International Scholars Journals

Advanced Journal of Environmental Science and Technology ISSN 2756-3251, Vol. 16 (4), pp. 001-006, April, 2025. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Toxicity of *Erythrophleum suaveolens* Saponin on *Lanistes lybicus*: A Study on Freshwater Snail Tissues

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Accepted 14 January, 2025

The study investigated the activity of saponin from ethanolic extract of *Erythrophleum suaveolens* stem bark against freshwater snail, *Lanistes lybicus*. The crude saponin (4 g) was separated by silica gel using gradient elution with dichloromethane in methanol (100:0 to 0:100) followed by thin layer chromatography using precoated silica gel 60 F_{254}. Fractionated saponins (90:10, 80:20 and 70:30) were employed for snail toxicity using fresh water snails, *L. lybicus.* The biochemical changes were evaluated in haemolymph, muscle, intestine and hepatopancreas of fresh water snails exposed to sub-lethal dose of fractionated saponins. Elevation of activities of acid and alkaline phosphatase in the intestine and hepatopancreas, haemolymph and total protein level were observed. The activity of acetylcholinesterase was inhibited in the haemolymph, muscle, hepatopancreas and intestine of the snails. The activity of saponin was observed to be dose dependent as mortality increased with relative increase in the saponin concentrations. The study provides considerable scope in exploiting local indigenous plant resources for control of fresh water snails and monitor water pollution.

Key words: *Erythrophleum suaveolens,* saponin, molluscicidal activity, *Lanistes lybicus,* pollution, hepatopancreas.

INTRODUCTION

The control of harmful fresh water snails through synthetic as well as plant extracts has been extensively studied by several investigators (Agarwal and Rastogi ., 1974). Many species of fresh water snails act as intermediate hosts of trematodes, parasitic worms causing endemic diseases such as schistosomiasis, filariasis, etc. (Aladesanmi, 2007), and as pests in overpopulated water or farm where they decimate a population of live plants (Anon, 2001). Different strategies have been used to control snail populations but biological control stands to be a better alternative to the chemical controls. Synthetic molluscicides have been widely used for the effective control of vector snails (Clarke et al., 1997). However, these molluscicides are considered toxic to non target animals and may have long-term detrimental effects on the aquatic environment (WHO, 1985; Massoud and Habib, 2003).

The search of herbal preparations, that do not produce any adverse effects in the non-target organisms and which are easily biodegradable, remains a top research issue for scientists associated with alternative mollusci-cides control. Medicinal plants represent the oldest and most wide spread form of medication known to man and have become the focus of attention as source of mollu-scicidal agents, since they are less expensive and less hazardous to the environment compared to their synthetic counterparts (WHO, 1985).

The use of plant molluscicides has received increased interest, primarily because it could be an appropriate and inexpensive technology for snail control in endemic poor nations of the world (Aladesanmi, 2007).

Saponins extracted from many sources have been reported to exhibit molluscicidal properties; they include

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*Phytolacca dodecandra (*Lemma*,* 1970*)*, *Tetrapleura tetraptera* (Leguminosae) (Adewunmi, 1984; 1991) and *Balanite aegptica* (Marston and Hostettmann, 1985).

Erythrophleum suaveolens is a perennial tree of about 30 m in height, slightly buttressed, often low-branching and producing a dense spreading crown. It is referred to by various names by natives. These include *obo* and *erun* (Yoruba), *inyi* (Igbo), *baska* (Hausa), *aba* (Akan-Asante, Ghana), *digpende* (Bassari –Togo), *teli* (Koranko - Sierra Leone), etc. It is often referred to in English as sassy, sasswood, red water tree and ordeal tree (Burkill, 1985). Studies have shown that the plant *Erythrophleum* species are extremely toxic to livestock especially sheep and cow. In Savannah regions, the cattle herders are always very careful not to allow their animal to graze along the routes where the trees of these species are known to grow (Nwude, 1981; Nwude and Chineme, 1981).

In folk medicine, the stem bark decoction is used as emetic and purgative, as an anesthetic, anthelminitic, treatment of malaria, analgesic and disinfectant. The extracts are utilized in cases of skin disease, oedemas, gangrenous wound, rheumatism and arthritis. It is also reported to be used as poison or repellant against rodent, insects and some aquatic animals and also in tanning hides and as dye (Aiyegoro et al., 2007; Burkill, 1985; Dongmo et al., 2001)

As such, this study investigated and reported the activity of saponin from ethanolic extract of *E. suaveolens* stem bark for control of fresh water snails (*Lanistes lybicus)* and monitoring water pollution.

