedicine has demonstrated its contribution to the reduction of excessive mortality, morbidity, and disability dues to diseases such as HIV/AIDS, malaria, tuberculosis, sickle cell anaemia, diabetes, mental disorders (Elujoba, Odeleye, and Ogunyemi 2005), and microbial infections (Iwu, Duncan, and Okunji 1999). The viscid nonmilky sap is used for treating sore eye troubles and stomach pains in Ivory Coast (Burkill 1997). The sap is used to arrest bleeding in Ghana (Abbiw 1990). The present study therefore aimed at evaluation of the molluscicidal potency of *F. exasperata* against both juvenile and adult *B. pfeifferi*.

Material and methods

Field studies

Snail collection

Adult snails were collected from Lake Eleyele, Ibadan, Oyo State, Nigeria (7°23′49″N, 3°52′2″E). It is a man-made lake and there is no report to indicate that the lake has been treated with molluscicide recently. The snail collection was done between the early hours of 8.00 a.m. to 12.00 noon, with the aid of a flat dip-net scoop following the methods of Richie, Radke, and Ferguson (1962) and Demian and Kamel (1972). Hand picking of snails with gloves and forceps was also adopted especially for the shallow edge of the lake, since many snails were found by turning over the surface of leaves, while some resided on stony or woody substances and other materials found on the lake. Collected snails (in soaked cotton wool with the lake water) were transported to the Parasitology Research Unit, Department of Zoology, University of Ibadan, for identification.

Collection of plant samples

Fresh leaves, seeds, roots and bark of natural *F. exasperata* were collected from the wild around Umuchu, Anambra State, Nigeria (5°55′59″N, 7°7′59″E) in December 2011. The collection was done between the early hours of 7 a.m. to 10 a.m. in the morning. The plants were sorted into the parts (leaves, roots, bark and seeds) and rinsed to remove dust, sand, and unwanted materials. Afterwards, the collected plant parts were taken to the Botany Department Herbarium, University of Ibadan, for identification. The authentication was done at Forest Reserve Institute of Nigeria with voucher specimen number FHI. 109550. The plant parts were air-dried at room temperature (26 °C) for four weeks and grounded into powder-like form with the aid of a local blender.

Phytochemical screening

The powdered plant parts were subjected to preliminary photochemical screening to test for the presence or absence of some phytochemical constituents. This was done by following the methods outlined by Harborne and Harborne (1998) and Evans (2002).

Laboratory studies

Maintenance of the snails

The collected snails were identified to species level, using the snail identification key by WHO (1971) and Danish

Bilharzias Laboratory keys constructed by Christensen and Frandsen (1985) and employed by Otarigho and Morenikeji (2012). Infected snails were identified using the shedding method described by Frandsen and Christensen (1984). The snails that shed cercariae were gathered in one circular glass trough, half filled with dechlorinated tap water. The healthy snails were maintained in aquaria of circular glass troughs with 12 cm depth and 30 cm diameter and with a capacity of 6 l.

Rearing of snails in the laboratory

The snails were reared following the methods described by Mello-Silva et al. (2006, 2007), with a little modification. Snails were placed in 30 l polyethylene aquaria with dechlorinated tap water. Three times a week the aquaria were cleaned and the snails were fed with both fresh and dried grounded water leaf plants instead of the usual lettuce leaves (*Lactuca sativa*) (El-Kamali, El-Nour, and Khalid 2010; Otarigho 2012; Otarigho and Morenikeji 2012).

Cold water extraction

Cold water extraction was performed following the method described by Adenusi and Odaibo (2008, 2009) with little modifications. For each plant part, a stock extract was prepared by soaking hundreds of grams of pulverized plant parts (400 ml for roots and bark, 200 ml for seeds and leaves) in dechlorinated tap water for five days at room temperature. The suspensions were occasionally shaken every 24 h. The suspensions were filtered through Whatman filter paper (No. 1) and cold dechlorinated tap water was added to adjust the filtrate at a specific volume so as to replenish any water loss (Rawi, Al-Hazmi, and Seeif Al Nassr 2011). The filtrates were used to prepare a series of concentrations that were used to calculate the lethal concentrations (LC_{50} and LC_{90}) according to Hashem (1999) and Bakry (2009a).

Organic solvent extraction

Plant parts were packaged in glass jars and stored at 4°C until extraction (Ojiako and Akubugwo 1997). Later the grounded plant part samples were subjected to extraction. One hundred grams of each finely powdered sample were separately put in a themble and placed in a Soxhlet extractor. 150 ml of each solvent were poured in a Soxhlet flask. The extraction apparatus was attached to the water source for cooling. The temperature of the electric heater was also adapted according to the boiling range of each solvent. The powdered dry material of the plant parts was extracted continuously for 12 h in a Soxhlet using ethanol solvent.

