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Phytochemical analysis and toxicity evaluation of *Polyscias fulva* stem bark extract using gas chromatography-mass spectrometry, fourier transform infrared spectroscopy, in silico, and in vivo studies in wistar rats

Kenedy Kiyimba[1,2,3] · Ayaz Ahmed[4] · Muhammad Iqbal Choudhary[5] · Samuel Baker Obakiro[2,3] · Yahaya Gavamukulya[2,6] · Eric M. Guantai[1] · Were Lincoln Munyendo[7]

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Abstract

Scientific research on *Polyscias fulva* has demonstrated the plant's pharmacological properties, including antitumor, antibacterial, and hypoglycemic effects, but there is a paucity of data concerning the phytochemical components responsible for these effects as well as the safety of *Polyscias fulva*. This study aimed to profile the phytoconstituents in the different solvent fractions of the ethanolic extract of *Polyscias fulva* stem bark using GC–MS and FTIR analysis, and subsequently evaluate acute and sub-acute toxicity effects in Wistar albino rats. GC–MS analysis identified 19 major phytocompounds. In silico toxicity prediction revealed the potential skin sensitization, eye corrosion, and respiratory toxicity effects of some of the identified compounds. *Polyscias fulva* ethanolic stem bark extract showed minimum acute toxicity in Wistar albino rats, with an LD_{50} exceeding 5000 mg/kg. Sub-acute toxicity testing did not show any significant biochemical or hematological toxic effects. However, dose dependent histopathological toxic effects were noted in the lungs with the lower doses (200 and 400 mg/kg) causing mild bronchitis with mononuclear cell infiltration, and the highest dose (800 mg/kg) causing marked peri-bronchiolar and perivascular lymphoid cell accumulation. These findings suggest the presence of pharmacologically active phytochemicals in *Polyscias fulva* with no major acute toxicity, biochemical or hematological toxic effects, but with lung specific dose-dependent histopathological toxic effects, and predicted skin and eye sensitivity reactions. Further research is needed to identify the specific phytochemicals responsible for its pharmacological activities and potential toxic effects, to inform its safe use in managing human ailments.

Keywords *Polyscias fulva* · Acute toxicity · Sub-acute toxicity · GC–MS · FTIR

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✉ Yahaya Gavamukulya, gavayahya@yahoo.com | [1]Department of Pharmacology, Clinical Pharmacy and Pharmacy Practice, Faculty of Health Sciences, University of Nairobi, P.O. Box 19676-00202, Nairobi, Kenya. [2]Natural Products Research and Innovation Centre, Busitema University, P.O. Box 1460, Mbale, Uganda. [3]Department of Pharmacology and Therapeutics, Faculty of Health Sciences, Busitema University, P.O. Box 1460, Mbale, Uganda. [4]Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD), International Centre for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan. [5]International Center for Chemical and Biological Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi 75270, Pakistan. [6]Department of Biochemistry and Molecular Biology, Faculty of Health Sciences, Busitema University, P.O. Box 1460, Mbale, Uganda. [7]School of Pharmacy & Health Sciences, United States International University-Africa, P. O. Box 14634–00800, Nairobi, Kenya.

1 Introduction

The growing global interest in natural remedies underscores the need for rigorous scientific evaluation of traditional medicinal plants to establish their safety and efficacy profiles [1]. Phytochemical profiling, acute, and sub-acute toxicity studies are crucial components of this evaluation process, which provide essential information on the potential risks associated with short-term and prolonged exposure to plant extracts [2]. These studies help determine safe dosage ranges and identify adverse effects that may pose a risk to human health.