MATERIALS AND METHODS

Plant materials

Dried stem-barks of *E. suaveolens* (Guill. & Perr.) were obtained from the Central Local Market (Oja Tuntun) in Ile-Ife, Osun State, Nigeria. The identification and authentication of the plant was done by Dr. H .C. Illoh, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

Reagents and chemicals

All the reagents used were of analytical grade and procured from British Drug House (BDH) Poole, U.K., Sigma Chemical Company, Louis, U.S.A., Fluka Chemical Company, USA and Pharmacia Fine Chemicals, Uppsala, Sweden. Silica gel 60 F_{254} precoated-plate (20 × 20 cm) was a product of Alumgram, Germany. Silica gel 60 (50 to 200 mesh) was obtained from Lab. Tech. Chemicals, U.S. Aluminium Oxide was from Associated Chemical Enterprises, South Africa.

Fresh water snails

Adult fresh water snails (*L. lybicus)* with mean length 2.3±0.2 cm and average weight of 39±2.0 g were collected from the gutters behind Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria. The snails were acclimatized in laboratory conditions for two weeks. They were kept in a plastic aquaria containing stream water at 25°C and fed with fresh leaves of *Telfairia occidentalis* and *Talium triangularie*.

Isolation and fractionation of crude saponin

Ethanolic extract of the stem bark of *E. suaveolens* was prepared according to the procedure described by Oyedapo and Amos (1997). Typically, powdered (900 g) dried stem bark was suspen-ded in 4.2 L of 80% (v/v) ethanol for 72 h at room temperature. The suspension was filtered through two layers of cheese-cloth. The extraction process was repeated several times until the extract became clear. The filtrates were combined and concentrated under reduced pressure on rotatory evaporator (Edward Vacuum Co-operation, Crawley, England) at 35°C to give a coffee brown resi-due which was stored in the dessicator until required for further processing.

Crude saponin mixture was isolated according to a procedure that was based on the methods described by Abdel-Gawad et al. (1999) and Wagner et al. (1984). The ethanolic extract (10 g) was washed twice with chloroform (50 ml × 2) and, also twice with ethyl-acetate (50 ml × 2). The residue was dissolved in 50% (v/v) metha-nol. The water-methanol solution was extracted three times (100 × 3) with n- butanol. On evaporation, a syrupy residue was obtained which was taken up in methanol (50 ml, 50% v/v), followed by the addition of diethylether (100 ml) to precipitate crude saponins. The precipitate was further purified by repeated dissolution in methanol (50 ml × 5) and precipitation with diethylether (50 ml × 5) until a cream light brown precipitate termed crude saponins was obtained.

The crude saponin was fractionated on silica gel (Kiesel gel 60; 60 to 200 mesh) column chromatography using gradient elution with mixture of dichloromethane in methanol (DCM: MeOH) 100:0 to 0:100 as described by Lin et al. (2008). Fractions were collected for each of the solvent mixtures and evaporated to dryness at 40°C on rotatory evaporator to give various fractions. The active fractions were subjected to thin layer chromatography on precoated silica gel 60 F_{254} plates DCM: MeOH (95:5) as mobile phase. The chromatograms were sprayed with spraying reagent (0.5% p-anisaldehyde in MeOH: acetic acid: conc. H_2SO_4 (17:2:1 v/v/v)) followed by incubation at 110°C for 3 min and visualized with iodine vapour.

Molluscicidal activity assay

Toxicity studies of the fractionated saponin mixture

The fresh water snails were treated with saponins, using the guideline for evaluation of molluscicidal potency of extracts. Six aquaria of five (5) snails each with an average weight of 39±2 g in each aquarium were set up in duplicate using river water. The saponin mixtures (10, 20, 30 40 and 50 µg/ml concentrations) were prepared using DMSO/H_2O, 1:1 (v/v). Each of the concentrations was diluted with river water to make up 500 ml in each aquarium. Mixture of DMSO/H_2O was applied to the sixth aquarium which served as the control. Period of 48 h exposure was allowed, followed by 48 h recovery period. Death of the snails was confirmed by lack of reaction to irritation of the foot, with a blunt wooden probe to elicit typical withdrawal movements. Control snails were subjected to the same condition. The LD_{50} was estimated using probit analysis (Finney, 1971).