Molluscicidal potency assay of plant extracts against adult snails

This potency assay was tested against adult and juvenile *B. pfeifferi*, as outlined by WHO (1965, 1971) and employed by Adenusi and Odaibo (2008, 2009), Sharma, Singh, and Vijayvergia (2009), and Singh, Yadav, and Singh (2010). The different volumes of 0.0 (control), 0.0, 0.2, 0.4, 0.6, 0.8, and 1 ml from the stock solution of the extracts and purified fractions of each plant parts were added to the equal volume of dechlorinated tap water of 200 ml in plastic trough containers (10 cm depth × 17 cm diameter), to have a working solution. Then the concentrations of each solution were calculated in ppm: 0.0, 0.25, 0.50, 0.62, 0.87, and 1.25 ppm, respectively. 10 healthy and active adult snails of uniform size and weight were immersed in each trough containing the solution (Patole and Mahajan 2010). In each set-up, the snails were prevented from crawling out of the trough by means of a fine mesh white cloth tied to the trough and fastened with a rubber band (Giovanelli et al. 2002; Sermsart et al. 2005; Mello-Silva et al. 2006; El-Sherbini, Zayed, and El-Sherbini 2009).

The snails were not fed during the experiment (Vasconcellos and Amorim 2003; Sermsart et al. 2005; Adetunji and Salawu 2010). Reports have shown that healthy snails can live up to five days or more without food (Sermsart et al. 2005; Adetunji and Salawu 2010; Salawu and Odaibo 2011), provided other environmental conditions remained at the optimum level. The snails in the control (untreated) groups were equally maintained under the same experimental conditions. After 24 h of exposure to the plant extracts, the snails were transferred to fresh dechlorinated tap water for another 24 h for recovery. Each test concentration was duplicated and determination of the concentration mean values were done and recorded. Deaths of the snails were determined by the lack of reaction to irritation of the foot and viability of the snails was confirmed with needle stimulation, as live snails would close their opercula. The dead snails protruded out their soft tissue without retraction or lost their opercula. They were motionless. Similarly, the absence of heartbeat was observed under the microscope (El-Sherbini, Zayed, and El-Sherbini 2009; Rawi, Al-Hazmi, and Seeif Al Nassr 2011), and mortality counts recorded. According to El-Sherbini, Zayed, and El-Sherbini (2009) any plant extract that causes no mortality at 1000 ppm should be considered inactive and further investigation should be discontinued.

Consequently, some snails' digestive tracts exposed to different extracts were removed from their shells 24 h after treatment for histopathological study. The same process was applicable to the control snails' digestive tracts.

Molluscicidal potency assay of plant extracts against juvenile snails

The different volumes of 0.0 (control), 0.1, 0.3, 0.5, 0.7, and 0.9 ml from the stock solution of both extracts of each plant were each added to the equal volume of dechlorinated tap water of 100 ml in plastic trough containers (10 cm depth × 17 cm diameter) to have a working solution. Then, the corresponding concentration of

each solution was calculated in part per million (ppm): 0.0 (as control), 0.55, 0.58, 0.15, 0.15, and 0.25 ppm. About 10 juvenile snails of uniform size were immersed in each trough containing the solution. After 24 h of exposure to different plant extracts, the juveniles were transferred to fresh dechlorinated water and maintained there for another 24 h for recovery. Molluscicidal tests with each plant extract dose were separately repeated twice without feeding. Thereafter, mortality counts were recorded after careful observation under the microscope.

Histological study of some snails

A histological study involved collection of digestive glands of treated and control snails. The specimens were fixed in Bruin's fluid for five hours and were later transferred to 70% alcohol. Further procedures included dehydration in 100% alcohol, clearing in xylol and paraffin embedding followed suit. The snails were stained with haematoxylin and eosin. The histological changes in tissue sections of snails were observed under a polarized light microscope (Mohamed and Saad 1990; Kim, Ashton-Alcox, and Powell 2006; Bakry 2009a; Ming et al. 2011).

Statistical analysis

Analysis of data obtained from the molluscicidal test was subjected to probit analysis using Biostat software. The results of this analysis were plotted on a probit graph. A regression line was obtained.

Results

The preliminary phytochemical screening was carried out on the powdered forms of different parts of *F. exasperata* (Table 1). The screening test revealed the presence of alkaloids, cardenolides, anthraquinones, saponins, and tannins and the absence of flavonoids in the leaves. In the roots, alkaloids, cardenolides, saponins, and flavonoids were present whereas anthraquinones and tannins were absent. In the bark, alkaloids, cardenolides, saponins, and tannins were present while anthraquinones and flavonoids were absent. Alkaloids, cardenolides, anthraquinones, saponins, tannins, and flavonoids were all present in the seeds.