Polyscias fulva, a species of flowering plants in the Araliaceae family, commonly known as the Parasol Tree and locally called Setaala (Luganda) in Uganda, is an evergreen or deciduous tree widely distributed in tropical Africa. It is renowned for its ethnobotanical and ethno-medicinal applications [3]. Traditional African medicine has long utilized various parts of *Polyscias fulva* to treat a wide range of ailments. In West Africa, particularly Cameroon, a decoction of the stem bark is orally administered to manage venereal infections, while the leaves are pounded and applied topically to treat skin conditions [4]. In Kenya, especially among the Nandi community, both the aerial and stem bark parts are used to manage diabetes mellitus and digestive issues, including stomach aches, diarrhea, dysentery and obesity [5]. In the Kivu region of Eastern Democratic Republic of Congo, a decoction or infusion of the bark is traditionally used to manage malaria, fever, mental illness and epilepsy [6]. In Uganda, the stem bark is used in traditional medicine for several therapeutic purposes. It is administered as an enema and purgative to treat colic [7]. Pulverized bark is inhaled as an anodyne and is also employed in the treatment of coughs, hemoptysis, and tuberculosis [8]. The plant is also believed to stimulate appetite and support digestive health. In some cultures, *Polyscias fulva* is utilized as a tonic to strengthen the immune system and promote overall vitality and well-being [3].

Scientific studies have validated the anticancer [9], antioxidant, anti-inflammatory [10], antibacterial [11], antifungal [4], hypoglycemic [12], and anti-tubercular properties [6] of both the aerial, and stem bark parks of the plant. These effects are attributed to a variety of biologically active phytochemical compounds such as triterpenes glycosides (alpha-hederin), phenolics like Quercetin-3-O-D-glucopyranoside, steroids (such as 3-O-β-D-glucopyranosyl-sitosterol, β-sitosterol and 3-o-β-D-glucopyranosyl-stigmasterol), terpenoid saponins and others compounds like Lichexantone, 3-α-L-arabinopyranosyl-hederagenin (cauloside A), and kalopanax-saponin B, saponins (oleanane-type aglycones such as oleanolic acid) [4, 13, 14].

While *Polyscias fulva* has long been used in traditional medicine for its therapeutic purposes, scientific evidence on its comprehensive phytochemical composition particularly regarding its bioactive compounds, remains limited. Furthermore, despite some studies on its pharmacological effects, there is a notable lack of in-depth toxicity profiling, especially in relation to its chemical constituents, as well as a gap in experimental validation of its safety in animal models. This study aims to address these gaps by employing Gas Chromatography Mass Spectroscopy (GC–MS) and Fourier Transform Infrared Spectroscopy (FTIR) to identify and characterize the bioactive compounds in various solvent-based crude extracts of *Polyscias fulva*.

Additionally, we utilized in silico toxicity profiling approaches to predict the potential risks associated with these compounds, and validated the findings through in vivo testing in Wistar albino rats. This research seeks to fill the existing knowledge gap and provide a scientific basis for its safe use in African traditional medicine. The outcomes of this study will contribute to a broader understanding of the phytochemical composition and toxicological properties of *Polyscias fulva*, guiding its safe application and informing future more advanced medicinal chemistry, pharmacological and toxicological investigations.

2 Materials and methods

2.1 Sample collection, authentication and processing

In June 2023, *Polyscias fulva* stem bark was collected from Mabira Forest, Buikwe District, Central Uganda (N02° 05′18″ E034°03′49″). Permission to access the field sites was granted by the local area administration, as the plant specimens were collected from individual home gardens rather than from a protected forest area. The plant material was identified by a taxonomist (Dr. Carol Kawuma), and a voucher specimen was deposited at and deposited at the Makerere University Herbarium, Department of Plant Sciences, and Microbiology and Biotechnology with ID (KK/001/2023). Proper harvesting and conservation practices were adhered to during the collection process. The collected materials were carefully

packed in zip-lock bags and transported to the Natural Products Research and Innovation Centre (NaPRiC), Busitema University for processing. At the laboratory, the materials were sliced into small pieces and shade-dried for 28 days at room temperature. Once dried, the plant materials were ground into a powder using an electric grinder (NutriBullet® 600 Series) and stored in clean, labeled paper envelopes at room temperature until extraction.

