

## Biochemical and histopathological profile of toxicity induced by saponin fraction of *Erythrophleum suaveolens* (Guill. & Perri.) bark extract

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### Abstract

In this study the toxic effect of saponin fractions from the stem-bark of the *Erythrophleum suaveolens* on some biochemical parameters in white albino rats, haemolytic effect and membrane stabilizing activities exposed to both heat and hypotonic-induced lysis using bovine red blood cells were investigated. The result revealed that saponin fractions (90:10,80:20 and 70:30) haemolysed red blood cells in varying degrees and stabilizes bovine erythrocytes at low concentration except for= 70:30 fractions which protected at all concentration used. The combined saponin fractions (90:10+80:20 + 70:30) caused hepatic damage, renal impairment and metabolic derangement in the treated rats followed by significant elevation of plasma and liver aspartate and alanine aminotransferases, total bilirubin, total protein, plasma creatinine, urea, total sugar and significant reduction in plasma and liver alkaline phosphatase, gamma glutamyl transferase, muscle creatinine and liver glycogen. Moreover, the saponin mixture caused progressive degeneration of the liver, intestine and kidney of treated animals as revealed by histopathological examination of the organs. Thus *E.suaveolens* stem bark saponins exhibits toxic and haemolytic activities.

**Keywords:** *Erythrophleum suaveolens*; Haemolytic activity; Membrane stabilizing activity

### Introduction

*Erythrophleum suaveolens* is a perennial tree of about 30m in height, 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-ogo), *тели* (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).

Phytochemical constituents of *E. suaveolens* stem-bark include alkaloids, saponins, tannins, glycosides and flavonoids (Adeoye and Oyedapo, 2004). The stem-bark extracts of *E. suaveolens* have been reported to show anesthetic (Mgbeka and Ejiofor, 1999) and convulsant effects (Harborne and Baxter, 1993), procyanidins from the stem was implicated to possess potent anti-inflammatory and analgesic properties (Dongomo *et al.*, 2001), alkaloidal fractions markedly alter the serum metabolites and activities of the hepatic enzymes (Adeoye and Oyedapo, 2004), diterpenoid alkaloids (amide norcassaide and norerythrosua-veolide) showed potent antimicrobial activities against bacteria and yeasts (Ngounou *et al.*, 2005, Aiyegoro *et al.* (2007).

In folk medicine, the stem bark decoction is used as emetic and purgative, as an anesthetic, anthelmintic, 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 repellent against rodent, insects and some aquatic animals and also in tanning hides and as dye (Burkill, 1985; Dalma, 1970; Neuwinger, 1996). Saponins are surface-active, high molecular-weight and chemically complex group of compounds that occur naturally in plants, to a lesser extent in lower marine organisms and bacteria (Hostettman and Martson, 1995). Several important biological properties such as antimicrobial, piscicidal, anti-inflammatory, spermicidal, adaptogenic, antipyretic, analgesic, antifeedant, antiviral, anticancer and immunostimulant activities have been attributed to saponins. (Price *et al.*, 1987; Riguera, 1997; Yoshiki *et al.*, 1998).

This study reported the isolated and the bioactivity of crude and fractionated saponin mixture from *E. suaveolens* stem bark on white albino rats with view to expanding the available information on toxic principles of stem-bark of *E. suaveolens* and its contribution to the overall toxicity of *E. suaveolens* stem-bark.

## Materials and Methods

### *Plant Materials*

Dried stem-barks of *Erythrophleum suaveolens* (Guill & Perr) were obtained from the Central Local Market (Oja Tuntun) in Ile-Ife, Osun State, Nigeria (127.49 N, and 4.07E). Plant identification and authentication 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 obtained mainly from the following Chemical Manufacturing Companies: British Drug House (BDH) Poole, U.K., Sigma Chemical Company, Louis, U.S.A., Fluka Chemical Company and Pharmacia Fine Chemicals, Sweden. Reagent diagnostic Kits for the assay of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, plasma  $\gamma$ -glutamyltransferase (GGT), urea and bilirubin were products of Randox Laboratory Ltd, UK. Germany, Silica gel 60 (50-200

mesh), were obtained from Lab. Tech. Chemicals, U.S.A, aluminium oxide was from Associated Chemical Enterprises, South Africa. Acetaminophen tablets (500 mgs) non-steroidal anti-inflammatory drugs) were purchased from a pharmaceutical shop at Obafemi Awolowo University, Ile-Ife, Nigeria. All the solutions, reagents and buffers were prepared with glass distilled water.

### ***Experimental Animals***

Fifteen, adult inbred Wistar rats of both sexes (*Rattus norvegicus*) with average weight  $142 \pm 3.0$  g and sixteen mice (*Mus musculus*) of average weight  $23.7 \pm 0.9$  g were obtained from the Animal House, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. The animals were fed with Standard rat chow (Ladokun Feeds, Ibadan, Nigeria) and watered *ad libitum*. The animals were maintained under normal laboratory conditions of humidity, temperature ( $25 \pm 1$  °C) and light (12-hr day: 12-h night), in the Department of Biochemistry Animal House for at least a week, before the commencement of experiments. The “principle of laboratory animal care” (NIH publication No 85 – 23) guidelines and procedures were followed in this study (NIH publication revised 1985).