Table 2 shows the LC_{50} and LC_{90} values of the ethanolic extracts of *F. exasperata* roots, bark, leaves, and seeds on juvenile and adult snails exposed for 24 h. The LC_{50} values of the root, bark, leaf, and seed ethanolic extracts were 0.5584, 0.7395, 0.7395, and 0.3187 ppm, respectively, for the juveniles, and 0.6919, 1.1804, 1.0642, 0.6919 ppm, respectively, for the adults. The LC_{90} values of ethanolic extracts of roots, bark, leaves, and seeds of the same plant were 0.9327, 1.0248, 1.0248, and 0.7555 ppm, respectively, for the juveniles, and 1.9160, 1.7972, 2.0117, and 1.9160 ppm, respectively, for the adult snails. Statistically, bark ethanolic extract was the most potent for juvenile *B. pfeifferi* when compared to other plant part extracts ($\chi^2 = 7.01$, df = 3; and $p < 0.05$). The second most potent was the leaf ethanolic extract ($\chi^2 = 1.82$, df = 3; $p < 0.05$). The leaf ethanolic extract was the most potent for adult snails ($\chi^2 = 6.05$, df = 3; $p < 0.05$), while seed extract was the least potent ($\chi^2 = 9.00$, df = 3; $p < 0.05$) for adult snails.

The LC_{50} values of the root, bark, leaf, and seed aqueous extracts were 0.8409, 2.3811, 0.8749, and 0.5332 ppm, respectively, for the juveniles, and 1.4392, 4.8393, 1.7216, and 4.0963 ppm, respectively, for the adults. The LC_{90} values of the root, bark, leaf, and seed aqueous extract of the same plant were 2.5102, 6.7434, 1.8701, and 1.0150 ppm, respectively, for the juveniles, and 6.6956, 16.6481, 5.4663, and 17.9510 ppm, respectively, for the adult snails. The leaf aqueous extract had a very high potency against juvenile *B. pfeifferi* ($\chi^2 = 8.03$, df = 3; $p < 0.05$), while the seed aqueous extract was the least potent among the different parts used for exposure with the LC_{50} value of 6.63 ppm ($\chi^2 = 5.0$, df = 3; $p < 0.05$) for adult snails. The slope values of the regression relationship between egg mortality and treatment with different concentrations of the two extracts showed a linear increase in mortality of *B. pfeifferi* in two stages from low to higher concentrations (Figures 1–4).

In general, no mortality was observed in all the control groups used during the course of the experiment for both ethanol and aqueous extracts. The 'distress syndrome' and escape mechanism were observed during the exposure of snails to the extracts. As a result, some snails tried to crawl out of the container but they were prevented by covering the container with a sizeable mesh net. About 10 min after the introduction of the extract

Table 1. Phytochemical analysis of *F. exasperata* plant parts.

Compounds	Alkaloids	Cardenolides	Anthraquinones	Saponins	Tannins	Flavonoids
	D, M, W	K-K, K	C/A	F	FC	A/C
Leaf	+, +, +	+, –	+	+	+	–
Root	+, –, +	+, +	–	+	–	+
Bark	+, +, +	+, +	–	+	+	–
Seed	+, +, +	+, +	+	+	+	+

Notes: +, present; –, absent; where: D, Dragenduff's test; M, Meyer's test; W, Wagner's test; K-K, Keller-Killiani test; K, Kedde test; C/A, chloroform/ammonia test; F, $FeCl_3$ test; FC, ferric chloride test; A/C, ammonia/conc. H_2SO_4 test.

Table 2. Toxicity of *F. exasperata* extracts against *B. pfeifferi*.

Plant parts	Extracts	Stages	Regression equation	Lc_{50} (ppm)	Lc_{90} (ppm)	χ^{2*}	R^2
Root	Aqueous	Juvenile	$y=0.2494+0.1222x$	1.4392	6.6956	0.5395	0.9875
		Adult	$y=0.5711x-0.0114$	0.8409	2.5102	0.3065	0.9660
	Ethanolic	Juvenile	$y=0.1560+0.3686x$	0.6919	1.9160	0.2155	0.9292
		Adult	$y=0.8657x-0.0202$	0.5584	0.9327	0.3762	0.9376
Bark	Aqueous	Juvenile	$y=0.0106+0.0983x$	4.8393	16.6481	0.0885	0.9894
		Adult	$y=0.1786x-0.0347$	2.3811	6.7434	2.3463	0.8794
	Ethanolic	Juvenile	$y=0.4457x-0.0571$	1.1804	1.7972	1.6234	0.9421
		Adult	$y=0.8095x-0.1180$	0.7395	1.0248	615.1223	0.9867
Leaf	Aqueous	Juvenile	$y=5.9855x-0.4256$	1.7216	5.4663	1.1046	0.9730
		Adult	$y=0.5925x-0.0529$	0.8749	1.8701	0.1016	0.9594
	Ethanolic	Juvenile	$y=0.4257x-0.0155$	1.0642	2.0117	0.2993	0.9674
		Adult	$y=0.8095x-0.1180$	0.7395	1.0248	615.1223	0.7291
Seed	Aqueous	Juvenile	$y=0.1194x-0.0096$	4.0963	17.9510	1.6098	0.9468
		Adult	$y=0.4029x-0.0202$	0.5332	1.0150	0.2565	0.9867
	Ethanolic	Juvenile	$y=0.1560+0.3686x$	0.6919	1.9160	0.2155	0.9292
		Adult	$y=1.6714x-0.0679$	0.3187	0.7555	2.4835	0.8339

Note: $^{*}p<0.05$.