2.2 Preparation and extraction of plant materials

Analytical grade solvents (ethanol, methanol, ethyl acetate and n-hexane) purchased from Sigma-Aldrich (Germany), were used to extract the plant materials. Initially, 500 g of *Polyscias fulva* powder were macerated in 1500 ml of 70% ethanol for 72 h with continuous shaking to obtain the crude ethanolic extracts. The mixture was then double-filtered, first through cotton wool and subsequently using Whatman No. 1 filter paper. The filtrate was concentrated to a minimal volume using a rotary evaporator (DLAB: RE100-Pro) at 40 °C under reduced pressure (70-100mBar). The concentrated crude extracts were dried to a constant weight in a desiccator over anhydrous copper (II) sulfate at room temperature. The extracts were stored in clean, labeled bottles in a refrigerator at 4 °C for further use.

The crude *Polyscias fulva* ethanolic extracts (PFE) were fractionated through sequential extraction with solvents of increasing polarity: starting with n-hexane, followed by ethyl acetate, and then methanol. The extracts were fractionated using a soxhlet apparatus (maintained at 40 °C for 2–3 h). Briefly, the ethanolic extracts were wrapped in muslin cloth and placed in the soxhlet thimble; n-hexane was added to the distillation flask and heated to 40 °C. The resulting vapors condensed in the thimble holder, dissolving the dried crude extract. When the condensed n-hexane in the extraction chamber reached the overflow level, it was siphoned back into the distillation flask. This cycle continued until all n-hexane-soluble compounds were extracted. The dried extract was then removed, unwrapped, and air-dried. This process was repeated with ethyl acetate and methanol. The resulting n-hexane, ethyl acetate, and methanol fractions were separately concentrated using a rotary evaporator, and the dried residues from each fraction were subjected to GC–MS analysis and FTIR analysis. The percentage yield of extraction for each solvent was calculated using the following formula;

$$Percentage\ yield\ (\%) = \frac{Mass\ of\ the\ extract(g) \times 100}{Mass\ of\ the\ Plant\ powder(g)}$$

2.3 Qualitative phytochemical screening

Standard qualitative methods were used to test for the presence of major phytocompounds including; flavonoids, terpenoids, tannins, alkaloids, steroids, saponin and phenolics, as described previously [15–18]

2.4 Gas chromatography-mass spectrometry (GC–MS) analysis

The GC–MS analysis of the different solvent extracts of *Polyscias fulva* was performed using an Agilent GC-7890A/MS-5975C GC–MS system equipped with an HP-5MS column (30 mm length × 0.32 mm diameter × 0.25 µm thickness of film) (Agilent Technologies, Santa Clara, CA, USA) Approximately 1 µL of the extracts (ethanolic, methanolic, ethyl acetate and n-hexane) were each separately injected into the GC–MS using a micro syringe and scanned for 90 min with a split flow of 60 mL/min. The oven conditions were programmed as follows: Oven temperature: 40 °C (1 min), 10 °C/min up to 260 °C, 260 °C (5 min). The injector temperature was initially set at 200 °C, while the detector temperature was 250 °C. Helium (He) was the carrier gas at a constant flow of 1.0 mL/min. The system was operated with a detector hydrogen flow of 35 mL/min, a detector air flow of 350 mL/min, and a detector nitrogen flow of 30 mL/min. The mass spectrum was referred to a computer-fed mass spectra data bank. The separated components were translated into mass spectra peaks and identified by comparing them with mass spectra chromatograms in the built-in National Institute of Standards and Technology's (NIST) GC–MS library software. A NIST library probability score of at least 50% matching in GC–MS analysis was used to select a compound from the hit list.

2.5 FT-IR analysis

Approximately 10 mg of the PFE was encapsulated into a pellet by mixing it with half a microspatula of chromatographic-grade potassium bromide (KBr). The powdered sample was then loaded into an FT-IR spectrometer (Shimadzu, Kyoto, Japan) with a resolution of 4 cm^{-1} and a scanning range of 400–4000 cm^{-1} [19]. Prior to sample analysis, the spectrometer was calibrated using standard reference materials, ensuring accurate wavelength assignments and baseline correction. The method was validated by analyzing known samples with established chemical compositions. To ensure reproducibility, multiple measurements of the same sample were performed ensuring consistent peak positions, intensities, and spectral shapes were generated.