### ***Collection and Preparation of bovine erythrocytes***

Fresh bovine blood was collected into an anticoagulant (containing 3.8% trisodium citrate) in a clean sterile bottle, mixed by inversion and kept in the refrigerator until used. The blood was centrifuged at 4000 rpm for 10 min. at room temperature. The supernatant was carefully decanted and the packed red blood cell was re-suspended in normal saline followed by centrifugation. The process of washing and centrifugation was repeated three times. On each occasion, the clear supernatant was carefully decanted and the packed red blood cells were kept in the refrigerator from which 2% v/v red blood cells was prepared in normal saline.

### ***Preparation of Saponin mixture***

Isolation of crude saponin was carried out according to a procedure that was based on the methods described by Abdel-Gawad *et al.* (1999) ; Oyedapo and Amos (1997) and Wagner *et al.* (1984). Typically, ethanolic extract of the stem bark of *E.suaveolens* was prepared with 80% (v/v) ethanol using 900g powdered stem to give coffee brown residue. The ethanolic extract (10g) was washed twice with chloroform (50 ml) and, also twice with ethylacetate (50 ml). It was dissolved in 100 ml 50% (v/v) methanol. The water-methanol solution was extracted three times (100 x 3) with butanol. The butanol fraction on evaporation yielded a syrupy residue which was taken up in MeOH (50ml). Then diethylether (100ml) was added to precipitate crude saponin. The precipitate was further purified by repeated dissolution in methanol and precipitation with diethylether (5 times) until a cream light brown precipitate termed crude saponins was obtained.

### ***Fractionation of E.suaveolens stem-bark saponin mixture***

The saponin mixture was fractionated on Silica gel (Kiesel gel 60; 60-200 mesh) column chromatography using gradient elution mixture of dichloromethane with methanol (DCM: MeOH, 100:0 - 0:100) according to the modified method of Lin *et al.* (2008). Fractio-

ns were collected for each of the solvent mixtures and evaporated to dryness at 40°C on rotatory evaporator to give various fractions. The resulting residue of each fraction was reconstituted in MeOH and subjected to thin layer chromatography on pre-coated silica gel 60 (Merck, Germany) using solvent mixtures (DCM : MeOH (95:5) as mobile phase. The chromatograms were sprayed with spraying reagent (0.5% *p*-anisaldehyde in MeOH: acetic acid: Conc. H<sub>2</sub>SO<sub>4</sub> (17:2:1 v/v/v) followed by incubation at 110°C for 3 min and visualized with iodine vapour.

The TLC analysis of the obtained fraction indicated that only fraction F<sub>2</sub> (90:10), F<sub>3</sub> (80:20) and F<sub>4</sub> (70:30) gave better resolutions without interference of colouring matter. These three fractions were shown to contained mixture of saponins with prominent spots and were kept in dessicator and used for further analyses

### ***Haemolytic activity***

The assessment of haemolytic activities of both fractionated saponins was based on the procedure earlier reported by Oyedapo *et al.* (2004) with slight modification. The assay involved pipetting 0, 0.25, 0.5, 0.75, and 1 ml of saponin mixture (1.5 mg/ml) in triplicates into clean dried test tubes. The volumes were adjusted to 1.0 ml with distilled water. To each of the tubes was added 2.5 ml of 0.1M phosphate buffered saline (PBS), pH 7.0 followed by the addition of 0.5 ml of freshly prepared 2% (v/v) red blood cells. The reaction mixture was incubated at 37°C for 1 hr followed by centrifugation at 3,500 rpm for 15 min on visible spectrophotometer (Spectrumlab 23A). The supernatants were collected and the absorbance was read at 540nm. The red blood cells treated with 0.5% Triton X-100 served as control and represented 100% lysis. Percentage haemolysis was calculated using the expression

### ***Membrane stabilizing activity***

The membrane stabilizing activity assay was carried out by procedure that was based on those as previously described by Sadique *et al.*, 1989 and Oyedapo *et al.* 2010. The assay mixture consisted of hyposaline (0.25 % (w/v) NaCl, 2 ml), 0.15 M sodium phosphate buffer, pH 7.4 (1 ml), varying volumes of saponins fractions (0.0 -1.0 ml of 1.5mg/ml), erythrocyte suspension (2 % v/v, 0.5 ml) and isosaline (0.85 % (w/v) NaCl) to give total assay volume of 4.5 ml. The control (4.5 ml) was prepared as above except the drugs were omitted, while drug control (4.5ml) did not control erythrocyte suspension. The reaction mixtures were incubated at 56°C for 30 min. The tubes were cooled under running water followed by centrifugation at 3,500 rpm for 10 min on visible spectrophotometer (Spectrumlab 23A) . The supernatants were collected and the absorbance was read at 560nm against reagent blank. The percentage membrane stability was estimated from the expression:

$$100 - (\text{Abs. of saponin sample} - \text{Abs. of drug control}) \times 100 / (\text{Abs. of blood control})$$

Blood control represented 100 % lysis or zero percent stability.

### ***Estimation of LD<sub>50</sub>***

Acute toxicity studies and LD<sub>50</sub> determination was carried out according to the procedure described by Lorke (1983). Fractionated saponin mixture of *E. suaveolens* (10,

100 and 1000 mg/kg body weight) was administered to 3 groups (three rat per group) of rat one after the other orally in a single dose using DMSO and H<sub>2</sub>O (1:1) as vehicle. The animals closely monitored for behavioral and general sign of toxicity within the first 24 hr. The second phase of the test was performed after 24 h based on the observation and number of survivors noted in first test. This was done by administration of 1600, 2900 and 5000 mg/kg body weight of the saponin mixture to another set of rat, 3 groups (one rat per group) of rats, and one after the other intraperitoneally in a single dose. The visual changes, behavioral pattern and mortality of mice were monitored for 48 h. The numbers of survivors was noted after 48 hr for each animal. The LD<sub>50</sub> value was estimated from the plot of % mortality versus concentration.