Collection and preparation of tissue homogenates

The shells of the active snails were broken after 48 h recovery period in fresh water. The tissues were dissected using forceps and scissors. The muscle, hepatopancreas, haemolymph and intestine

of each snail were collected separately. The combined haemo-lymph from each group was centrifuged on Bench Centrifuge Model 800D (Microfield Instrument England, UK) at 3000 rpm for 10 min at room temperature. The supernatants were transferred into vials and kept wet-frozen in deep freezer (Royal Chest Freezer) until analyzed biochemically.

The homogenization of muscle, hepatopancreas and intestine was carried following the methods reported by Olagunju et al. (2000). The muscle, hepatopancreas and intestine were weighed. Each of the muscles (average weight of 1.2 g) in each group was homogenized using 10 ml of normal saline. The hepatopancreas per snail (average weight of 0.66 g) in each group was homo-genized using 5.0 ml of normal saline. The intestine per snail (average weight of 1.5 g) in each group was homogenized using 10 ml of normal saline. The homogenization was carried out using mortar and pestle aided with washed fine sand. The homogenates were centrifuged at 3000 rpm for 10 min. The supernatant in each tube was collected into labeled vials and kept in the deep-freezer until analyzed.

Biochemical analyses of tissue homogenates

The following biochemical analyses were carried out on the snail's haemolymph and tissue homogenates.

Estimation of total protein: The total protein concentrations were determined by simplified method for the quantitative assay of small amount of protein in biological material as described by Shacterk and Pollack (1973). Typically, 1.0 ml of each of the homogenates was pipetted into clean test-tubes in triplicates, 1.0 ml of alkaline copper reagent (10% Na_2CO_3, 0.1% K-Na tartarate and 0.05% $CuSO_4$. $5H_2O$) was added and mixed thoroughly. The mixtures were left to stand undisturbed for 10 min at 25°C. Then, 4.0 ml Folin-Ciocalteu's Phenol reagent (1:10 dilution) was added forcibly and rapidly. The blank was also prepared using distilled water instead of the homogenates. The mixture was then incubated at 55°C for 5 min, removed and cooled under running water. The absorbance was read at 650 nm against the blank. The protein concentrations were calculated using the expression:

Concentration of protein (g/dl) = Abs. of sample/ Abs. of standard × df × concentration of standard protein.

Where, Abs is absorbance at 650 nm and df is dilution factor.

Assay of acetylcholinesterase activity: The assay was carried out by a procedure that was based on the method of Ellman et al. (1961) with slight modifications. The reaction mixtures consisted of 25 µl of haemolymph and 50 µl each of the other homogenates in triplicates. Then, 50 µl (5, 5'-dithiobis-2-nitrobenzoic acid (DNTB) in 5 mM sodium phosphate buffer, pH 8.0, containing 17.74 mM $NaHCO_3$) was added followed by 1.45 ml of 5 mM sodium phos-phate buffer, pH 8.0. The reaction mixtures were mixed properly and transferred into a cuvette followed by the addition of 1.45 ml of 5mM sodium phosphate buffer, pH 8.0. The initial absorbance was read at 412 nm on SpectrumLab 752S uv/vis spectrophotometer. The reaction was initiated by the addition of 50 µl substrate (12.5 mM acetylcholine iodide) and stirred. The absorbance was read at 30 s interval for 4 min. The change in absorbance per time (slope) for each experimental data was determined. The activity of acetyl-cholinesterase was estimated using the following expression:

Enzyme activity (µmol SH hydrolyzed min/mg protein) = $\Delta A \times TV / \varepsilon_{DTNB} \times \ell \times SV$,

Where ΔA = change in absorbance/min; TV = total volume of the assay; SV = sample volume (25 µl/50 µl); ℓ = path length of cuvette (1 cm); ε_{DTNB} = molar extinction co-efficient of DTNB (1.36×10^4 $M^{-1}cm^{-1}$).