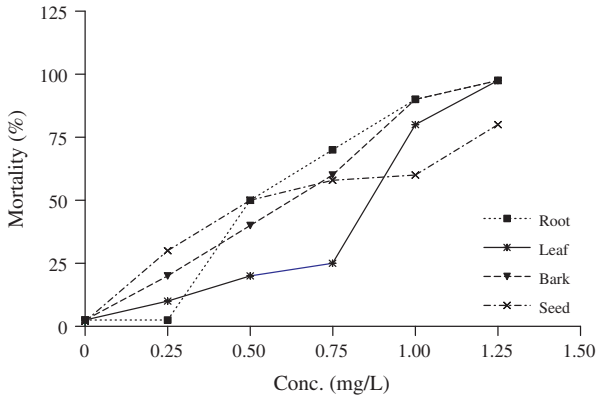

Figure 1. Toxicity of ethanolic extract of *F. exasperata* against adult *B. pfeifferi*.

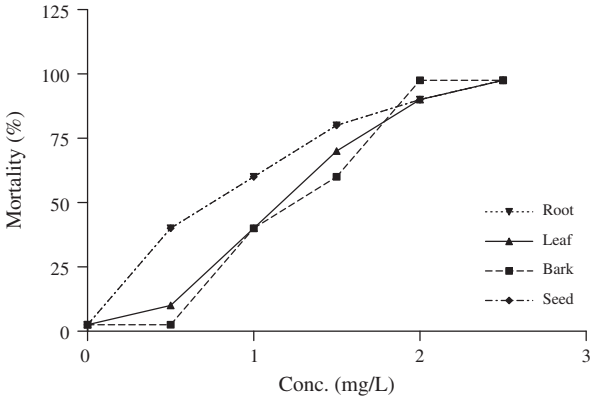

Figure 3. Toxicity of ethanolic extract of *F. exasperata* against juvenile *B. pfeifferi*.

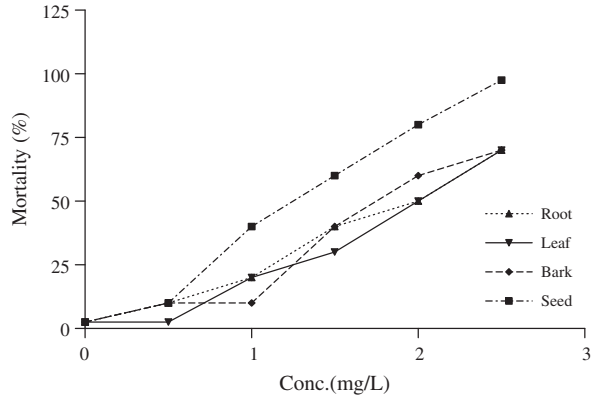

Figure 2. Toxicity of aqueous extract of *F. exasperata* against adult *B. pfeifferi*.

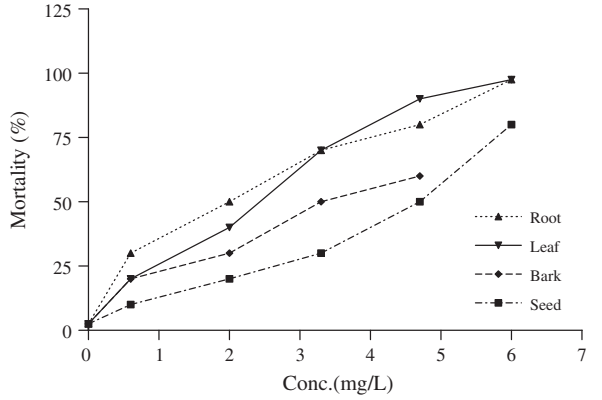

Figure 4. Toxicity of aqueous extract of *F. exasperata* against juvenile *B. pfeifferi*.

(mainly at higher concentrations), there was extension of the cephalopedal mass from the shell aperture. Some snails were seen shedding excessive mucus. An hour later, the swelling of their body ensued and this was accompanied by a motionless state in the snails and then death.

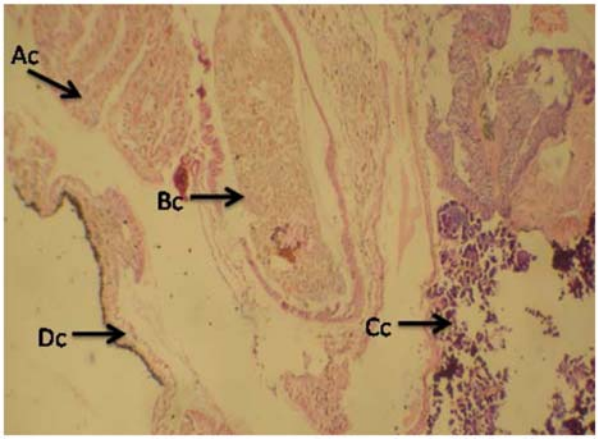

Plate 1. Control showing longitudinal section of digestive tract of adult *B. pfeifferi* snail. Ac = Damaged Epithelial lining, Bc = Erupted mitochondrial and endoplasmic reticulum normal nuclei cells, Cc = Atrophied cell nuclei, Dc = Digestive glands (Dg) of control group showing affected calcium cells (Ca), A = Damaged Epithelial lining, B = Erupted mitochondrial and endoplasmic reticulum normal nuclei cells, C = Atrophied cell nuclei, D = Digestive glands (Dg) of control group showing affected calcium cells (Ca).