2.6 In silico toxic risks prediction

We employed computational approaches to predict the potential toxic effects of the 19 most abundant compounds in the stem bark of *Polyscias fulva*. We used six online platforms to evaluate the potential toxic effects of the compounds and these include ADMET Lab 3.0 https://admetlab3.scbdd.com/documentation/#/, SWISS ADME (https://www.swissadme.ch/index.php), admetSAR server (https://lmmd.ecust.edu.cn/admetsar2), pkCSM platform (https://biosig.lab.uq.edu.au/pkcsm/), PreADMET (https://preadmet.webservice.bmdrc.org/), and ProTox 3.0 in silico toxicity risk prediction softwares. The toxic risks assessed included cardiotoxicity, human hepatotoxicity, mutagenicity, carcinogenicity, rat oral acute toxicity, skin sensitivity, eye irritation, eye corrosion and respiratory toxicity. The risk of toxicity was graded as: (+) low potential, (+ +) medium risk, (+ + +) high risk and no risk detected (NRD).

2.7 Experimental animals and handling

Ethical approval (ICCBS-ASP-02–2024-05) was obtained from the Ethical Committee of Animal Research, Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD), International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, Pakistan. Female, healthy, inbred Wistar rats (100–200 g) aged 8–10 weeks old were obtained from the National Facility for Laboratory Animal Research and Care (NFLARC) at PCMD. The animals were kept in standard propylene cages (25 cm × 40 cm × 50 cm) with wood chips for bedding and nipple tipped water bottles, fed standard rodent pellets and water ad libitum. They were allowed to acclimatize to the experimental room conditions for 14 days prior to the experiments. The animal beddings were changed three times a week, and the animal facility was kept within a temperature range of 23–27 °C, with a light–dark cycle of 12 h. After the study period, the animals were sacrificed under ketamine-xylazine anesthesia (100 mg/Kg BW, Intraperitoneal). The carcasses were collected in a biohazard bag, stored at – 20 °C for approximately 24 h before incineration. All experiments were conducted in accordance with standard laboratory animal care protocols [20]. Wistar rats were selected for evaluating the toxicological effects of *Polyscias fulva* due to their well-defined physiology, metabolic similarities to humans, and established reference data for comparison. While in silico and in vitro models offer preliminary toxicity insights, they cannot fully assess systemic effects, metabolism, or organ-specific toxicity. Since no reliable non-animal alternatives can replicate the complex interactions within a living organism, an in vivo model was necessary to obtain comprehensive toxicity data.

2.8 Acute oral toxicity evaluation

Female, healthy, nulliparous, non-pregnant Wistar rats were used for acute oral toxicity testing of PFE. The limit test was used to ascertain acute oral safety of PFE [21]. PFE was purposively selected for this activity to replicate the traditional extraction process used by local medicinal practitioners in preparing herbal decoctions. Additionally, it is considered a safer solvent with higher extraction efficiency and greater regulatory acceptance compared to the other solvents evaluated in this study. A total of six rats (n = 6) were randomly divided into two treatment groups in Group I: 2000 mg/kg, Group II: 5000 mg/kg (3rats/group). These were orally administered the determined doses of the PFE using a feeding cannula depending on their body weight after overnight fasting. After administration of

the PFE, observations were made for acute signs of toxicity at intervals (hr); 0.5, 1, 2, 3, 4, 6, 9, 12, and subsequently monitored for 14 days for any delayed toxicity in surviving animals. The injectable volume administered was calculated using the equation;

$$Volume\ of\ extract\ administered(ml) = \frac{Body\ weight\ (g) \times Dose\left(\frac{mg}{kg}\right)}{concentration\left(\frac{mg}{ml}\right) \times 1000}$$