Assay of alkaline and acid phosphatases: The assays of alkaline and acid phosphatases were carried out by the method described by Sanni and Van Etten (1978) as reported with slight modification by Oyedapo (1996). The reaction mixture contained 50 µl haemo-lymph (or 50 µl of muscle/intestine/ hepatopancreas homogenates) in triplicates and incubated at 37°C for 3 min. The blank was simi-larly prepared using water instead of the liver homogenates or plasma samples. The reaction was initiated by the addition of 1.0 ml of substrate (5 mM p-nitro phenyl phosphate, Na salt) in appropriate buffer. The reaction mixtures were incubated for additional 15 min, followed by the addition of 2.0 ml 0.02 M NaOH to terminate the reaction. The reaction mixtures were allowed to cool down to room temperature. The absorbance was read at 410 nm against the reagent blank. The activities of the phosphatases were calculated using the following expression:

$$\frac{\text{Absorbance}}{\varepsilon \times l} \times 10^6 \times \frac{1}{t} \times \frac{TV}{SV}$$

Where ε = molar extinction co-efficient (1.88×10^4 $M^{-1}cm^{-1}$); l = path length of cuvette (1.0 cm); TV = total assay volume; SV = sample volume (25 µl/50 µl) and t = incubation time (15 min)

Statistical analysis

Data for the biochemical assays were expressed as mean ± SEM. Statistical analysis was performed by one way ANOVA followed by Turkey-Kramer multiple comparison test to ascertain differences between treatment groups. All analysis was performed using GraphPad Instat 3 (Version 1.1, 2007).

RESULTS

There was no mortality recorded in the 0 to 30 g/ml concentration, 60% mortality was recorded in the 40 g/ml concentration while 100% mortality was recorded when 50 g/ml was applied. Using Probit analysis, LD_{50} was estimated to be 38.74 g/ml.

The effects of sub-lethal concentration of saponin fractions of *E. suaveolens* on total protein, acid phosphatase, alkaline phosphatase and acetylcholine esterase in the muscle, hepatopancreas, haemolymph and intestine of *L. lybicus* are summarized in the Table 1. There was significant increase ($p \leq 0.05$) in muscle and haemolymph total protein concentration. In intestine and hepatopancreas, alkaline phosphatase activities increased significantly ($p \leq 0.5$). It was noted further that acid phosphatase activity in the intestine and hepatopancreas increased and the increase was significant at 30 g/ml only in hepatopancreas. In the haemolymph, the activity of the enzymes reduced significantly as saponin concentration increases. Moreover, acetylcholinesterase activity was significantly ($p \leq 0.05$) inhibited in intestine, while the inhibition of the enzyme in the muscle, hepatopancreas, and Haemolymph were not statistically significant.

Table 1. Changes in total protein concentration and enzyme activities in the haemolymph muscle, hepatopancreas and intestine of *L. lybicus* after 96 h of exposure to fractionated saponin mixture.

Parameter	Tissue	Control	10 (µg/ml)	20 (µg/ml)	30 (µg/ml)	40 (µg/ml)
Total protein (mg/dl)	Haemolymph	0.561 ± 0.004	1.357 ± 0.017*	1.324 ± 0.002*	1.346 ± 0.001*	1.346 ± 0.001*
	Muscle	0.121 ± 0.028	0.202 ± 0.014*	0.214 ± 0.019*	0.264 ± 0.015*	0.250 ± 0.004*
	Hepatopancreas	0.211 ± 0.036	0.262 ± 0.014	0.257 ± 0.019	0.297 ± 0.015*	0.293 ± 0.004
	Intestine	1.341 ± 0.031	1.339 ± 0.003	1.351 ± 0.004*	1.352 ± 0.001	0.894 ± 0.006*
Alkaline phosphatase (mole/min/mg protein)	Haemolymph	0.023 ± 0.001	0.022 ± 0.001	0.027 ± 0.000*	0.016 ± 0.000*	0.01 ± 60.000*
	Muscle	0.152 ± 0.029	0.059 ± 0.010*	0.127 ± 0.014	0.149 ± 0.015	0.147 ± 0.071
	Hepatopancreas	0.184 ± 0.023	0.230 ± 0.068	1.762 ± 0.351*	0.142 ± 0.026	0.126 ± 0.009
	Intestine	0.120 ± 0.004	0.221 ± 0.004*	0.224 ± 0.009*	0.192 ± 0.007*	0.223 ± 0.007*
Acid phosphatase (mole/min/mg protein)	Haemolymph	0.129 ± 0.003	0.122 ± 0.003*	0.122 ± 0.001*	0.121 ± 0.003*	0.114 ± 0.002*
	Muscle	0.086 ± 0.004	0.045 ± 0.023	0.114 ± 0.027	0.116 ± 0.018	0.210 ± 0.023
	Hepatopancreas	0.160 ± 0.021	0.175 ± 0.024	0.211 ± 0.021	0.319 ± 0.075*	0.471 ± 0.045
	Intestine	0.220 ± 0.001	0.254 ± 0.002*	0.240 ± 0.001*	0.237 ± 0.002*	0.225 ± 0.001*
Acetylcholin-esterase (µmole/min)	Haemolymph	17.99 ± 0.353	18.89 ± 0.240	18.27 ± 0.970	15.96 ± 0.144	13.71 ± 0.120
	Muscle	0.583 ± 0.138	0.479 ± 0.051	0.427 ± 0.089	0.345 ± 0.119	0.142 ± 0.069
	Hepatopancreas	0.591 ± 0.155	0.570 ± 0.138	0.302 ± 0.039	0.173 ± 0.050	0.101 ± 0.036
	Intestine	9.700 ± 0.314*	1.170 ± 0.023*	1.000 ± 0.064*	0.463 ± 0.027*	0.388 ± 0.034*