### ***Toxicity studies of fractionated saponins***

A total of fifteen (15) Wistar albino rats of both sexes with mean weight of  $98 \pm 5.0$  g were randomly divided into 3 groups of five animals each. Groups II and III received 125mg/kg/bwt and 250 mg/kg/bwt saponin every other day for a period of 28 days while group I(control) received vehicle solution at the dose of 10 ml/kg/ bwt. The animals were weighed before and after completion of saponin administration. Daily visual observations were made and recorded systematically as performed in acute toxicity study. On the 29th day, all the animals were anesthetized with inhaled chloroform and

Blood samples were collected by cardiac puncture into centrifuge tubes containing 3.8% trisodium citrate using 21 gauge (21G) needles mounted on a 5 ml syringe (Anhui Kangda Medical Products Co., Ltd, Anhui, China) and centrifuged at 3,000 rpm for 10 min. to obtain plasma used for biochemical studies. The animals were sacrificed and selected internal organs such as liver, kidney and intestine were removed aseptically sections of the organs were and preserved in 10% buffered formal saline solution for histological examination. Liver homogenates were prepared as described by Olagunju *et al.* (2000).

### ***Biochemical Assays***

The blood plasma and liver homogenates were subjected to biochemical analyses: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using Randox assay kit, based on standard methods of Reitman and Frankel (1957). Alkaline phosphatase activity was carried out by the method described by Sanni and Van Etten (1978) as reported with slight modification by Oyedapo (1996). The assay of plasma urea was based on Berthelot (1967) colorimetric method using a commercially available kit by Randox Laboratories Ltd, Antrim, UK. The creatinine concentration was estimated using Jaffe's-alkaline picrate method as described by Chawla (1999) with slight modification. The protein concentrations were determined by simplified method for the quantitative assay of small amount of protein in biological material described by Shacterk and Pollack (1973). Albumin (bromocresol purple) concentration was determined by the modified method of Pinnell and Northam (1978). Liver and muscle glycogen were isolated and estimated according to the methods of Jermyn (1975) and Cowgill and Pardee (1966) as modified by Oyedapo and Araba (2001). The sugar concentrations in plasma and liver homogenate was estimated using the phenol/sulphuric acid reaction method as described by Dubois *et al.* (1956) as modified by Oyedapo and Araba (2001).

The following biochemical analyses were carried out on the snails' haemolymph and tissue homogenates; acid phosphatase activity by the method described by Sanni and Van Etten (1978) as reported with slight modification by Oyedapo (1996), acetylcholinesterase by the method of Ellman *et al.* (1961) as described by Hangen and Ekhlom (1976) while alkaline phosphatase activity and total sugar concentration were carried out by the method used in plasma analyses.

### ***Histopathological studies***

Histopathological examination liver, kidney and intestine of rats were conducted. The organs were fixed in 10% formaline, dehydrated in gradual ethanol concentration (50-100%) and were embedded in paraffin blocks. From the embedded paraffin blocks 4-5 $\mu$ m section of each tissue was prepared and then stained with heamatoxylin-eosin (H-E) dye. These were examined under a photomicroscope (x16- x40 objectives) connected with a host of computer (Drury and Wallington, 1973)

### ***Statistical Analysis***

Data for the biochemical assays were expressed as mean $\pm$ 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**

Chromatographic analyses (column and thin layer) and chemical tests (haemolytic and Lieberman-Burchard) revealed that F<sub>2</sub> (90:10), F<sub>3</sub> (80:20) and F<sub>4</sub> (70:30) contained the s the summary of the heamolytic activities of fractionated saponin (F<sub>2</sub>, F<sub>3</sub> and F<sub>4</sub>), crude saponin (Crd) and Quillaja saponin (Std). The results showed that the greatest haemolysis w-

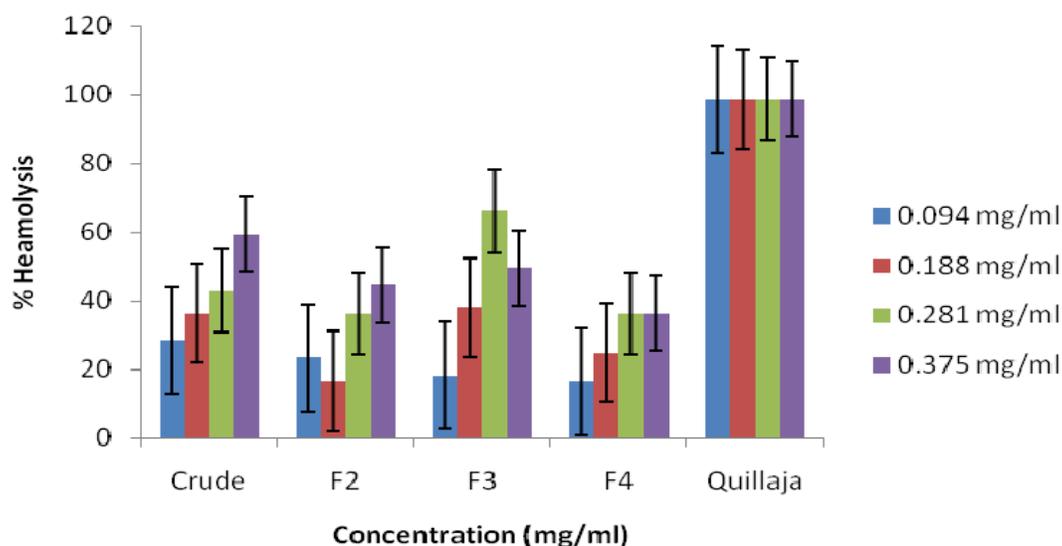


Figure 1. Percentage hemolytic activities of crude saponin mixture, 90:10 (F<sub>2</sub>), 80:20 (F<sub>3</sub>), 70:30 (F<sub>4</sub>) and Quillaja (standard saponin).