2.9 Sub-acute oral toxicity

The OECD 407 guidelines for oral repeated toxicity testing of compounds in rodents were followed to assess the subacute toxicity profile of the PFE (20). Forty (40) young adult Wistar rats of both sexes were randomized based on their body weights to four groups (200, 400, 800 mg/kg and distilled water (DW)); 10 rats (5 female and 5 males) per group. The doses selected for the sub-acute toxicity study correspond to 1/10th, 1/5th, and 1/2.5th, of the 2000 mg/kg dose used in the acute toxicity test, which was identified as the maximum tolerated dose, respectively. The PFE was orally administered using an oral gavage tube on a daily basis for a period of 28 days. Observations for signs and symptoms of toxicity were made once a day throughout the study period and twice daily for any mortality. Prior to the administration of the test agents, the body weights of the animals were recorded on Day 1 and then every week up to the last day of the treatments. Upon commencement of the treatment, food consumption was determined on a weekly basis by first weighing and recording the food put in each cage and then weighing the leftovers the next day. The difference between the initial weight of the food given and the leftovers was used to calculate the daily food intake (g/rat/day). On the 29th day, the rats were sacrificed under anesthesia by intraperitoneal administration of ketamine-xylazine (100 mg/kg BW). Blood samples were collected for hematological and biochemical analysis. Tissues were collected for histopathological evaluation.

2.10 Hematological and biochemical analysis

Blood samples were obtained from each rat via cardiac puncture using syringes and collected into heparinized vacutainers for hematological indices and non-heparinized vacutainers for biochemical analysis. The Sysmex 1000i analyzer was used for hematological analysis of different parameters. These included white blood cells (WBC), basophils (BASO), lymphocytes (LYMP), neutrophils (NEUT), monocytes (MONO), eosinophils (EO), red blood cells (RBC), hematocrit (HCT), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count (PLT). For biochemical parameters, blood was centrifuged at 3000 rpm for 5 min to collect serum. The automated chemistry analyzer (HumaStar 200) was then used to analyze the concentrations of various biochemical parameters (Total proteins, bilirubin, triglycerides, total cholesterol, low-density lipoproteins (LDL), high-density lipoproteins (HDL), electrolytes (Na^+, K^+, Ca^{2+}, Cl^-, and H^+), liver enzymes (ALP, AST, and ALT). The Sigma-Aldrich test kits were used in accordance with the manufacturer's instructions to quantify the various parameters.

2.11 Gross pathologic observations and histopathologic studies

The rats were dissected to collect the vital organs (kidney, liver, pancreas, stomach, ileum, duodenum, heart, testes, uteri, and lungs) to assess histomorphological alterations, and their individual weights were measured using a digital scale. The excised organs were preserved in containers labelled and filled with 10% (v/v) buffered formalin for 72 h. Following fixation, the tissues were processed using an automated tissue processor (Leica 40). Initially, they were dehydrated in graded alcohol solutions (70, 80, 90, and 96% v/v), followed by clearing in xylene baths to remove the alcohol. Subsequently, the tissues were impregnated with molten wax and allowed to solidify. Sections were then cut using a rotary microtome at a thickness of 5 µm and stained with hematoxylin and eosin (H and E). Prepared slides were examined using a research light microscope connected to a Leica LB2—computerized image analyzer). Photomicrographs were captured and evaluated for histopathological changes by two independent pathologists.

2.12 Statistical analysis

The GraphPad Prism version 5.01 (GraphPad Software, San Diego, California, USA) was used to calculate the means and standard errors of the means of all the quantitative data. Results were displayed as means ± standard error of the mean. Statistical significance was assessed through one-way analysis of variance (ANOVA) and/or Student's *t*-test, followed by Tukey's post-hoc test; statistical significance was set at $p < 0.05$.

3 Results

3.1 Percentage yield

The following were the percentage yield of the different solvent extracts of *Polyscias fulva;*

1. PFE; Percentage yield = (85/500)*100 = 17%
2. PFHE; Percentage yield = (1/85)*100 = 1.2%
3. PFEA; Percentage yield = (4.3/84)*100 = 5.1%
4. PFME; Percentage yield = (57.6/79.7)*100 = 72.3%