Each value represent mean ± SEM of n = 3. * Values were taken to be statistical significant at P ≤ 0.05 by using analysis of variance (ANOVA). Acethylcholinesterase activity, AchE (mol SH hydrolyzed/min/mg); acid/alkaline phosphatase activity (mole-p-nitrophenyl/min/mg); protein concentration (mg/dl).

DISCUSSION

The treatment of *L. lybicus* with varying concentrations of saponins caused inhibition of acetylcholinesterase activity in the haemolymph, muscle, hepatopancreas and intestine of the snails in a dose-dependent manner. Acetylcholinesterase (AChE) is a key enzyme in the nervous system of animals. The enzyme occurred in the outer basal lamina of nerve synapses, neuromuscular junction and in certain other tissues (Guyton and Hall, 2006). AchE is responsible for the termination of cholinergic impulses by the hydrolysis of acetylcholine released during synaptic transmission, inhibition of acetylcholi-nesterase thus permits accumulation of the synapses when concentration rises several folds in comparison to the normal levels, leading first to paralysis and then eventually to death (Singh and Singh, 2003). Muscle fatigue may result from impaired excitation-contraction mechanisms, neurotransmission failure or both (Kuei et al., 1990). Singh and Agarwal (1983) reported a dose dependent response of AchE as presumably due to conversion to more toxic metabolites in the body of fresh water snail (*Lymnaea acuminate*). The organophospho-rous and carbamate pesticides, the most frequently used due to their high insecticidal activity, are acutely neuro-toxic. These pesticides are effective inhibitors of the enzyme acetylcholinesterase located at neuromuscular junctions in the central and peripheral nervous system of the organisms (Walker et al., 2001). Acetylcholinesterase activity index has been widely used to indicate exposure of both vertebrate and invertebrate species to organo-phosphorous and carbamate pesticides (Van Erp et al., 2002) including freshwater bivalves (Moulton et al., 1996; Doran et al., 2001). Exposure of *L. lybicus* to *E. suaveolens* stem-bark saponin mixture produced a significant inhibition in AChE activity in their tissues. These responses might be useful indicators of the saponin mixture toxicity in fresh water snails. Thus, it might be suggested that the saponin mixture was neurotoxic to fresh water snails.

Decrease in the activity of muscle alkaline phosphatase was observed in the muscle of treated snails compared to the control snails. In the intestine, there was increase in the enzyme activity with increasing concentration in treated snails when compared to the control snails. However, in the haemolymph, the activity decreased with increa-sing concentrations of saponin except in 20 g/ml treated snails in which the activity was higher than that of the control snails. In the hepatopancreas, there was an incre-ase in the enzyme activity from 0.160 mole/min/mg in control snails to 0.2 3 and 1.76 mole/min/mg in 10 and 20 g treated snails respectively, while the activity in 30 and 40 g treated snails reduced to 0.142 and 0.126 mole/min/mg respectively. Alkaline phosphatase breaks down ester compounds of orthophosphate acids under alkaline conditions between pH 9.2 and 9.8 (Adolph and

