

Research

Assessment of oral and dermal toxicity of selected ethno-medicinal plants used to treat bacterial skin infections in Uganda using Wistar albino rats

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Abstract

Introduction *Erlangea tomentosa* S. Moore, *Plectranthus caespitosus* Lukhoba and *Psorospermum febrifugum* Spach are used in traditional medicine for management of skin and other bacterial infections in Uganda. Unlike their efficacies, the toxicity profiles have not been investigated. Therefore, this study investigated the dermal and oral toxicities of these plants in Wistar albino rats.

Methods Acute and repeated oral doses of the aqueous extracts (2000 and 5000 mg/kg bw; 200 and 400 mg/kg bw respectively) and dermal doses of the organic extracts (8000 and 10,000 mg/kg bw; 2000 and 5000 mg/kg bw respectively) were administered to Wistar albino rats following OECD guidelines with slight modifications. The occluded dermal irritation and modified mouse ear swelling tests were conducted to evaluate skin irritation and sensitization potentials of the medicinal plants. Throughout the study, clinical observations were recorded, and body weights were monitored periodically. Biochemical parameters, organ weights, and histopathological analyses of the liver, kidneys, heart, stomach, and small intestines were performed to detect any signs of systemic toxicity. Additionally, phytochemical screening was carried out to identify the major classes of phytochemicals in the selected plants.

Results The leaf extract of *E. tomentosa* showed no signs of toxicity with acute oral administration at doses of 2000 and 5000 mg/kg bw. However, repeated oral exposure at 400 mg/kg bw resulted in liver injury. Whereas no acute dermal toxicity was observed for *E. tomentosa*, repeated dermal exposure at 200 and 400 mg/kg bw caused necrotizing liver hepatitis. Acute oral exposure to 5000 mg/kg bw of *P. febrifugum* aqueous extract increased liver weight and temporarily elevated respiration, urination, and mobility, all of which resolved within 24 h. In contrast, repeated oral exposure at 400 mg/kg bw led to reduced liver and kidney weights, with biochemical markers indicating liver injury, although histopathology revealed no significant lesions. Dermal exposure to the organic extract did not cause skin irritation or sensitization. However, repeated dermal application at 2000, 5000 and 8000 mg/kg bw led to increased bilirubin, creatinine, and AST levels, alongside decreased urea and ALP levels, suggesting cholestasis and impaired kidney function. *P. caespitosus* demonstrated no signs of toxicity with acute oral administration at 2000 and 5000 mg/kg bw, and repeated oral exposure at 200 and 400 mg/kg did not cause organ toxicity. No acute dermal toxicity was observed, even with repeated exposure.

Conclusion & recommendation No mortality or acute toxicity was observed with oral or dermal administration of *E. tomentosa*, *P. febrifugum*, and *P. caespitosus* extracts in Wistar albino rats, though repeated exposure to some extracts

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indicated potential liver and kidney toxicity. Phytochemical analysis revealed the presence of anthraquinones, coumarins, saponins, steroids, and tannins, suggesting further investigation is needed to fully understand their toxicological profiles.

Keywords Toxicity · Plant extracts · Histopathology · Biochemical parameters · Skin irritation · Skin sensitization · Phytochemistry

Abbreviations

OECD	Organization for Economic Co-operation and Development
WHO	World Health Organization
IC ₅₀	Half-maximal inhibitory concentration
BIL	Bilirubin
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
GGT	Gamma glutamyl transferase
ALB	Albumin
Na	Sodium
K	Potassium
Cl	Chloride

1 Introduction

Bacterial skin infections are a burden to many rural populations in developing countries. In such countries, the management of these infections is usually not integrated in conventional national health care systems [1, 2]. Moreover, due to the high costs of antimicrobial drugs coupled with their limited access to people in remote areas, the WHO emphasizes the need for medicinal plant remedies [3]. In rural areas of Uganda, medicinal plants are largely used for management of skin infections. Various ethnobotanical reports have highlighted the popularity of medicinal plants in the treatment of bacterial infections [4–6]. Nevertheless, there is limited information on the toxicity of most of the plants used.

One other reason that people are turning to plant based antibacterial products is bacterial resistance [7]. The rise in antimicrobial resistance has been driven by several factors, such as misuse and overuse of antibiotics, poor infection prevention and control practices, limited access to quality healthcare, weak regulatory frameworks, lack of public awareness, high burden of infectious diseases, and antibiotic contamination in the environment.

Previously, we reported promising antibacterial activities of *Erlangea tomentosa*, *Plectranthus caespitosus* and *Psorospermum febrifugum* against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhi* with minimum inhibitory concentrations ranging between 3.12 and 12.5 mg/ml. In the same study, the plants exhibited good antioxidant activity (IC₅₀ 25.04–57.01 mg/ml) and good sun protection factor ranging from (SPF, 3.08–37.84) [8].

E. tomentosa, also synonymous to *Bothriocline longipes* (Oliv & Hiern) Wild & G.V Pope (Asteraceae) is an erect woody herb that grows to about 50 cm high with opposite petiolate and pubescent leaves. The plant is present in East Africa, as well as Zimbabwe, Zambia, Angola and Malawi. In Uganda, the plant is locally known as ‘ekyoganyanja’ in Runyankore and ‘kisula’ in Luganda [9], and it is used traditionally to treat skin infections [10]. An infusion or water decoction of the plant is usually administered either topically or orally depending on the ailment being treated. The leaf extracts of *E. tomentosa* were investigated against *S. typhi*, *E. coli*, *K. pneumoniae* and *S. aureus*. The hexane extract showed no inhibition for any of the bacterial strains, the dichloromethane extract showed varying effectiveness, with the highest inhibition against *K. pneumoniae* (17.3 mm) and moderate inhibition against *S. typhi* (10 mm). The ethyl acetate extract showed significant antimicrobial activity across all tested bacteria, particularly effective against *S. aureus* (16.0 mm) and *K. pneumoniae* (17.03 mm) while the methanol extract showed consistent antimicrobial activity, most notably against *S. aureus* (15.0 mm) and *E. coli* (14.7 mm). Isolation of the ethyl acetate extract yielded Eriodictyol-7-O-β-glucoside and 5, 7, 3', 4'-tetrahydroxy-7-O-[6''-O-(acetyl)-β-D-glucopyranosyloxy]-flavanone, and the dichloromethane extract yielded di-(2-ethylhexyl) phthalate which is reported to have a moderate activity against *Bacillus subtilis*, *S. aureus*, *S. equosemens*, *E. coli* and *P. aeruginosa* [11]. The biological property of eriodictyol-7-O-glucoside has not been well established. In contrast

to eriodictyol-7-*O*-glucoside, eriodictyol has been associated with a variety of biological properties in vitro and in vivo such as antioxidant, antimicrobial, anti-inflammatory all of which play a vital role in healing various skin infections [12–14].

P. caespitosus (Lamiaceae), synonymous with *Equilabium caespitosum* is a perennial aromatic clump-forming suffrutescent with several stems arising from a large fleshy rootstock. It stands up to 0.5 m tall with ascending petiolate thick textured elliptic leaves. The plant is present in Uganda, Kenya and Tanzania [15]. Savina et al. [16] reported the use of the plant to treat skin allergy among other illnesses [17]. Other *Plectranthus* species such as *P. barbatus* and *P. amboinicus* have been reported elsewhere as having antibacterial properties [18, 19] and are used in the treatment of skin infections [20, 21]. Studies on related *Plectranthus* species identified various bioactive compounds such as flavonoids and terpenoids, especially the abietane type, which are known to possess antimicrobial, anti-inflammatory and anti-oxidant properties. The antibacterial compounds isolated from species of *Plectranthus* include; forskolin, apigenin, luteolin, rosmarinic acid, carvacrol among others [22].