Table 1. Percentage hemolytic activities of crude saponin mixture, 90:10 (F2), 80:20 (F3), 70:30 (F4) and standard saponin

Conc.(mg/ml}	Crude sap.%	F2%	F3%	F4%	Std saponin %
0.094	0.63±16.28	3.17±1.16	17.42±7.18	14.57±4.41	95.66±11.11
0.188	13.08±10.78	17.37±6.18	40.77±5.53	28.19±7.84	95.98±7.58
0.282	35.48±10.69	38.99±18.22	66.11±1.79	34.30±4.77	95.34±13.07
0.375	33.58±15.39	42.14±10.47	52.49±8.91	41.50±18.17	89.64±23.31

as exhibited by Quillaja saponin, followed by crude saponin, F<sub>3</sub>, F<sub>2</sub>, while F<sub>4</sub> gave least haemolytic activity.

The membrane stabilizing potentials exhibited by crude saponin mixture, F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub>, standard saponin(Quillaja) and acetaminophen on red blood cells exposed to both heat and hypotonic induced lyses are as shown in Fig 2.0(a-f). It was observed that crude saponin, F<sub>2</sub> (90:10) and F<sub>3</sub> (80:20) did not exhibit membrane stabilizing activity. However F<sub>4</sub> (70:30) exerted some degree of protection to red blood cells against heat and hypotonic lyses. As shown, F<sub>2</sub> (90:10) caused rbc to lyse at all concentrations (b), while, F<sub>3</sub> (80:20) c and F<sub>4</sub> (70:30) (d) adequately protected the rbc against induced lyses. The standard anti-inflammatory drug (acetaminophen) and standard saponin (Quillaja) also exhibited adequate protection of bovine erythrocytes exposed to both lyses. The percentage membrane stability exhibited at highest concentration by F<sub>3</sub>, F<sub>4</sub>, Quillaja and acetaminophen were 47.80±2.88%, 90 ± 0.04%, 21.85±2.00% and 22.5 ± 3 respectively.

### Toxicity studies and behavioral effects

Within 2 min of administration of the fractionated saponin mixture, all the mice injected (intraperitoneally) with 1000 mg/kg died and those injected with 100 mg/kg/bwt all died an hour after administration. No death was recorded in those mice injected with 10 mg/kg/bwt saponin. In second phase of the test, all the mice administered 160 and 80 mg/kg/bwt of saponins died about 25 min and 36 min respectively after administration while those mice administered 20 and 40 mg/k/bwt saponins survived ( Table 1). The median lethal dose value (LD<sub>50</sub>) of the fractionated saponin mixtures of *E. suaveolens* determined to be 61 mg/kg/bwt body weight within 95 % confidence limits.

When administered to the rats orally, no death was recorded in the first and second phase of the oral administration of 10, 100 and 1,000 mg/kg/bwt (first phase) and 1,600, 2,900 and 5,000 mg/kg/wt (second phase). However, higher doses (2,900 and 5,000 mg/kg) caused very slow movement, weakness and freezing of the rats. Thus, the median lethal dose value (LD<sub>50</sub>) of orally administered *E. suaveolens* saponin mixture was greater than 2,900 mg/kg body weight.

The control rats did not exhibit any toxicity symptoms throughout the study period while toxic symptoms like restlessness and increased water intake were noticed in groups II and III rats. From 20 day onwards the faeces of the treated rats were very hard and dark compared with those of control rats. Table 2 showed the effects of combined fractionated saponins of *E. suaveolens* stem bark treatments on rats blood plasma parameters. The effects

Table 2. Effects of combined fractionated saponins of *E. suaveolens* stem bark treatments on rats blood plasma parameters.

Parameters	Group 1 (Control)	Group II (125 mg/kg)	Group III (250mg/kg)
Alkaline phosphatase ( $\mu\text{mol}/\text{min}/\text{mgprotein}$ )	458.08 $\pm$ 1.67	455.37 $\pm$ 1.1	449.32 $\pm$ 1.62*
ALT (U/L)	3.20 $\pm$ 0.15	6.63 $\pm$ 0.41***	6.96 $\pm$ 0.45***
AST (U/L)	17.75 $\pm$ 0.78	15.23 $\pm$ 0.55*	21.35 $\pm$ 1.82*
GGT (U/L)	1.55 $\pm$ 0.41	1.52 $\pm$ 0.45*	0.38 $\pm$ 0.06***
Total Protein (mg/dl)	1.70 $\pm$ 0.10	3.52 $\pm$ 0.22***	2.27 $\pm$ 0.17*
Total Bilirubin (g/dl)	3.43 $\pm$ 0.06	3.73 $\pm$ 0.06*	3.79 $\pm$ 0.08**
Direct Bilirubin (g/dl)	1.91 $\pm$ 0.05	3.73 $\pm$ 0.12*	1.25 $\pm$ 0.14**
Urea (mmol/L)	4.71 $\pm$ 0.23	4.81 $\pm$ 0.41	4.78 $\pm$ 0.22
Albumin (g/ml)	0.26 $\pm$ 0.01	0.30 $\pm$ 0.01*	0.20 $\pm$ 0.01**
Creatinine (mg/dl)	7.93 $\pm$ 0.08	7.83 $\pm$ 0.09*	8.71 $\pm$ 0.30**
Total sugar ( $\mu\text{g}/\text{ml}$ )	73.94 $\pm$ 3.00	95.62 $\pm$ 1.58**	158.87 $\pm$ 6.16***

of combined fractionated saponins of on liver homogenates biochemical parameters are shown in Table 3.