The result of the histopathology test showed that the control group maintained normal mitochondrial and endoplasmic reticulum, normal nuclei cells, normal calcium cells in their digestive tract and epithelial lining as shown in Plate 1.

Plates 2–4 show the longitudinal section of the digestive tract of adult *B. pfeifferi* snails exposed to *F. exasperata* root, seed, and bark ethanolic extracts. The result revealed that there was a visible degeneration of digestive epithelial and glandular cells except in snails exposed to the bark ethanolic extract (Plate 4) where severe necrosis of germinal tissue and digestive gland were seen. In all snails, eruption of mitochondria and endoplasmic reticulum were evident, the cell nuclei were

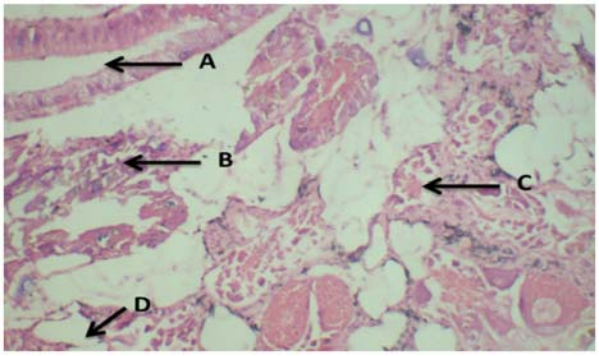

Plate 2. Longitudinal section of digestive tract of adult *B. pfeifferi* exposed to ethanolic root extract. Ac = Damaged Epithelial lining, Bc = Erupted mitochondrial and endoplasmic reticulum normal nuclei cells, Cc = Atrophied cell nuclei, Dc = Digestive glands (Dg) of control group showing affected calcium cells (Ca), A = Damaged Epithelial lining, B = Erupted mitochondrial and endoplasmic reticulum normal nuclei cells, C = Atrophied cell nuclei, D = Digestive glands (Dg) of control group showing affected calcium cells (Ca).

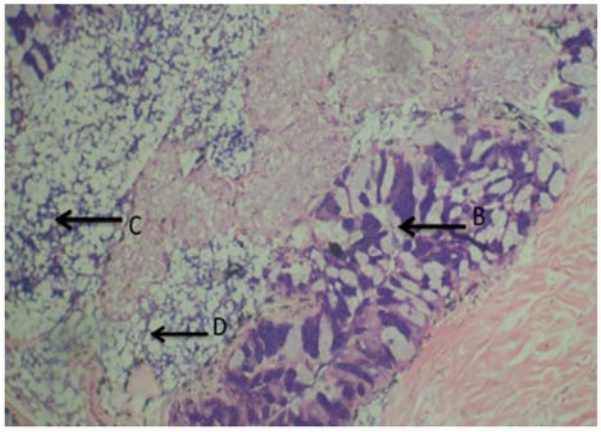

Plate 3. Longitudinal section of digestive tract of adult *B. pfeifferi* exposed to ethanolic seed extract. Ac = Damaged Epithelial lining, Bc = Erupted mitochondrial and endoplasmic reticulum normal nuclei cells, Cc = Atrophied cell nuclei, Dc = Digestive glands (Dg) of control group showing affected calcium cells (Ca), A = Damaged Epithelial lining, B = Erupted mitochondrial and endoplasmic reticulum normal nuclei cells, C = Atrophied cell nuclei, D = Digestive glands (Dg) of control group showing affected calcium cells (Ca).

atrophied and calcium cells in the digestive gland were affected.

Plates 5–8 show the longitudinal section of the digestive tract of adult snails exposed to aqueous extracts of bark, leaves, root, and seeds of *F. exasperata*. In case of exposure to bark aqueous extract (Plate 1), damage in the epithelial lining was seen and calcium cells were also affected in the digestive gland. Leaf aqueous extract damaged epithelial lining; this was accompanied by the eruption of mitochondrial and endoplasmic reticulum of the normal nuclei cells (Plate 6). This eruption was also found in case of exposure to root aqueous extract (Plate 7), while seed aqueous extract equally damaged epithelial lining,