P. febrifugum (Hypericaceae) is a small sized tree found in the tropical regions of Africa, Madagascar and South America. It grows in open woodland over a wide range of altitudes with fragrant and inconspicuous flowers, small berries of 6 mm in diameter and bright red when mature. The plant is commonly called Christmas berry in English and “akanzironziro” in Luganda/Lusoga [5] and “nangoso” in western Kenya [23]. The genus *Psorospermum* has been used by indigenous African populations for treatment of skin diseases and subcutaneous wounds such as treatment for leprosy and skin sores in HIV/AIDS patients [5, 24–26]. Among the array of biological activities, the stem bark extracts of *P. febrifugum* have been shown to possess antibacterial properties, effectively targeting a broad spectrum of bacteria, including both Gram-positive (*S. aureus* and *S. pyogenes*) and Gram-negative (*K. pneumoniae*, *S. typhi*, *E. coli*, and *P. aeruginosa*). *P. febrifugum* on the other hand has been studied to a fairly greater extent than *E. tomentosa* and *P. caespitosus*. Various phytochemicals including xanthenes, anthraquinones, terpenoids and alkaloids have been isolated from the plant. Antimicrobial compounds isolated from the stem bark of *P. febrifugum* include; febrifuquinone, carvacrol, adamabianthrone, caryophyllene oxide, among others [25].

This study therefore compliments the previous study [8], by examining the oral and dermal toxicity profiles of *E. tomentosa* (ET), *P. caespitosus* (PC) and *P. febrifugum* (PF) extracts upon acute and sub-acute exposure to Wistar albino rats.

2 Materials and methods

2.1 Collection and preparation of plant material

The plant collection was conducted between April and June and prepared as previously reported by Namukobe et al. [8]. The leaves of *E. tomentosa*, *P. caespitosus* and the stem bark of *P. febrifugum* were collected from Mpanga Central Forest Reserve (0° 12' 29.5" N, 32° 17' 32.4" E) and Mabira forest (0° 24' 3.6" N, 33° 2' 10.3" E) respectively. Plant sample collection procedures followed local and national regulations. Permission was obtained from the respective forest reserves offices prior to sample collection commenced. The plants were authenticated by Mr. Rwaburindore Portase (taxonomist) at the Makerere University herbarium, Department of Botany where voucher specimens (AS 056, AS 003 and JN38) were stored for future reference. The samples were chopped into small pieces and air dried under shade for 28 days. The dry samples were then ground into fine powder, sealed in air-tight polythene bags and stored in a cool dry place.

Aqueous extracts were used for only oral toxicity testing since these medicinal plants are traditionally consumed as water-based decoctions. Organic extracts were therefore excluded from oral toxicity assessments as they do not reflect typical consumption methods. For dermal toxicity evaluations, organic extracts were selected since the plants are commonly applied to the skin, often mixed with vehicles such as petroleum jelly. Aqueous extracts were therefore not chosen for dermal/topical toxicity assessments in this study.

2.2 Sample extract preparation

Finely powdered plant material was extracted with water and organic solvents (Dichloromethane: Methanol 1:1 v/v) separately. The aqueous extracts were obtained by warm maceration (40 °C) while the organic extracts were obtained by cold maceration. The extracts were filtered and either freeze dried (aqueous extract) or concentrated on a rotary evaporator at 40 °C to obtain a dried extract. The dried extracts were stored in tightly stoppered sample bottles in a refrigerator awaiting further analysis.

2.3 Toxicity studies

The study utilized rats aged 10–12 weeks. The animals were maintained under controlled environmental conditions, with humidity levels maintained between 60–70% and temperatures regulated at 24 ± 1 °C. Prior to initiating toxicity tests, the rats were acclimatized for a period of 7 days.

2.3.1 Determination of acute toxicity of the extracts

Acute oral and dermal toxicity studies were carried out according to OECD guidelines with slight modifications [27, 28]. For oral toxicity, five groups of rats (four test groups and one control group) were used while nine groups (eight test groups and one control group) ($n=3$ per group) were used to study dermal toxicity.

Organic and aqueous extracts from three plants were used for dermal sensitization testing, while aqueous extracts from four plants were administered for acute oral toxicity testing. Animals were fasted overnight and weighed before extract administration. Organic extracts were dissolved in Tween 20, and aqueous extracts in distilled water. Test groups received a single dose of 5000 mg/kg bw, while control groups received an equivalent volume of either Tween 20 or distilled water.

For oral toxicity, both the test and control groups received the dissolved herbal extracts orally using a 5-inch gavage feeding tube. Food was provided after 4 h of administration. After administration, the animals were placed in clean and transparent observation chambers and individually observed for clinical signs of drug toxicity during the first 30 min, then on hourly basis and then on 24 h basis for 21 days. The body weights of the animals were recorded weekly during the treatment period.

At the end of the treatment period, rats were subjected to overnight fasting and then euthanized with an intraperitoneal injection of pentobarbital (150 mg/kg bw). Blood was collected by cardiac puncture for hematologic and biochemical analyses before the organs (including the heart, kidneys, small intestines, liver and the stomach) were harvested, weighed and collectively suspended in formalin for preservation prior to histopathological analysis.

For acute dermal toxicity, the extract was put in place with a porous gauze dressing and a non-irritating tape on a previously clipped dorsal area throughout the exposure period. After exposure, the application site was thoroughly cleaned with sterile distilled water. Cage side/top observations were frequently done for changes in behaviour patterns, skin reactions or corrosion of test samples, body weight and death if any. The rats were monitored for 14 days.

The method for determination of doses used for dermal application was as follows. The rat's skin surface area was estimated using the formula;

$$\text{Surface area (cm}^2\text{)} = \kappa \times \text{Body weight (g)}^{2/3}, \kappa = 10.5 \text{ for rats.}$$

The total dose was determined by multiplying the desired dose (mg/kg bw) by the rat's weight (kg). This total dose was then converted to a dose per unit area (mg/cm²) by dividing by the surface area. The volume of the solution needed per unit area was calculated by dividing the dose per unit area by the concentration of the solution (mg/mL). Finally, the total volume to apply was obtained by multiplying the volume per unit area by the surface area.

2.3.2 Determination of repeated dose toxicity

Repeated dose oral and dermal toxicity studies were done according to OECD guidelines [29, 30] with some modifications. Male and female (nulliparous and non-pregnant) Wistar albino rats were repeatedly dosed orally for 28-days and dermally for 21 days. The doses selected for repeated dose dermal toxicity were 200 and 400 mg/kg bw, and 2000 and 5000 mg/kg bw for oral toxicity.

For oral toxicity, the animals were randomly divided into five groups (4 test, 1 control; $n=3$) while the dermal toxicity group had nine groups (eight test, one control, $n=3$). Oral administration of the extracts was done using a 5-inch infant feeding gavage tube while a porous gauze dressing soaked with the extract was wrapped on the clipped dorsal area of the rat to study dermal toxicity.

Both treatments were done daily, and body weights of the animals taken weekly around noon time for 28 days. The animals were also observed for clinical signs of drug toxicity. At the end of the treatment period, blood was collected by

cardiac puncture for hematologic and biochemical analyses. The rats were euthanized as described above and dissected to obtain the kidneys, liver, heart, stomach, skin tissue and small intestines which were then weighed and preserved in formalin for further analysis.