### Histopathological studies

Histology of the liver of rats administered 125mg/kg body weight of fractionated *E. suaveolens* saponins mixture revealed mild cytolysis of the hepatocytes affecting predominantly the periportal region, nuclear vacuolation and prominent nuclei and sinusoidal congestion (Fig 3B) while the liver of rats administered 250mg/kg body weight showed hepatocytes architectural disarray with extensive necrosis (Fig 3C). The kidney section of rat administered 125 mg/kg bwt showed focal tubular atrophy and necrosis with epithelial cells vacuolation (Fig 3E) while kidney of the rat administered 250 mg/kg bwt showed acute tubular necrosis more prominent in the proximal tubules (Fig 3F). Acute and chronic inflammatory cells and lymphoid follicles were observed in section of group II rat intestine (Fig 3H) while mucosa and sub mucosa of group III rat intestine section showed lymphoid hyperplasia with increased inflammatory cells in the lamina propria. (Fig 3I).

### Discussion

Earlier studies have speculated alkaloids to be responsible for the toxicity of *Erythrophleum* species (Nwude and Chineme, 1980; Nwude, 1981; Adeoye and Oyedapo, 2004). Some saponins have also been reported to be highly toxic. Among the toxic pharmacological properties elicited by saponins include gastroenteritis, cellular damage, and modification of tissue permeability and induction of irritation of mucous membranes (Attele *et al.*, 1999; D'Mello, 1997). Such findings raised the possibilities of exploiting saponins contribution to the overall toxicity of *E. suaveolens* stem-bark.

Phytochemical screening of *E. suaveolens* crude saponin mixture was confirmed positive by frothing and Liebermann-Burchard reaction test. The ability of the samples to haemolyse red blood cells was confirmed that the samples are saponins. As shown in Table 1 crude

Table 3. Effects of combined fractionated saponins of *E. suaveolens* stem-bark treatment on liver homogenates biochemical parameters

Parameter	Group I (Control)	Group II (125mg/kg bwt)	Group III (250mg/kg bwt)
Alkaline phosphatase (µmole/min/mg protein)	457.08 ± 0.70	453.18 ± 0.69*	451.05 ± 1.74**
ALT(U/mg protein)	21.24 ± 0.72	19.88 ± 0.34*	37.00 ± 0.49***
AST (U/mg protein)	7.15 ± 0.06	11.27 ± 0.08***	27.39 ± 1.65***
GGT(U/mg protein )	2.37 ± 0.01	1.81 ± 0.18**	1.86 ± 0.04*
Total Protein(mg/0.5g liver wet weight)	0.55 ± 0.02	0.61 ± 0.02	0.58 ± 0.02
Total Bilirubin(mg/0.5g/liver wet weight)	0.35 ± 0.02	0.9910 ± 10***	1.23 ± 0.10***
Direct Bilirubin(mg/0.5g/liver wet weight)	0.30 ± 0.01	0.32 ± 0.08	0.47 ± 0.01
Total sugar (µg/0.5g liver wet weight)	95.97±7.09	92.48 ± 3.56*	133.80±6.16***
Glycogen (µg/0.5g liver wet weight)	132.91 ± 3.20	109.00 ± 6.42*	94.78 ± 5.99***
Creatinine(mg/dl/0.5g muscle wet weight)	7.74 ± 0.052	7.62 ± 0.020*	7.50 ± 0.030*
Glycogen(µg/0.5g muscle wet weight)	106.06 ± 6.47	139.52 ± 6.47***	117.43 ± 5.20*

Each value represented mean ± SEM. Data shown with are

\* Significantly different from control (p≤0.05)

\*\* Significantly different from control (p≤0.01)

\*\*\* Significantly different from control (p≤0.001)