Table 1 Phytochemical composition of *Polyscias fulva* ethanolic extract

S/n	Compound	Test	Result
1	Flavonoids	Ferric chloride test	+
		Zinc-HCl test	+
		Lead acetate test	+
2	Steroids	Salkowski test	+
		Liebermann -Burchard test	+
3	Terpenoid test	Diterpenes	–
		Monoterpenes	–
4	Tannins	Ferric chloride test	+
		Gelatin test	+
		lead acetate test	+
5	Glycosides	Kellar-Kiliani test	+
		Sulphuric acid test	
		Molisch's test	+
6	Saponin test		
7	Alkaloids	Mayer's test	+
		Wagner's test	+
		Dragendorff's	+
8	Phenolics	Ferric chloride test	+
		Ellagic acid test	+
9	Quinone		–
10	Lignin	Labat test	–
		Lignin test	–
11	Coumarins		–
12	Phlobatannins		–
13	Anthraquinones	Borntrager's test	+
		Sulphuric—ammonia test	+

Key: (+) represents present; (–) represents absence of a particular phytochemical

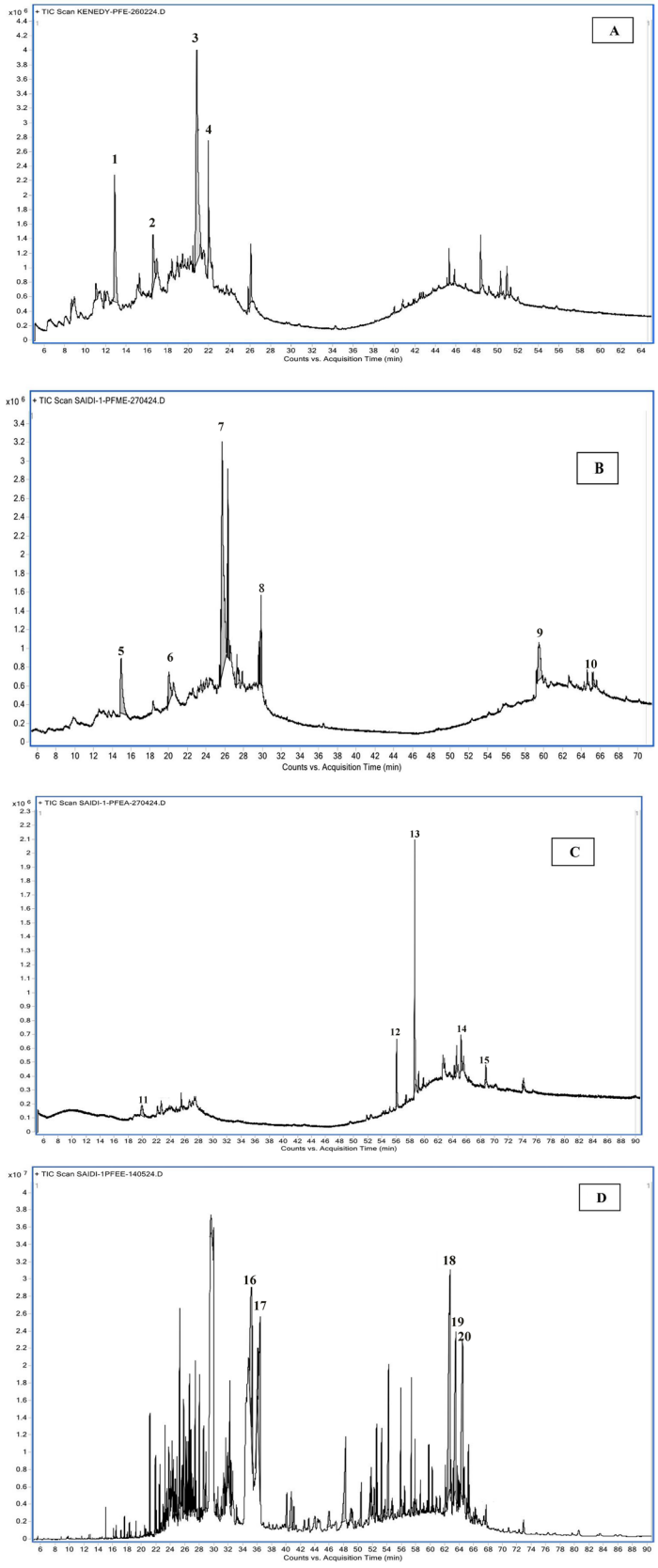


Fig. 1 GC–MS chromatogram of the different solvent extracts of *Polyscias fulva*. The spectra display multiple peaks representing various phytochemical constituents, with major compounds labeled 1–20, indicating the most abundant or significant metabolites identified in the extracts. **a**; PFE, **b**: PFMF, **c**: PFEF, **d**: PFHF. The compounds identified are summarized in Table 2