2.3.3 Histopathology

Organ tissues were taken and processed using an automated tissue processor (Biorex, TRX 7070, Germany). After processing, the tissues were sectioned to a thickness of 5 µm using a rotary microtome and dried overnight in an oven at 37 °C. The sections were stained with hematoxylin and eosin (H&E) and examined microscopically for signs of toxicity [31].

2.3.4 Determination of biochemical parameters

Biochemical analysis was done by a fully automated biochemistry analyzer (COBAS 6000). Hepatic and renal toxicity was determined by measuring parameters including direct and total bilirubin (BIL), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT) and albumin (ALB) for the liver as well as total protein, creatinine, urea, sodium, potassium and chlorides for the kidneys [32].

2.3.5 Skin irritation test

The degree of dermal irritation of the plant extracts was determined using the occluded dermal irritation test method as described by Teshome et al. [33]. Three rats were used per test group. About 10% of total body surface area equivalent of the back hair was clipped. The extract (0.5 g), mixed in tween 20 (10 mL), was evenly applied to the right-side of the shaved area and the skin was covered with a gauze patch, plastic sheet and a non-irritating adhesive plaster. The rats were exposed to the test extract for 24 h after which the bandage was removed. The area was rinsed with distilled water before returning the rat into its cage. The rats' right and left side (control) shaved area were examined for the presence of erythema according to the Draize dermal irritation (Table 1) scoring system at 1-, 24-, 48- and 72-h intervals. The primary irritation index (PII) was calculated and the extracts classified according to the Draize method of classification [34].

2.3.6 Dermal sensitization test

The skin sensitization test was done according to [33] with modifications. Wistar albino rats aged 10–12 weeks were utilized, and tape stripping was applied as a mechanical technique to enhance immune responses. Solvent selection was based solely on solubility, with 70% ethanol chosen as it effectively solubilized the extract.

In the preliminary range-finding test, eight female mice were allocated into three groups, each receiving different concentrations of the extract. The test solution was applied to the abdomen and ears, and irritation was evaluated on Day 4. The concentration causing mild irritation (4 mg/mL) was selected for induction, while the highest non-irritant concentration (5 mg/mL) was chosen for the challenge phase.

For the primary test, ten mice were randomly divided into test and control groups. On Day 0, the abdomen was clipped and treated with either the extract solution (4 mg/mL) or vehicle (70% ethanol). This treatment was repeated on Days 1, 3,

Table 1 Draize scoring criteria for erythema classification

Endpoint measured	Grade
No perceptible erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema to scar formation	4
Primary Irritation Index (PII)	
Non-irritant	PII < 1.5
Irritant	1.5 < PII < 3
Moderately irritant	3 < PII < 5
Severe irritant/corrosive	PII > 5

and 5. The mice were then left undisturbed until Day 10. On Day 10, mice from the test group and one control group were challenged with 20 μ L of the extract (5 mg/mL) applied to both the dorsal and ventral surfaces of the left ear, while the right ear received the vehicle. Ear thickness was measured on Days 11 and 12.

On Day 17, a re-challenge was conducted using a higher concentration of the extract (10 mg/mL). Thickness measurements were recorded on Days 18 and 19. The percent ear swelling (% Δ) was calculated by comparing the treated ear's thickness to that of the control ear. Additionally, the percent responder rate was determined by the proportion of mice exhibiting a positive response (% $\Delta > 20$). These metrics were used to evaluate and compare sensitization levels across test and control groups.

2.3.7 Qualitative phytochemical screening

The preliminary qualitative phytochemical screening was carried out as described by [35]. Finely ground plant samples were extracted into diethyl ether, methanol and distilled water for analysis. The samples were analysed for presence steroids, alkaloids, anthraquinones, coumarins, flavonoids, tannins, anthocyanins and saponins.

2.4 Statistical analysis

The numerical data was expressed as mean $\pm$ standard deviation and analysed for significant statistical differences using the IBM SPSS Statistics 20. One-way analysis of variance (ANOVA) followed by post hoc Tukey's HSD and Dunnett's test for multiple comparisons were used to determine statistical significance. Differences were considered statistically significant at $P \leq 0.05$.

2.5 Ethical approval for animal studies

This study was done after approval from the Institutional Animal Care and Use committee of the School of Veterinary Medicine and Animal Resources, Makerere University Ref. No. SVAR_IACUC/73/2021 and the Uganda National Council for Science and Technology (UNCST) approval number NS306ES.

3 Results

3.1 Clinical signs and observations against the acute exposure to the plant extracts

There was a marked sustained increase in mobility and hyperventilation after 24 h in both *E. tomentosa* and *P. caespitosus* plant extracts, however this subsided after 24 h only in the case of *P. caespitosus* (Table 2). There was an increased urination at 5000 mg/kg bw for *E. tomentosa* extract.

3.2 Skin irritation and dermal sensitization

There was no mortality, significant body weight changes or skin damage observed during both the acute and repeated dose dermal toxicity period of study. The results also showed no irritation as the Draize scoring criteria (Table 3). Furthermore, there was no skin sensitization observed after conducting the ear swelling test as shown in Tables 4 and 5.

3.3 Effects of the extracts on body and organ weights

The body weights of the animals generally increased throughout the study period and the weights at the 14th day showed no statistically significant differences as showed in Fig. 1.

3.4 Effects of the extracts on biochemical parameters

3.4.1 Effects of the extracts on biochemical parameters during the oral toxicity study

In the oral toxicity study (Table 6), repeated administration of *E. tomentosa* aqueous extract at both 200 and 400 mg/kg bw did not lead to any significant alterations in the biochemical parameters when compared to the negative

Table 2 The effect of aqueous extracts on the behaviour of rats in acute oral toxicity studies

Time periods	Dosage (mg/kg bw)	Negative control		<i>E. tomentosa</i>				<i>P. caespitosus</i>			
				< 3 h		> 24 h		< 3 h		> 24 h	
				2000	5000	2000	5000	2000	5000	2000	5000
Coma	A	A	A	A	A	A	A	A	A	A	A
Convulsions and tremors	A	A	A	A	A	A	A	A	A	A	A
Eyes	N	N	N	N	N	N	N	N	N	N	N
Faeces consistency	N	N	N	N	N	N	N	N	N	N	N
Fur and skin	N	N	N	N	N	N	N	N	N	N	N
Itching	A	A	A	A	A	A	A	A	A	A	A
Mortality	A	A	A	A	A	A	A	A	A	A	A
Mucous membrane	N	N	N	N	N	N	N	N	N	N	N
Respiration	N	↑	↑	↑	↑	↑	↑	↑	↑	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N	N
Somatomotor activity and behavior pattern	N	↑	↑	N	N	N	N	N	N	N	N
Urination	N	N	N	N	↑	N	↑	N	↑	N	N
Mobility	N	↓	↓	↑	↑	↑	↑	↑	↑	N	N

A Absent, N Normal, ↑ increased activity, ↓ decreased activity

Table 3 Draize scoring criteria for erythema of the extracts

Medicinal Plant	Negative control	<i>E. tomentosa</i>		<i>P. caespitosus</i>		<i>P. febrifugum</i>	
		Aqueous	Organic	Aqueous	Organic	Aqueous	Organic
Day 0	0	0	1	0	0	0	0
Day 1	0	0	1	0	0	0	0
Day 2	0	0	0	0	0	0	0
Day 3	0	0	0	0	0	0	0
Day 8	0	0	0	0	0	0	0
Day 16	0	0	0	0	0	0	0
Primary Irritation Index	0	0	0.33	0	0	0	0
Interpretation	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Non-irritant

control. Conversely, *P. febrifugum* at 200 mg/kg bw primarily affected total bilirubin and AST levels, with no significant changes observed in other parameters. At a higher dose of 400 mg/kg bw, *P. febrifugum* stem bark extract notably increased both total and direct bilirubin levels, indicating potential disturbances in bilirubin processing or liver function.