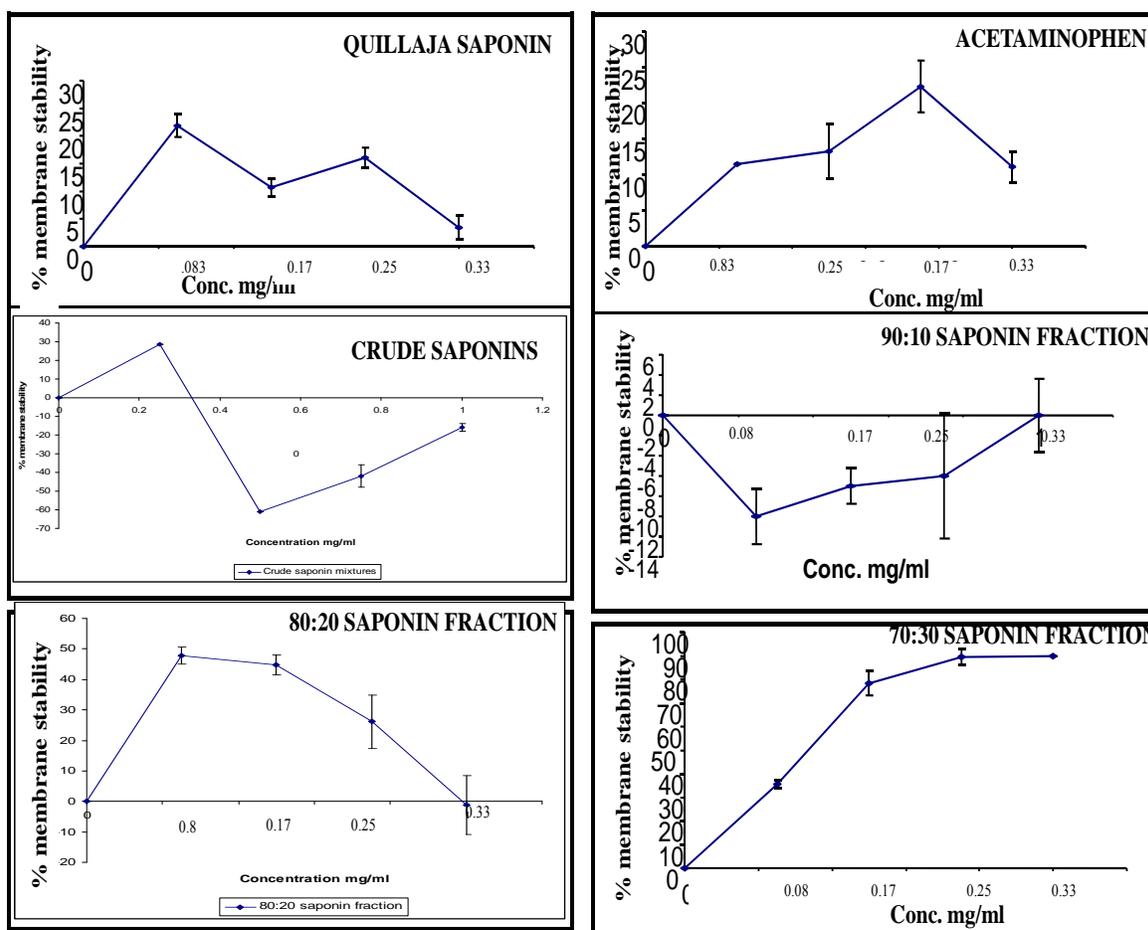


Figure 2. Membrane stabilizing activities profile of Quillaja saponin, acetaminophen, crude saponins, 90:10, 80:20 and 70:30 saponin fractions on bovine red blood cells.

compared to fractionated saponins while *Quillaja* saponins (standard) had 98 % haemolysis at all concentration used. It could be adduced that *E. sauveolens* could be another plant source of haemolytic saponin in addition to known plant saponins such as *digitonin* from the temperate *digitalis* seeds and *Quillaja* saponin (Daniel, 2006; Hassan *et al.*, 2010). Thus it could be employed for laboratory haemolysis in medical (human and veterinary) research.

Plant derived drugs have been demonstrated to contain principles that possessed ability to facilitate the stability of biological membranes when exposed to induced lyses (Sadique *et al.*, 1989; Oyedapo and Amos, 1997; Oyedapo *et al.*, 2004). Membrane stabilizing profile of crude saponins (Fig 2a) showed biphasic mode of stability. Crude saponin mixture was observed to protect red blood cells only at the lowest concentration used and did not exert any form of protection as the concentration increases. F<sub>2</sub> 90:10 showed that the fraction did not protect red blood cells at all the concentration used while 80:20 fraction showed biphasic membrane stabilizing profile i.e. fraction protected rbc at lowest concentration but the percentage membrane stability decreased as the concentration increased. F<sub>4</sub> (70:30) adequate-