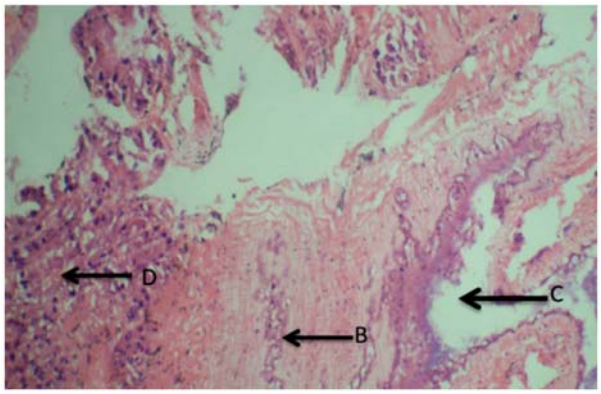

Plate 4. Longitudinal section of digestive tract of adult *B. pfeifferi* exposed to ethanolic bark extract. Ac = Damaged Epithelial lining, Bc = Erupted mitochondrial and endoplasmic reticulum normal nuclei cells, Cc = Atrophied cell nuclei, Dc = Digestive glands (Dg) of control group showing affected calcium cells (Ca), A = Damaged Epithelial lining, B = Erupted mitochondrial and endoplasmic reticulum normal nuclei cells, C = Atrophied cell nuclei, D = Digestive glands (Dg) of control group showing affected calcium cells (Ca).

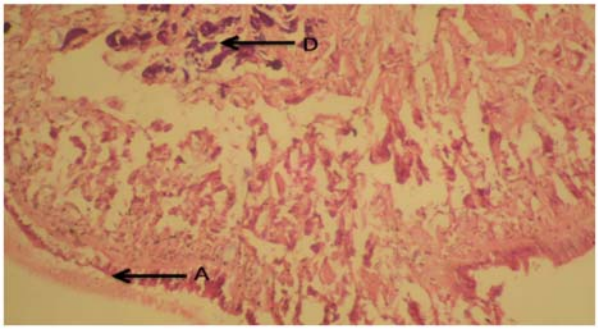

Plate 5. Longitudinal section of digestive tract of adult *B. pfeifferi* exposed to aqueous bark extract. Ac = Damaged Epithelial lining, Bc = Erupted mitochondrial and endoplasmic reticulum normal nuclei cells, Cc = Atrophied cell nuclei, Dc = Digestive glands (Dg) of control group showing affected calcium cells (Ca), A = Damaged Epithelial lining, B = Erupted mitochondrial and endoplasmic reticulum normal nuclei cells, C = Atrophied cell nuclei, D = Digestive glands (Dg) of control group showing affected calcium cells (Ca).

caused eruption of mitochondrial and endoplasmic reticulum as well as affected calcium cells.

Discussion

The preliminary phytochemical tests carried out on different parts of *F. exasperata* (Vahl) showed that the plant is very rich in phytochemicals. The phytochemical analysis of the different parts (roots, bark, leaves, and seeds) were screened and revealed some slight differences in their constituents based on the different methods used. Some phytochemical components detected were alkaloids, cardenolides, anthraquinones, saponins, tannins, and flavonoids, and all these were responsible for molluscicidal properties of the plant. This corresponds with the work of Ram and Mehrotra (1976) who reported that *Ficus* spp. contain the following constituents: flavonoids,

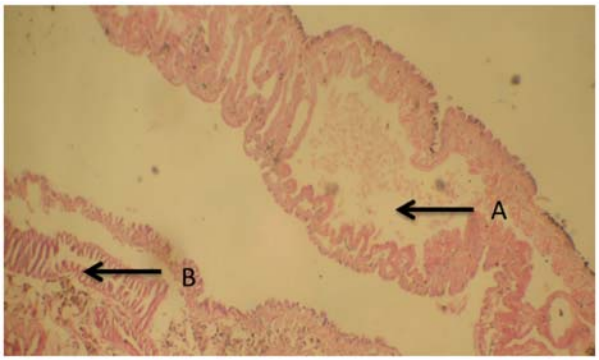

Plate 6. Longitudinal section of digestive tract of adult *B. pfeifferi* exposed to aqueous leaf extract. Ac = Damaged Epithelial lining, Bc = Erupted mitochondrial and endoplasmic reticulum normal nuclei cells, Cc = Atrophied cell nuclei, Dc = Digestive glands (Dg) of control group showing affected calcium cells (Ca), A = Damaged Epithelial lining, B = Erupted mitochondrial and endoplasmic reticulum normal nuclei cells, C = Atrophied cell nuclei, D = Digestive glands (Dg) of control group showing affected calcium cells (Ca).