3.4.2 Effects of the extracts on biochemical parameters during the dermal toxicity study

During the dermal toxicity study, *E. tomentosa* aqueous extract at 2000 and 5000 mg/kg bw significantly reduced levels of ALT, ALP, and urea. Additionally, bilirubin levels were elevated at the 2000 mg/kg bw dose. No significant changes in biochemical parameters were observed at the higher acute doses of 8000 and 10,000 mg/kg bw. In contrast, *P. febrifugum* exposure at 2000 and 5000 mg/kg bw led to significant increases in AST, bilirubin, and creatinine levels, with corresponding reductions in urea and ALP levels. At the 5000 mg/kg bw dose, *P. febrifugum* raised bilirubin and creatinine levels while decreasing AST, ALP, and urea levels. Acute exposure to 8000 mg/kg bw resulted in increases in sodium and potassium levels, while the highest dose of 10,000 mg/kg bw did not produce significant changes in any biochemical parameters compared to the negative control.

Table 4 Average ear swelling measurements of the skin sensitization test for the different plants during the challenge stage

	Reading after 24 h (mm)		% Δ	Reading after 48 h (mm)		% Δ	% Ear swelling after 24 h	% Ear swelling5 after 48 h
	Left ear (A)	Right ear (B)		Left ear (A)	Right ear (B)			
Control group								
1	0.250	0.248	0.81	0.254	0.253	0.400	100.64	100.51
2	0.252	0.251	0.4	0.248	0.247	0.400		
3	0.285	0.283	0.71	0.282	0.280	0.710		
Sum	0.787	0.782	0.64	0.784	0.780	0.510		
<i>E. tomentosa</i>								
1	0.240	0.238	0.84	0.250	0.249	0.400	100.81	100.55
2	0.241	0.239	0.84	0.265	0.264	0.380		
3	0.265	0.263	0.76	0.220	0.218	0.920		
Sum	0.746	0.740	0.81	0.735	0.731	0.550		
<i>P. caespitosus</i>								
1	0.233	0.234	-0.4	0.236	0.235	0.430	100.55	100.26
2	0.249	0.246	1.22	0.273	0.274	-0.360		
3	0.250	0.248	0.81	0.254	0.252	0.790		
Sum	0.732	0.728	0.55	0.763	0.761	0.260		
<i>P. febrifugum</i>								
1	0.271	0.268	1.12	0.270	0.268	0.750	100.93	100.62
2	0.233	0.231	0.87	0.275	0.274	0.360		
3	0.256	0.254	0.79	0.271	0.269	0.740		
Sum	0.760	0.753	0.93	0.816	0.811	0.620		

Table 5 Average ear swelling measurements of the actual skin sensitization test for the different plants during the re-challenge stage

	Reading after 24 h (mm)		% Δ	Reading after 48 h (mm)		% Δ	% Ear swelling after 24 h	% Ear swelling after 48 h
	Left ear (A)	Right ear (B)		Left ear (A)	Right ear (B)			
Control group								
1	0.255	0.253	0.79	0.251	0.250	0.400	100.52	100.41
2	0.264	0.263	0.38	0.262	0.261	0.380		
3	0.249	0.248	0.4	0.224	0.223	0.450		
Sum	0.768	0.764	0.52	0.737	0.734	0.410		
<i>E. tomentosa</i>								
1	0.263	0.261	0.77	0.253	0.252	0.400	100.91	100.54
2	0.232	0.230	0.87	0.228	0.227	0.440		
3	0.283	0.280	1.07	0.266	0.264	0.760		
Sum	0.778	0.771	0.91	0.747	0.743	0.540		
<i>P. caespitosus</i>								
1	0.225	0.222	1.35	0.280	0.278	0.720	100.70	100.63
2	0.234	0.233	0.43	0.254	0.252	0.790		
3	0.257	0.256	0.39	0.270	0.269	0.370		
Sum	0.716	0.711	0.7	0.804	0.799	0.630		
<i>P. febrifugum</i>								
1	0.276	0.274	0.73	0.246	0.245	0.410	100.72	100.64
2	0.288	0.286	0.7	0.263	0.260	1.150		
3	0.281	0.279	0.72	0.278	0.277	0.360		
Sum	0.845	0.839	0.72	0.787	0.782	0.640		

Fig. 1 Effect of 2000 and 5000 mg/kg bw doses of the aqueous extracts on the body weights of Wistar albino rats during the acute oral toxicity study. Results are presented as mean $\pm$ standard deviation n=3. ET-*E. tomentosa*, PF-*P. febrifugum*, 2000/5000—dose (in mg/kg bw)

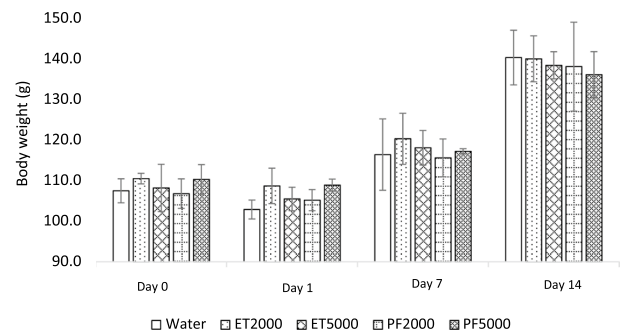
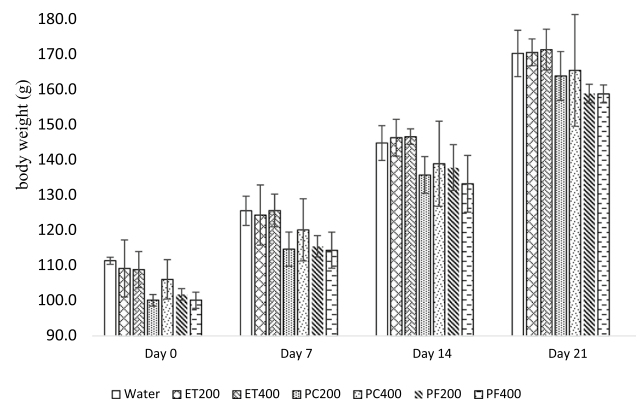


Table 6 Biochemical parameters of the rats in repeated dose oral toxicity studies of the selected aqueous extracts