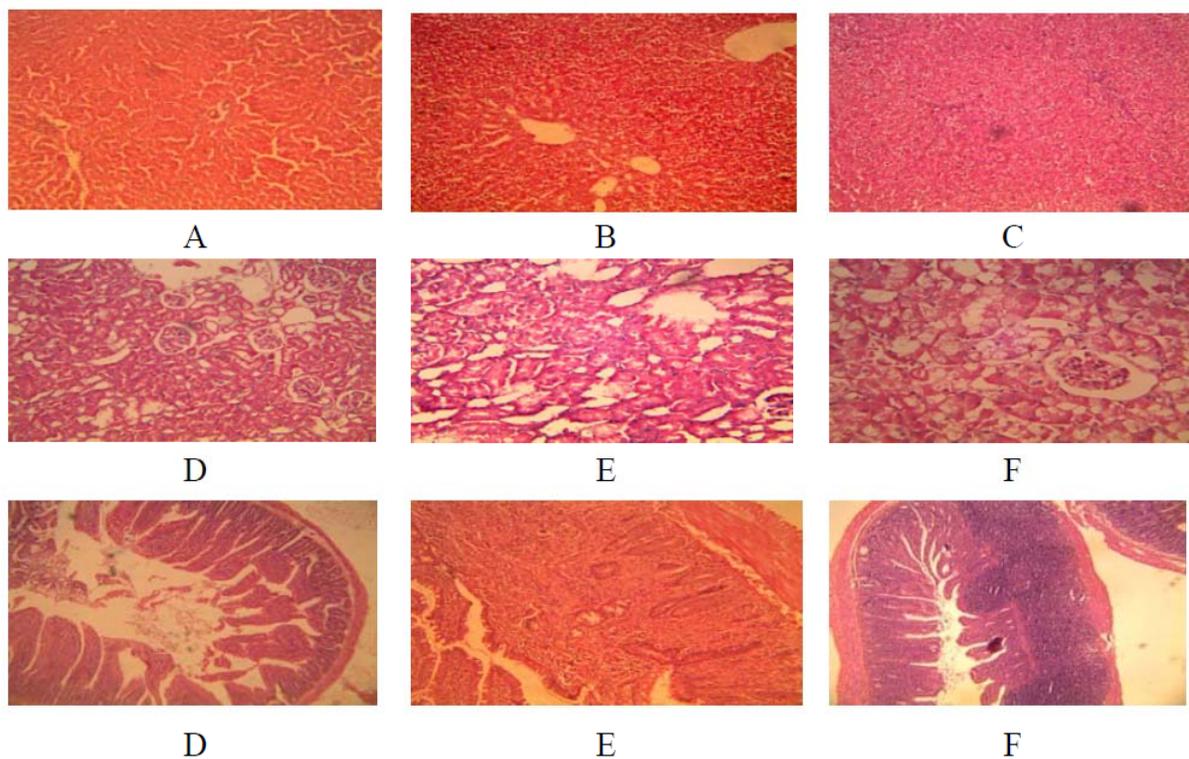


Figure 3. Histopathological analysis of H & E: Section of the liver tissue showing: (A) Normal hepatocytes (Control) x16 objective; (B) mild-moderate necrosis, sinusoidal congestion, prominent nuclei and nuclear vacuolation x16 objective (125mg/ml) and (C) hepatocytes disarray, with extensive necrosis. x16 objective (250 mg/ml). Section of the kidney showing: (D) normal tubules and glomeruli (Control) x16; (E) focal tubular atrophy and necrosis with epithelial vacuolation and prominent nucleoli (Note, upper left corner) x25 125 mg/ml) and (F) severe tubulorrhexis and epithelial vacuolation x40 objective (250 mg/ml). Section of the intestine showing: (G) normal rat intestine (Control) x6.3; (H) acute and chronic inflammatory cells in the widened lamina propria. X6.3 objective (125 mg/ml) and (I) lymphoid hyperplasia in the mucosa and submucosa with increased inflammatory cells in lamina propria x6.3 objective (250 mg/ml).

ely protected the red blood cells more than acetaminophen (a standard anti-inflammatory drug) at all concentrations. The mode of action of the fraction was monophasic i.e. it was concentration dependent.

The symptoms of toxicity manifested by animals during acute toxicity studies of the saponin mixture include restlessness, freezing, writhing, spasmodic jerks of the limbs during locomotion, quickened respiration and convulsion which resulted eventually in death. These symptoms agreed with scientific reports by Bruton and Pye (1876) in their investigations on physiological action of aqueous extract of *E.suaveolens* stem-bark in cats and dogs. The 24 hour median lethal dose (LD<sub>50</sub>) value of combined fractionated saponins (90:10, 80:20 and 70:30) of *E. suaveolens* stem-bark obtained was 61 mg/kg body weight towards mice when administered intraperitoneally implicated toxicity of *E.suaveolens* saponin mixture. The LD<sub>50</sub> value when administered orally in rats was very high, more than 2,900 mg/kg body weight was obtained based on the toxic symptoms (very slow movement, weakness and freezing) manifested because no mortality was recorded after 48 hour observation. It had been reported that absorption of most saponins through an intact mucous membrane was usually very poor because of the large size of the saponins molecules which are not readily absorbed by the intestines. Thus, oral doses usually produce only local effects but systemic intoxications are rare. Studies have also found that continued ingestion of sub-lethal levels can lead to corrosion of the intestinal mucosa, allowing increased absorption which facilitates absorption of toxic quantities (Petrovsky and Aguilar, 2004). Thus, the obtained acute toxicity result of oral administration of *E.suaveolens* stem-bark in rats agreed with literature reports (Price *et al.*, 1987).

Liver plays a major role in metabolism and has a number of functions in the body these includes glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production and excretion of waste materials. Most injected agents pass through the liver before entering the general circulation (Matoni *et al.*, 1993). Liver damage could be confirmed by changes in the activities of hepatic enzymes in serum or plasma by their increased or decreased synthesis, released from damaged cells and extra-hepatic tissue (Chawla, 1999). Significant changes in the activities of plasma and liver aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase,  $\gamma$ -glutamyl transferase (GGT) and bilirubin levels are indices good for detecting liver damage (Boelsterli, 2003).

In this study, the plasma alanine aminotransferase (ALT) activity increased significantly ( $p \leq 0.05$ ) in groups II and III treated rats respectively. Likewise, in the liver of group III treated rats but decreased in that of group II treated rats. However, there was significant ( $p \leq 0.05$ ) decrease in plasma aspartate aminotransferase (AST) activity in group II treated rats and increased in group III treated rats while the enzyme activity increased in the liver of the treated rats with increase in saponin concentration.

Changes observed in transaminases activities in liver and plasma of the treated rats is speculated to be a reflection of liver damage occasioned by ingestion of *E.suaveolens* saponin. Significant decrease in alkaline phosphatase activity in both the plasma and liver of the treated rats was observed as the concentration of saponin mixture increases. Alkaline phosphatase is a marker enzyme for the plasma membrane and endoplasmic reticulum. It is often used to assess the integrity of plasma membrane and also related to the function of

hepatic cell (Akanji *et al.*, 1993). Decrease in plasma level of alkaline phosphatase could be found in severe anaemia, scurvy and kwashiorkor (Chawla, 1999). The reduction effect of alkaline phosphatase activity caused by the saponin mixture could be attributed to severe anemia which was an indication that the toxicity.