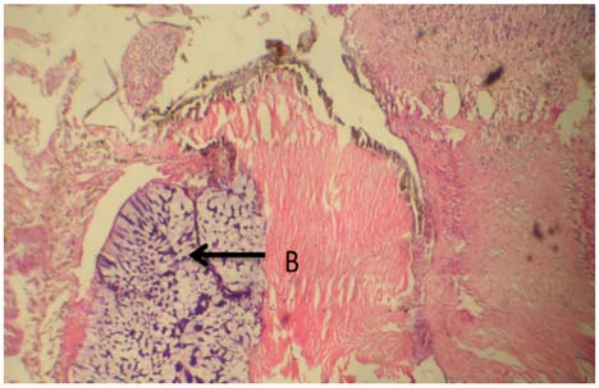

Plate 7. Longitudinal section of digestive tract of adult *B. pfeifferi* exposed to aqueous root extract. Ac = Damaged Epithelial lining, Bc = Erupted mitochondrial and endoplasmic reticulum normal nuclei cells, Cc = Atrophied cell nuclei, Dc = Digestive glands (Dg) of control group showing affected calcium cells (Ca), A = Damaged Epithelial lining, B = Erupted mitochondrial and endoplasmic reticulum normal nuclei cells, C = Atrophied cell nuclei, D = Digestive glands (Dg) of control group showing affected calcium cells (Ca).

glycosides, alkaloids, phenolic acids, steroids, saponins, coumarins, tannins, triterpinoids – oleanolic acid, rusolic acid, α-hydroxy ursolic acid, protocatechuic acid, maslinic acid. Their nonenzymatic constituents include phenolic compounds, flavanoids, and vitamin C. Ijeh and Ukwensi (2007) reported that the phytochemical screening result of *F. exasperata* revealed it being rich in alkaloids, tannins, and flavonoids, with the stems having higher concentrations of alkaloids and tannins while the leaves having high concentrations of flavonoids, saponins, and cyanogenic glycosides. The presence of tannins shows that the plant can be used as purgative. It also shows that this plant strongly supports its use in the

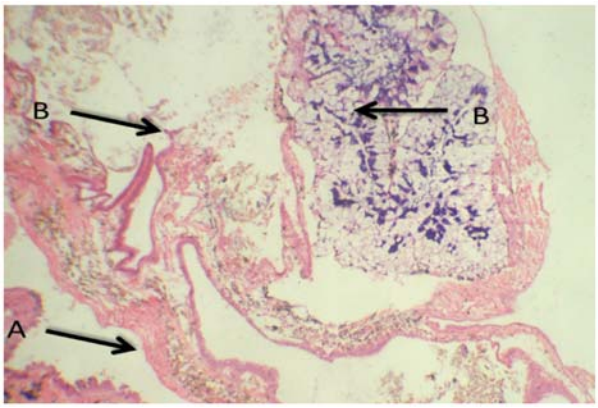

Plate 8. Longitudinal section of digestive tract of adult *B. pfeifferi* exposed to aqueous seed extract. Ac = Damaged Epithelial lining, Bc = Erupted mitochondrial and endoplasmic reticulum normal nuclei cells, Cc = Atrophied cell nuclei, Dc = Digestive glands (Dg) of control group showing affected calcium cells (Ca), A = Damaged Epithelial lining, B = Erupted mitochondrial and endoplasmic reticulum normal nuclei cells, C = Atrophied cell nuclei, D = Digestive glands (Dg) of control group showing affected calcium cells (Ca).

treatment of wounds, burns, and haemorrhoids in herbal medicine (Doherty, Olaniran, and Kanife 2010). The biological function of flavonoids includes protection against allergies, inflammation, platelets aggregation, ulcers, and tumours. This may be the reason behind the use of the extracts in the treatment of intestinal disorders.

This study demonstrated the molluscicidal potency of all the tested preparations from root, bark, leaf, and seed extracts of *F. exasperata* (Vahl). The toxic activity was higher in ethanolic extracts when compared to aqueous extracts of different parts. This difference may be due to the extraction method used or the poor solvent nature of water in extraction. This study also revealed that *B. pfeifferi* juvenile and adult snails were all susceptible to ethanolic and aqueous root, bark, leaf, and seed extracts of *F. exasperata*. But the juveniles and adults displayed more susceptibility to ethanolic extracts than to aqueous extracts. This high susceptibility of juveniles when compared to adults might be attributed to the high sensitivity of juveniles to the external stimuli while the lower susceptibility of the adult snails could be due to resistance or adaptability to harmful environmental factors (Mohammed 2001).