Dose, mg/kg bw	Negative control	<i>E. tomentosa</i>		<i>P. febrifugum</i>		<i>P. caespitosus</i>	
		200	400	200	400	200	400
Total bilirubin, $\mu\text{mol/L}$	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	$4.80 \pm 0.44^*$	NR	NR
Direct bilirubin, $\mu\text{mol/L}$	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	$2.10 \pm 0.26^*$	NR	NR
ALT, U/L	135.47 ± 11.54	118.93 ± 24.35	138.70 ± 22.53	136.47 ± 33.93	136.53 ± 10.05	NR	NR
AST, U/L	162.40 ± 6.56	206.63 ± 20.07	245.97 ± 45.85	$341.43 \pm 107.80^*$	227.17 ± 31.52	NR	NR
ALP, U/L	439.17 ± 21.88	320.20 ± 65.24	381.20 ± 74.25	343.87 ± 2.84	336.87 ± 32.52	NR	NR
GGT, U/L	5.90 ± 2.01	6.27 ± 1.95	8.23 ± 2.44	7.67 ± 1.94	4.35 ± 0.35	NR	NR
Total protein, g/L	76.13 ± 1.07	87.80 ± 3.12	87.73 ± 7.05	78.30 ± 6.51	80.67 ± 3.04	NR	NR
ALB, g/L	32.07 ± 1.33	31.93 ± 1.55	31.53 ± 0.90	31.70 ± 0.46	33.67 ± 0.81	NR	NR
Creatinine, $\mu\text{mol/L}$	97.17 ± 5.62	101.47 ± 2.63	76.83 ± 10.50	101.50 ± 2.09	88.83 ± 1.40	NR	NR
Urea, mmol/L	4.16 ± 0.44	5.03 ± 0.15	5.28 ± 1.38	6.44 ± 0.41	5.10 ± 0.36	NR	NR
Sodium, mmol/L	139.10 ± 1.01	140.73 ± 3.07	138.23 ± 2.20	140.03 ± 1.06	139.67 ± 3.15	NR	NR
Potassium, mmol/L	5.00 ± 1.06	4.33 ± 0.47	3.98 ± 0.03	5.17 ± 0.35	4.29 ± 0.61	NR	NR
Chloride, mmol/l	105.93 ± 3.62	100.70 ± 1.31	103.62 ± 4.51	102.20 ± 2.38	100.83 ± 2.52	NR	NR

Results are presented as mean $\pm$ standard deviation, n=4, There are statistically significant differences ($P \leq 0.05$) between *labelled values and the negative control in the same row. NR=Not Reported

Fig. 2 Photomicrographs of histological sections showing the effect of 400 mg/kg bw on different organs for repeated dose oral toxicity



3.5 Effects of the plant extracts on organs histopathology

This was done to assess the effects of long-term exposure to aqueous extracts towards selected vital body organs. Histopathology done on the heart, small intestines, liver, kidney and stomach showed no difference between the control and *P. caespitosus* aqueous extract at 200 and 400 mg/kg bw dose. However, *E. tomentosa* at 400 mg/kg bw

Table 7 Phytochemical screening of the extracts

Medicinal plant Extraction solvent	<i>P. febrifugum</i> stem bark			<i>E. tomentosa</i> Leaves			<i>P. caespitosus</i> Leaves		
	Ether	MeOH	H ₂ O	Ether	MeOH	H ₂ O	Ether	MeOH	H ₂ O
Steroids	+	+	N/A	–	–	N/A	+	–	N/A
Alkaloids	–	–	–	–	–	–	–	–	–
Anthraquinones	+	+	N/A	+	+	N/A	–	–	N/A
Coumarins	–	+	N/A	–	–	N/A	+	–	N/A
Flavonoids	+	+	+	–	–	+	–	–	+
Tannins	N/A	+	+	N/A	+	+	N/A	+	+
Anthocyanin	N/A	–	N/A	N/A	–	N/A	N/A	–	N/A
Saponins	N/A	–	+	N/A	–	+	N/A	–	+

– absent, + present, n/a test not applicable in the extraction solvent, MeOH Methanol

Table 8 Organ weights (g) of rats after acute oral toxicity studies

Dose (mg/kg bw)	Negative control	<i>E. tomentosa</i>		<i>P. caespitosus</i>	
		2000	5000	2000	5000
Absolute weights (g)					
Liver	7.6 ± 2.2	6.5 ± 0.6	6.5 ± 1.0	8.0 ± 1.0	8.0 ± 0.3
Heart	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.9 ± 0.2	0.9 ± 0.1
Kidneys	0.7 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	1.0 ± 0.1
Relative weights (%)					
Liver	5.42 ± 0.20	4.65 ± 0.20	4.70 ± 0.06	5.80 ± 0.86	5.88 ± 0.66
Heart	0.57 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.65 ± 0.01	0.66 ± 0.00
Kidneys	0.50 ± 0.00	0.36 ± 0.00*	0.43 ± 0.01	0.58 ± 0.01	0.74 ± 0.00*

Results are presented as mean ± standard deviation, n=3. There are statistically significant differences between *labelled values and the negative control in the same row

caused intestinal hyperplasia of goblet cells and villi sloughing and mild to severe multifocal necrotizing hepatitis in the Liver (Fig. 2b). The heart, stomach and kidneys from all the study groups were normal and unaffected by the oral exposure to the studied extracts.

3.6 Qualitative phytochemical screening of the plant extracts

The phytochemical screening of the plants revealed the presence of anthraquinones, coumarins, flavonoids, saponins, tannins and steroids in *P. febrifugum*, anthraquinones, tannins and saponins in *E. tomentosa* and steroids, coumarins, tannins and saponins in *P. caespitosus* as shown in Table 7.

4 Discussion

4.1 *Erlangea tomentosa*

Acute oral exposure to the aqueous extracts of *E. tomentosa* at 2000 and 5000 mg/kg bw induced clinical signs of toxicity such as elevated respiration and reduced mobility which subsided in 24 h as shown in Table 2. Additionally, reductions in relative kidney weights were observed at both doses (Table 8). However, after repeated dose exposure of 400 mg/kg bw histopathology results revealed moderate to severe multifocal necrotizing hepatitis as well as intestinal hyperplasia of goblet cells and mild villi sloughing (Fig. 2b). This can be attributed to toxic plant extract exposure [36, 37]. Drugs have been shown to have a range of side effects along the gastrointestinal tract [38–40]. The severe intestinal hyperplasia caused by repeated exposure to 400 mg/kg bw of *E. tomentosa* aqueous extract could therefore result into ulceration of the small intestine. However, the stomach lining had no significant lesions in comparison with the control (Fig. 2a).

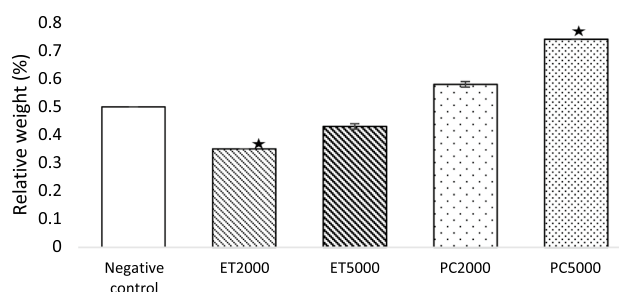


Fig. 3 Effect of 200 and 400 mg/kg bw doses of the different aqueous extracts on the body weights during the 21-day repeated dose oral toxicity study observation period. Results are presented as mean $\pm$ standard deviation $n=2$, ET- *E. tomentosa*, PF-P. *febrifugum*, PC—*P. caespitosus* 200/400—dose (in mg/kg bw)

Furthermore, the severe multifocal necrotizing hepatitis observed by histopathology was not corroborated with either the biochemical results (Table 6) as well as body weights for both acute and repeated dose studies (Fig. 3). The ALT: ALP ratio was below 10, and the levels of bilirubin, creatinine and ALP were not statistically different from the control group (Table 6). Rapid recovery of the biochemistry profile after withdrawing of necrotizing drugs is a common phenomenon and this could explain the mismatch in the results [41, 42].