Gamma glutamyl transferase (GGT) in plasma originates primarily from the hepatobiliary system. GGT have been reported to be the most sensitive enzymatic indicator of hepatobiliary disease and was elevated in all form of liver diseases (Teitz, 1987). In the present study GGT activity was found to reduce significantly in both the plasma and liver of the treated rats compared to the control. Decreased levels could be found in hypothyroidism, hypothalamic malfunction and very low levels of magnesium (Sahelian, 2009). Reduction in GGT activity occasioned by the saponin mixture confirmed its toxicity to the liver cells.

Significant elevation in both the liver and plasma total protein concentration was observed in treated rats compared with control rats. Emerson *et al.* (1993) reported that elevation in the level of serum proteins is an indication of tissue injury. It could be inferred that increase in protein synthesis due to saponin mixture ingested might be the means through which the treated rats compensated for the production of enzyme or protein lost as a result of tissue necrosis or means to meet increase demand to detoxify the saponin mixture. While the observed decreased in total protein concentration in group III treated rats in both plasma and liver could be attributed to inability of protein synthesizing machinery to be able to function properly as occasioned by saponin mixture at higher concentration.

Albumin is the major protein of the plasma and is responsible for the transportation of the fatty acids and bilirubin through the bloodstream to the liver. The synthesis of albumin is depressed in a variety of diseases, particularly those of the liver (Murray *et al.*, 2003). Since the plasma albumin in the treated rats was significantly affected, elevated in group II treated rats and reduced in group III treated rats when compared with control rats, it means that the transportation of materials within the blood stream might have been affected as well as the liver integrity.

Plasma bilirubin levels in the treated rats was slightly elevated in groups II and III rats respectively while the liver bilirubin level (more pronounced) increased in groups II and III treated rats respectively as concentration of saponins mixture increases. Bilirubin is converted into water soluble derivatives in the liver (Chawla, 1999). Elevation in bilirubin levels occasioned by the treatment with saponin mixture suggests inability of the liver to metabolize bilirubin further confirming liver dysfunction. Elevation of bilirubin might be caused by haemolysis of red blood cells (Chawla, 1999) and this support the claim of attributing reduction in alkaline phosphatase activity, caused by the saponin mixture, to severe anemia.

Plasma creatinine concentration was slightly elevated in group II and group III treated rats while it reduced as the saponin concentration increases in the muscle homogenate of group II and group III treated rats. Bazari (2007) reported that high creatinine level than normal might be due to kidney dysfunction while lower than normal levels indicates late muscular dystrophy and myastharia gravis. The changes observed in the creatinine levels of

both plasma and muscle of the treated rats indirectly suggests kidney damage and muscle dystrophy.

There was slight elevation in the plasma urea activities of the treated rats but the change was not statistically significant. Plasma urea level rarely rises above normal because of the high capacity of normal kidney to excrete urea. However a high plasma urea level is an indicative of renal impairment (Murray *et al.*, 2003). The elevation in the plasma urea level might probably be due to impairment in the excretory function of the kidney possibly as a result of ingested saponin mixture by the treated rats.

Significant elevation was observed in the plasma total sugar levels with concomitant reduction in liver glycogen level in treated rats as the concentration of saponin increased. Elevation of sugar and depletion of glycogen may be due to utilization of carbohydrates as a result of induced hypoxia (Oruc and Uner, 1998). Several studies have shown that the stress of hypoxia is accompanied by depletion of muscle and liver glycogen (Oruc and Uner, 1998). Thus, elevation of sugar and depletion of glycogen levels in this study suggest that the treated rats were under stress and need more energy in order to meet increase in energy demand imposed on them as a result of physiological stress occasioned by *E.suaveolens* saponins. Moreover, increased in muscle glycogen level was observed in saponin treated rats when compared with control rats with a concomitant decreased in the liver glycogen. The observation suggested activation of muscle glycogen phosphorylase by *E.suaveolens* saponins which results in increased gluconeogenesis.

The histology evidence from this study indicated the occurrence of a progressive degeneration of the liver and kidney tissues with the exposure time, justified the significant alteration of hepatorenal indices by *E. suaveolens* saponin mixture. It also elicited histology abnormalities in the intestine of the treated rats. Some investigators have reported untoward histopathological changes in rat /mice tissues following ingestion of saponin containing substances (Hostettmann and Marston, 1995, Gee *et al.*, 1993, Gee and Johnson, 1988,).

In conclusion the speculation by Adeoye and Oyedapo (2004) that alkaloidal fractions of *E.suaveolens* stem-bark might not be the only phytochemical responsible for the plant toxicity was confirmed by the result of the present study. Also, inspection of the enzymes and metabolites most often associated with hepatorenal damage were affected in the treated rats which were justified by histology evidence of a progressive degeneration of the liver and kidney tissues. Therefore there was possibility of *E. suaveolens* saponin mixture directly impairing liver and kidney functions. This may not be a surprise because liver and kidney are sites of xenobiotics metabolism and excretion respectively.

One of the *E.suaveolens* saponin fraction (70:30) was observed to protect bovine erythrocytes membrane against heat and hypotonic induced stress. The saponin fraction protected bovine erythrocyte more than acetaminophen at all concentrations used. Hence it could be surmised that the fraction in addition to its toxic properties exhibited very high anti-inflammatory activity which could be beneficial. However, further investigations on this fraction are hereby suggested.

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