El-Sherbin, Zayed, and El-Sherbini (2009) reported the following LC_{90} of ethanolic extracts of three species of *Solanum* against *B. alexandrina*: 5.95 mg/l was considered as the highest molluscicidal activity for *S. nigrum* leaves, 6.04 mg/l for *S. sinaicum*, and 8.95 mg/l for *S. villosum*. Hassan, Rahman, and Abd El Monem (2010) reported LC_{50} of 26.4 and 39.8 mg/l and LC_{90} of 70.8 and 79.4 mg/l for both *Meryta denhamii* flowers and fruits against *Lymnaea natalensis* and *Biomphalaria alexandrina* snails, respectively. *N*-butanol extract of *Solanum elaeagnifolium* roots and fruits on adult *B. pfeifferi* showed LC_{50} of 12 and 19 ppm (Larhini et al. 2010). Fresh leaf ethanolic extracts of *Euphorbia schimperiana* and *E. helioscopia* gave LC_{50} of 2.3 and 8.9 ppm, respectively (Al-Zanbagi 2005). Adetunji and Salawu (2010) reported the LC_{50} values for the ethanolic extract of *T. catappa* and *C. papaya* on *B. pfeifferi* and *B. globosus* as 864.1 and 1095.7 ppm and 716.3 and 619.1 ppm, respectively. Their lethal concentration results showed that *B. pfeifferi* was more susceptible to leaf ethanolic extracts of *C. papaya*. Adewunmi and Marquis (1981) found that *Tetrapleura tetraptera* (mimosicae) plant extracts killed 100% of all the snails tested at a concentration of 100 ppm with LC_{50} of 1.94 to 2.65 ppm. The results of Salawu and Odaibo (2011) showed that LC_{50} of the leaf ethanolic extract of *Hyptis suaveolens* on one-week eggs and three to four weeks old immature *B. globosus* as 0.614, 0.196, 0.161, and 0.077 ppm was potent.

The results of bark and leaf ethanolic extracts seem to be very close to those of niclosamide (Bayluscide) which showed LC_{50} and LC_{90} of 0.077 and 0.175 mg/l, respectively, for *B. glabrata*; and 0.08 and 0.22 mg/l, respectively, for *M. tuberculata* (Giovanelli et al. 2002).

The results of leaf aqueous extract on snail juveniles and adults in this present study were LC_{50} values of 0.45 and 0.60 ppm and LC_{90} values of 1.50 and 1.05 ppm, respectively. Schall et al. (1998) reported the aqueous latex extract of *Euphorbia splendens var. hislopii* against *B. glabrata* as LC_{90} of 0.13 and 4.0 ppm for *B. pfeifferi*. Azare, Okwute, and Kela (2007) reported the use of evaporated and unevaporated *Alternanthera sesselis* extracts from both fresh and dried leaves. Their result showed LC_{50} as 4.42 ppm (ranging within 35.15–46.47) for unevaporated crude water extract and 48.07 ppm (ranging within 42.81–54.28) for evaporated crude extract of dried leaves. Also, LC_{50} was 32.57 (27.15–39.08) of fresh leaves of unevaporated crude water extract and 45.00 ppm (39.09–51.79) of evaporated crude water extract. They equally discovered that the potency of the extract was dose dependent. The investigations of Otarigho and Morenikeji (2012) reported the molluscicidal activities of aqueous and ethanolic extracts of *C. citratus* leaves against different developmental stages (eggs, juveniles and adults) of *B. pfeifferi*. LC_{50} values were 73.27, 64.60, and 140.74 ppm for *C. citratus* aqueous extract while 42.85, 43.87, and 6.79 ppm for ethanolic extract, respectively. So, LC_{90} values for eggs, juveniles and adults were, respectively, 182.37, 244.42, and 254.92 ppm for *C. citratus* aqueous extract, and 113.20, 166.31, and 159.47 ppm for *C. citratus* ethanolic extract.

WHO (2003) stated that the LC_{50} values of plant extracts must be between 0.1 and 10 ppm before the extraction and can be considered as a strong molluscicidal candidate. In line with this, the results obtained with *F. exasperata* plant parts show that the plant is a promising molluscicide candidate which deserves further studies to see its effect on non-targeted species.

The hispathology results in this study showed that adult *B. pfeifferi* exposed to the ethanolic and aqueous bark, root, leaf, and seed extracts of *F. exasperata* sustained great damages in their digestive tract. Their epithelial lining was damaged, there was eruption of mitochondrial and endoplasmic reticulum in the nuclei cells, atrophied cell nuclei and calcium cells were all affected. These findings agree with the work of Bakry (2009b) who reported damages in the digestive gland of *B. alexandrina* after their exposure to methanolic extract of *Guayacum officinalis*, *Atriplex stylosa*, and *Euphorbia splendens*. The epithelial cells lost their regular shapes and appeared empty. The digestive tubules and connective tissue between shrinked acini were also damaged. It also agrees with the work of Adewunmi and Ogbe (1986) who reported that the overall appearance of the tissues of *Bulinus globosus*, *Bulinus glabrata*, and *Physa watterlotti* exposed to methanolic extract of *Tetrapleura tetraptera* caused swelling of the epithelial cells suggesting that the molluscicide may have either acted on the membranes of these cells in some way as to alter their permeability or interfered with the regulatory or metabolic processes within them.

Conclusion

The future status of molluscicides in control of schistosomiasis will depend on the type of the control strategy adopted, which, in turn, will be determined by the local ecological and socioeconomic conditions. Therefore, judicious molluscicide must remain among the methods of choice in any comprehensive schistosomiasis control programme. The use of *F. exasperata* (Vahl) as an extract demonstrated a promising molluscicidal activity against *B. pfeifferi*. Histopathological changes caused by this plant to the digestive tissue of the exposed snails show its high potency as a molluscicidal agent. This might go a long way in the control of schistosomiasis.

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