Regarding dermal exposure, the organic extract of *E. tomentosa* was neither a sensitizer nor an irritant on topical application. The extract had a primary irritation index below 1.5 (Table 3) and hence classified as non-irritant. The erythema observed reduced quickly after removing the gauze patch. This therefore may not only be attributed to the extract but also as an occlusion effect. Occlusion effects results when an impermeable film of a substance directly covers the skin. Skin occlusion usually produces profound changes in the dermis including reduced hydration, affects molecular and cellular processes, changes in epidermal lipid profile etc. This varied changes in the skin may provoke adverse skin reactions such as contact and allergic contact dermatitis [43, 44].

Nevertheless, repeated dermal exposure to the organic extract resulted in elevated respiration, sematomotor activity and reduced mobility. Biochemistry analysis revealed significant reduction in ALT, ALP (Fig. 4) and urea, with simultaneous increase in bilirubin levels. Bilirubin is made during normal breakdown of red blood cells into bile. The inability of the liver to clear bilirubin results in its build up which may be as a result of cholestasis, biliary obstruction, and intra-hepatocellular/extra-hepatocellular duct blockages [45, 46]. Reduction in levels of ALT and ALP are less indicative of liver damage and more indicative of plant hepatoprotective effects [47]. This result seems to support reports of toxicity of *E. tomentosa* flowers [47]. In that report, young leaves of the plant were determined to be toxic to a rabbit, with a fatal dose of 1160 g resulting in death within a week. A post-mortem examination showed considerable abdominal ascites and impacted dry ingesta in the stomach, indicating possible gastrointestinal obstruction or distress. Additionally, the liver, kidneys, and lungs were significantly congested, suggesting systemic toxicity and impaired organ function.

ALT and ALP are enzymes predominantly found in liver cells, with ALT primarily located in hepatocytes and playing a critical role in amino acid metabolism. When liver tissue is damaged, ALT is released into the bloodstream, serving as a marker for liver injury. Although ALP is also present in liver cells, it is found in other tissues as well, and elevated levels can indicate extra-hepatic tissue damage.

In a recent study, the repeated dose toxicity could be attributed to presence of tannins and anthraquinones in the aqueous extract. Studies have shown that oral exposure to tannin-rich containing plant extracts can cause mild disturbances in the liver and kidney functions. Tannins bind to epithelial proteins causing liver damage in form of necrosis in

Fig. 4 Effect of the various extract on the level of liver enzymes in the repeated dose oral toxicity studies (there is a statistically significant difference between * marked extract and the negative control)

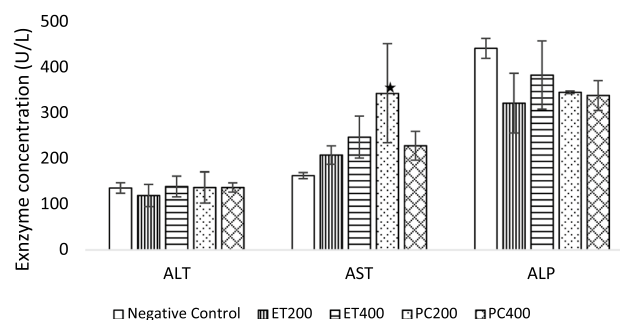


Fig. 5 Effect of the various extract on the level of liver enzymes in the repeated dose dermal toxicity studies (there is a statistically significant difference between * marked extract and the negative control)

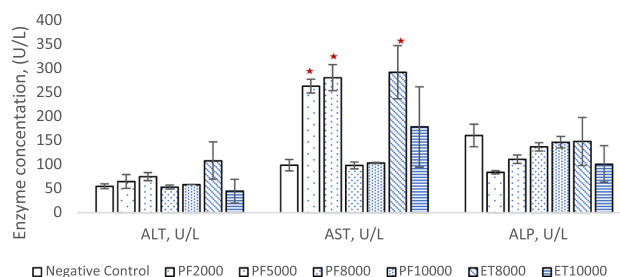
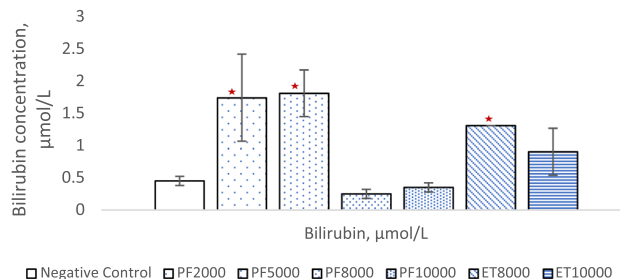


Fig. 6 Effect of the various extract on the level of bilirubin in the repeated dose dermal toxicity studies (there is a statistically significant difference between * marked extract and the negative control)



albino rats. [48–50]. In addition, anthraquinones have been shown to induce liver damage. [51–53]. On the other hand, saponins have been shown to possess hepatoprotective properties of chemical induced damage. [54, 55]. Saponins therefore could play an important role in ameliorating the adverse effects of necrosis caused by the tannins in this extract.

The methanol leaf extract of the plant was shown to be toxic against Wistar albino rats by Mboneye et al. [56]. The leaf extract exhibited toxicity at doses of 1600, 3100, and 5000 mg/kg, causing one death at the highest dose. At 800 mg/kg, there was increased kidney weight, urea, and ALT levels, decreased total protein and albumin, and caused liver and kidney damage and hematopoietic cell disruption. Unlike in this study, extract was safe at 400 mg/kg, although it was found to be toxic at 800 mg/kg, causing significant liver and kidney damage.

4.2 *Psorospermum febrifugum*

Acute oral exposure to the aqueous extract of *P. febrifugum* induced increased respiration, urination and mobility although these clinical signs subsided in 24 h (Table 2). There was normal increase of body weights throughout the study period, but acute exposure to 5000 mg/kg bw increased the liver weight compared to the control group, yet the liver and kidney were reduced by repeated exposure to 400 mg/kg bw (Figs. 1 and 2). A close inspection of biochemistry results however reveals a three-fold increase of the AST: ALT ratio in comparison to the control group (Table 6). Such an increase in the enzyme ratio, indicate significant implications for liver health in the context of toxicity from the plant extract. This elevation suggests that hepatocellular injury or dysfunction may be occurring as a result of exposure to the extract. The higher ratio typically indicates that AST, which is primarily found in the liver but also present in the heart, muscle, and other tissues, is released at a greater rate than ALT, which is more specific to the liver. This pattern signals potential liver damage or stress.

In this study, the significant increase in the AST: ALT ratio, alongside elevated AST and bilirubin levels (Figs. 5 and 6), suggests that the plant extract may be causing hepatocellular injury or obstructing bile flow. These findings raise concerns about the safety of the extract and highlight the need for further investigation into its hepatotoxic effects.

A simultaneous increase in both AST and bilirubin is usually indicative of either hepatocellular injury or biliary obstruction [57]. Table 9 indicate that *P. febrifugum*, especially at 400 mg/kg bw, significantly reduces both the absolute and relative weights of the liver. Such changes in organ weights have been reported to be adaptive mechanisms against hepatocellular injury [58, 59]. In addition, the liver has a tremendous ability to regenerate [60], which might explain why histopathology results show no significant lesions. However, the mismatch in biochemistry and histopathology results (Fig. 2c) might also be a sign of a slow onset of chronic toxicity. Phytochemical screening shows presence of saponins and tannins (Table 7). Tannins have been shown to be causative agents for hepatic injury whereas saponins possess hepatoprotective properties against chemical induced damage [47, 48, 50, 54, 55]. This would further explain the healthy histopathology state which seems contrary to the biochemistry and organ weight results.