Lorenz, 1981). It functions in the transport of metabolites across the membrane and also play active role in the formation of shell in mollusks as well as in glycogen metabolism (Gupta and Rao, 1974). The bioassay result showed increase in alkaline phosphatase activity in the intestine and hepatopancreas, and these two organ-systems are responsible for digestion and excretion in snail. The hepatopancreas, which is the main digestive gland, consists of three cells types, the digestive cells, the crypt cells and the excretory cells (Triebskorn and Kohler, 1992). Alteration in enzyme activity can be correlated with molluscicide induced changes in the ultra structure of the cells (Triebskorn, 1991). The enhanced activity of alkaline phosphatase in the hepatopancreas might be due to activation of intracellular energy consu-ming process because the alkaline phosphatase facilita-tes breakdown of ATP to ADP and inorganic phosphate, thereby making free energy available for metabolic processes (Botham and Mayes, 2003). It was possible that the synthesis of special enzymes was involved in detoxification of activated materials and other waste metabolic products, which was used in getting rid of the molluscicide from snail's body. Immediately after poison-ing with molluscicide, storage products in the cells, espe-cially in the crop, are reduced (Triebskorn and Kunast, 1990). This is because energy was released for the activated intracellular processes. The reduction in the storage product was preceded by a proliferation of the endoplasmic reticulum which showed high alkaline phosphates activity (Triebskorn, 1989). The increase in the hepatic alkaline phosphatase activity observed in this study could be attributed to cellular damages caused by saponin mixture or a response to overcome toxicity of the saponin mixture. Reduction observed in hepatopancreas alkaline phosphatase activity in the 30 and 40 g treated snails could be due to inability of the enzyme to carry out the functions afore stated as a result of toxicity elicited by saponin mixture at higher concentration.

Acid phosphatases are hydrolytic lysosomal enzymes and are released by the lysosomes for the hydrolysis of foreign material; hence it has a role in certain detoxification functions. It is responsible for catalyzation of reactions at the beginning of intracellular digestion shortly after the fusion of primary lysosomes with digestive vacuoles or secondary lysosomes (Lodish et al., 2000). Molluscicides have been reported to induce the fusion activity due to an impact on the cytoskeleton and there-fore, accelerate phosphatase catalysed primary digestive process (Triebskorn, 1991). It is known as inducible enzy-me whose activity in animal tissue goes up when there is a toxic impact and the enzyme begins to counteract. Subsequently, the enzyme activity may begin to drop either as a result of having partly or fully encountered the toxin or as a result of cell damage. In the present study, acid phosphatase activity in hepatopancreas was found to decrease as saponin concentration increases, likewise in the muscle. While in the intestine, lower doses were observed to increase the enzyme activity rather than higher doses, but in the haemolymph, the activity increased with increase in concentration. The increase in acid phosphatase activity in intoxicated animals as observed in the present investigation may be due to the destruction of the lysosomal membrane which resulted in the release of the enzyme. The pronounced increase in protease activity may be due to the damage caused to the lysosomal membrane, thus permitting the leakage of lysosomal enzyme into cytosol (Sherekar and Kulkurni, 1987).

There was significant increase in haemolymph, muscle and hepatopancreas protein concentrations with increasing saponin concentrations, while the intestine protein concentration was elevated in 20 and 30 g treated snails and reduced in 40 g treated snails. The elevation observed in protein turnover could be attributed to compensating production of protein lost as a result of tissue necrosis or to meet increased demand to detoxify the ingested saponins while reduction in total protein con-centration of 40 g treated snail could be attributed to inability of protein synthesizing machinery to be able to function properly.

Triterpenoids especially saponins and diterpepenoids have been reported to belong to groups of poisonous principles of African hunting poisons (Robert and Wink, 1998). Saponin bearing plants have been reported to be exclusively employed as fishing poisons, for example, plants with diterpenoid esters (Neuwinger, 1996). Pesticides are reported to considerably affect the intestine and hepatopancreas of the marine bivalves (Triebskorn, 1991). As observed from the results, these two organs were highly affected. Also, there was an indication that the toxic effect of *E. suaveolens* saponins was also mani-fested through the nervous system as it inhibits acetyl-choline esterase activity.

Hence, the toxicity of fractionated saponin mixtures from *E. suaveolens* stem-bark towards freshwater snail (*L. lybicus)* was ascertained. According to Fransworth et al. (1987), for a plant to be considered as molluscicide, it should be effective in concentration of about 100 g /L. Therefore, *E. suaveolens* stem-bark saponin could be considered as potential molluscicide. This study conclusively shows that *E. suaveolens* stem- bark saponin mixture satisfied the conditions which support its promising possibilities as a potent natural candidate molluscicide. It can be used for the control of fresh water snails and pollution.

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