Table 9 Organ weights (g) of rats after the repeated dose oral toxicity studies

Dose (mg/kg bw)	Negative control	<i>E. tomentosa</i>		<i>P. caespitosus</i>		<i>P. febrifugum</i>	
		200	400	200	400	200	400
Absolute weights (g)							
Liver	8.3±0.2	8.5±0.1	8.5±0.7	7.2±0.3	7.5±0.8	5.9±0.2*	5.7±0.5*
Heart	1.0±0.0	1.0±0.0	1.1±0.1	0.8±0.1	1.1±0.1	0.7±0.1	0.7±0.0
Kidneys	0.9±0.1	1.0±0.0	1.0±0.0	0.7±0.0	0.9±0.1	0.7±0.1	0.5±0.0*
Relative weights (%)							
Liver	4.88±0.01	4.99±0.02	4.96±0.02	4.40±0.05	4.53±0.01	3.72±0.03*	3.59±0.01*
Heart	0.59±0.00	0.59±0.00	0.64±0.00	0.49±0.01	0.67±0.00	0.44±0.00	0.44±0.09
Kidneys	0.52±0.01	0.59±0.00	0.61±0.01	0.42±0.01	0.54±0.00	0.44±0.04	0.31±0.00*

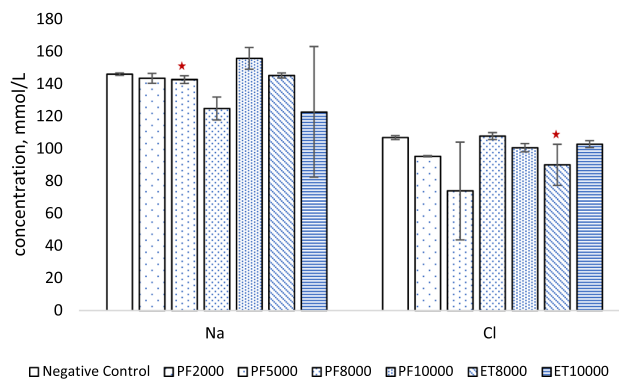
Results are presented as mean ± standard deviation, n=2, There are statistically significant differences ($P \leq 0.05$) between * labelled values and the negative control in the same row

Table 10 Biochemical parameters of the rats in repeated dose dermal toxicity studies of the selected aqueous extracts

Dosage (mg/kg bw)	Negative Control	<i>P. febrifugum</i>		<i>E. tomentosa</i>		<i>P. febrifugum</i>	
		8000	10,000	8000	10,000	2000	5000
ALB, g/L	37.40±0.14	40.00±1.41	38.55±0.78	38.77±2.25	21.10±8.51	38.07±3.09	49.00±1.73
Total protein, g/L	70.05±2.76	70.00±14.41	69.00±0.00	63.57±2.26	57.53±6.49	70.17±3.05	79.13±1.14
ALT, U/L	54.65±5.16	53.00±4.24	58.20±0.28	107.67±38.30	44.73±24.48	64.57±14.34	74.67±8.30
AST, U/L	98.50±11.88	97.95±7.14	103.10±1.27	290.37±54.79	177.37±83.14	262.00±14.34*	279.63±26.84*
Bilirubin, µmol/L	0.45±0.07	0.25±0.07	0.35±0.07	1.30±0.00	0.90±0.36	1.73±0.67*	1.80±0.36*
Creatinine, µmol/L	33.5±0.71	37.35±0.49	34.80±1.13	29.00±4.58	29.33±15.96	63.67±3.51*	87.60±4.03*
Urea, mmol/L	11.55±0.49	9.25±0.35	11.40±0.14	7.20±0.70	5.53±1.01	7.33±0.61*	7.40±1.64*
ALP, U/L	159.50±23.33	136.20±8.20	145.50±12.02	147.33±49.52	100.67±37.63	83.63±3.46*	110.80±8.83*
Na, mmol/L	145.50±0.71	124.50±7.07*	155.25±6.72	144.67±1.53	12,233±4.020	143.00±3.00	142.27±2.30
K, mmol/L	6.40±0.00	5.75±0.35*	6.21±0.30	6.97±0.85	10.96±5.97	7.36±0.29	7.77±0.87
Cl, mmol/L	106.55±1.20	107.50±2.12	100.30±2.55	89.75±12.66*	102.50±2.12	95.03±0.40	73.70±30.06

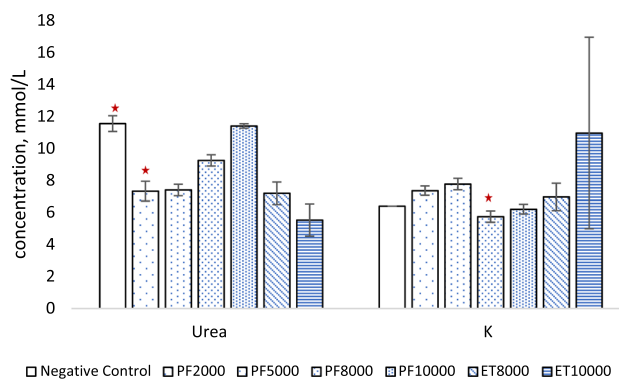
Results are presented as mean ± standard deviation, n=3, There are statistically significant differences ($P \leq 0.05$) between *labelled values and the negative control in the same row

Fig. 7 Effect of the various extract on the level of Na and Cl ions in the repeated dose dermal toxicity studies (there is a statistically significant difference between * marked extract and the negative control)



Topical application of the organic extracts did not irritate or sensitizes the skin. However, biochemistry results (Table 10) showed that at 2000 and 5000 mg/kg bw, the extract significantly increased bilirubin, creatinine and AST while decreasing urea and ALP levels in comparison with the control group. Elevated levels of AST and low urea levels points to drug-induced cholestasis [45, 61]. There was also a reduction in Na, K and Cl (Figs. 7 and 8) levels upon acute exposure to 8000 mg/kg bw of the organic extract although this was absent in the 10,000 mg/kg bw

Fig. 8 Effect of the various extract on the level of urea and K ions in the repeated dose dermal toxicity studies (there is a statistically significant difference between * marked extract and the negative control)



group. Elevation in levels of salt ions, low urea levels combined with elevated creatinine indicate impaired kidney functioning. [62, 63]. The organic extract contains anthraquinones, flavonoids and very high amounts of steroids (Table 6), which are known to affect the kidney in different ways. Steroids can induce or aggravate acute kidney injury, chronic kidney disease, and glomerular toxicity [64].

Agbogba and co-workers [65] showed that aqueous and ethyl acetate extracts of the root bark up to 2000 mg/kg, and daily doses of 200 mg/kg for 28 days, did not cause toxicity to the kidney, liver, or immune organs in vivo. Similarly, ethanol extract doses between 10 mg/kg and 5000 mg/kg showed no clinical toxicity in mice [66]. However, psorospermin 1 from *P. febrifugum* root was toxic in an in vitro KB cell culture ($ED_{50} = 10^{-1} \mu\text{g/ml}$), and methanol leaf extract was toxic in MRC-5 cell lines ($IC_{50} > 64 \mu\text{g/ml}$). Overall, root and bark extracts are safe orally. [67, 68]

4.3 *Plectranthus caespitosus*

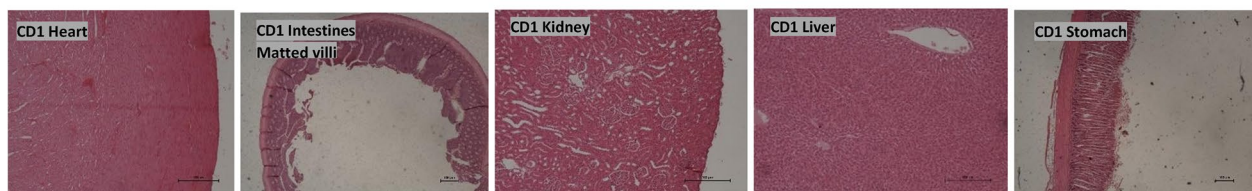
Oral exposure to the aqueous extract of *P. caespitosus* induced no changes in body or organ weights both in absolute or relative terms as compared to the control (Fig. 9). Histopathology results also showed no signs of toxicity (Fig. 2d). In addition, dermal application of *P. caespitosus* extract did not induce clinical signs of toxicity. There was neither skin irritation, nor sensitization inferred by results from Draize and mouse ear swelling tests respectively. Phytochemical screening of *P. caespitosus* revealed the presence of saponins, tannins, coumarins and steroids. The toxicity of these phytochemicals generally depends on various factors including concentration, the specific chemical structure, and synergies with other compounds. This could explain why *P. caespitosus* did not show any signs of toxicity.

Whereas, this is the first study of the toxicity of *P. caespitosus*, Plants in the same genus have shown similar results [69, 70]. Abu and co-workers [71] also reported similar results for a related species *P. neochilus*. The oral administration of the aqueous, ethanol, and acetone crude extracts of *P. neochilus* leaves on Wistar albino rats did not produce any toxic effects at 10, 100, 1000, 1500, 2250, 3500 and 5000 mg/kg bw. This was also shown to be the case in the hexane extract of *P. amboinicus* [72].

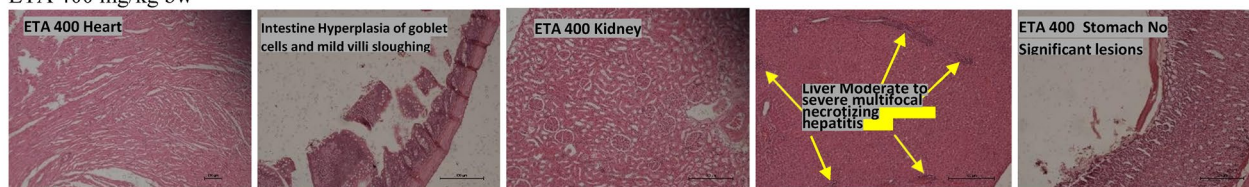
Steroids [73, 74] and tannins [75, 76] have all been reported to have both beneficial and harmful effects due to the diverse array of compounds under these classes of phytochemicals. The effects of tannins on liver health are concentration-dependent, presenting a dichotomy where low concentrations offer hepatoprotective benefits, while high concentrations can induce hepatotoxicity. At low to moderate levels, tannins act as antioxidants, neutralizing reactive oxygen species and protecting liver cells from oxidative damage. These concentrations have been shown to reduce liver inflammation, promote hepatic regeneration, and mitigate damage from various toxins [77, 78]. For example, tannins from sources such as green tea and grapes have demonstrated efficacy in reducing liver enzyme levels and improving histopathological outcomes in models of liver injury [79].

In contrast, high concentrations of tannins can precipitate proteins in the liver, disrupting cellular functions and leading to necrosis or apoptosis [77]. Excessive intake of tannins has been linked to elevated liver enzymes, inflammation, and histopathological changes indicative of liver damage. This hepatotoxicity is primarily driven by the generation of ROS, which overwhelms the liver's antioxidant defences, leading to oxidative stress and cellular injury [80, 81].

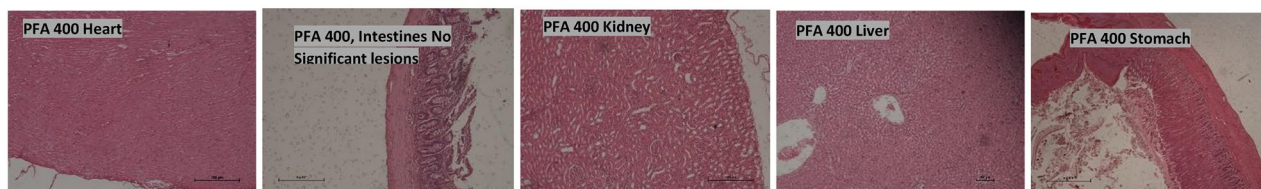
a) Negative controls



b) ETA 400 mg/kg bw



c) PFA 400 mg/kg bw



d) PCA 400 mg/kg bw

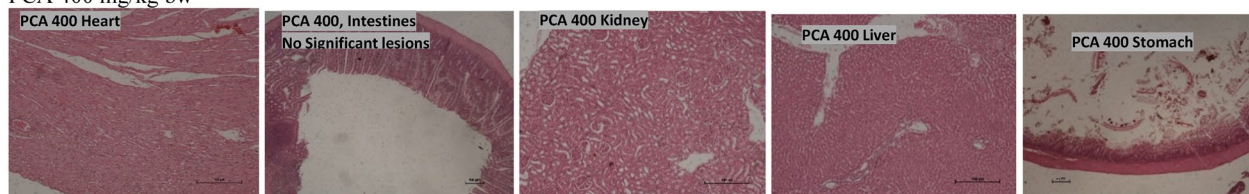


Fig. 9 Relative kidney weight in acute oral toxicity study (there is a statistically significant difference between * marked extract and the negative control)

5 Conclusion

The leaf extracts of *E. tomentosa* and *P. caespitosus* did not show any signs of acute oral toxicity when administered to Wistar albino rats at doses of 2000 and 5000 mg/kg bw. Repeated oral exposure of the aqueous extracts at 200 and 400 mg/kg of *P. caespitosus* did not cause any toxicity to the organs whereas *E. tomentosa* caused liver injury at 400 mg/kg. All plant extracts did not show any acute dermal toxicity including repeated exposure for *P. caespitosus*. However, repeated exposure caused necrotizing liver hepatitis with *E. tomentosa* extracts at 200 and 400 mg/kg bw while stem bark extracts of *P. febrifugum* were toxic to the liver and the kidney at doses of 400 mg/kg bw. Phytochemical analysis of the extracts revealed the presence of anthraquinones, coumarins, saponins, steroids and tannins. Toxicity of these extracts could be attributed to the quantity of some of the phytochemicals present especially the tannins.

6 Recommendation

Whereas *P. caespitosus* demonstrated no toxicity in Wistar albino rats, further research should focus on evaluating its constituent compounds for potential antibacterial use and integration into skin care products, while additional studies are necessary to identify and characterize the toxic compounds in *E. tomentosa* and *P. febrifugum*.

Author contributions J.N. conceptualized and designed the study M.M. and S.P. prepared chemical extracts and carried out the chemical analysis J.M. carried out the biochemical experiments, skin irritation & sensitization, histopathology and toxicity study. M.M. wrote the main

manuscript text and performed data analysis G.A. reviewed the manuscript S.A. reviewed the manuscript D.S.T. reviewed the manuscript R.B. reviewed the manuscript.

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Data availability Data is provided within the manuscript.

Declarations

Ethics approval and consent to participate The study protocols for both plants and animals do comply with relevant institutional, national, and international guidelines and legislation.

Consent for publication All the authors gave their consent for publication.

Competing interests The authors declare no competing interests.

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