3.2 Preliminary phytochemical screening

Qualitative phytochemical screening of the ethanolic stem bark extract of *Polyscias fulva* revealed the presence of several key classes of phytocompounds such as flavonoids, tannins, steroids, glycosides, alkaloids, phenolics and anthraquinones (Table 1).

3.3 Gas chromatography-mass spectrometry (GC–MS) analysis

The GC–MS chromatogram of the PFE showed a total of 22 peaks (Fig. 1a), each representing bioactive compounds that were identified by comparing their spectra to those of known compounds listed in the National Institute of Standards and Technology (NIST) library. Compounds accounting for more than 5% were classified as major peaks and selected for further analysis. Similarly, the methanolic fraction (PFMF) showed a total of 9 peaks, with 6 major peaks identified (Fig. 1b). The ethyl acetate fraction (PFEF) showed 13 peaks, of which 5 were considered major (Fig. 1c). The hexane fraction (PFHF) showed 121 peaks, also with 5 major compounds identified (Fig. 1d).

These compounds were identified by comparing their retention times, peak areas (%), heights (%), and mass spectral fragmentation patterns to those of known compounds listed in the National Institute of Standards and Technology (NIST) library (Table 2).

3.4 Fourier transform infrared spectroscopy

The results of FTIR spectra (Fig. 2) confirmed the presence of functional groups in the different solvent extracts of *Polyscias fulva* stem bark as summarized in Table 3

3.5 In silico toxic risks predictions of the most abundant phytochemical compounds identified in *Polyscias fulva* stem bark fractions

The in silico toxicity risk predictions for the various compounds identified in *Polyscias fulva revealed* potential toxicity concerns for certain compounds.

Overall, the compounds exhibited potential risks for skin sensitization, eye corrosion, and respiratory toxicity. Skin sensitization was predicted for most compounds, except for Compound 2, 12 and 20. Eye irritation risks were similarly predicted for the majority of the screened compounds. A significant risk of respiratory toxicity was identified for bioactive compounds 15, 16, 17, 18 and 19. Notably, only one compound was predicted to exhibit oral toxicity risks in rats, while no significant concerns regarding cardiotoxicity, human hepatotoxicity, mutagenicity, or drug-induced liver injury were identified (Table 4). Similarly, the ProTox 3.0 in silico toxicity risk predictions for the most abundant compounds in *Polyscias fulva* stem bark fractions did not reveal any significant toxicities as shown in Table 5, and Supplementary file 1.

3.6 Oral acute toxicity effects of *Polyscias fulva* ethanolic extracts on the wistar rats

Acute administration of PFE to the female Wistar rats led to decreased activity, lethargy, piloerection, and unusual vocalizations within 30 min of administration at both tested doses (2000 and 5000 mg/kg) (Table 6). These effects persisted for the next 12 h and over 50% of the animals showed signs of recovery after 24 h. Full recovery was observed in all experimental animals 48 h post-administration, and no mortalities occurred over the following 14 days. Notably, there were significant differences in food consumption between the test groups and the control animals ($p < 0.0001$). Additionally, significant differences in body weight were observed between the test groups vs. the control group ($p < 0.0001$) (Fig. 3a). However, macroscopic histomorphological analysis revealed no significant differences in the appearance and weight of the organ weights between the groups (Fig. 3b).

Table 2 The most abundant phytochemical compounds identified in the different solvent extracts of *Polyscias fulva* stem bark

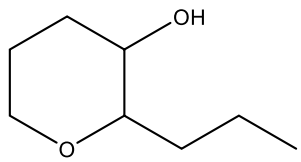
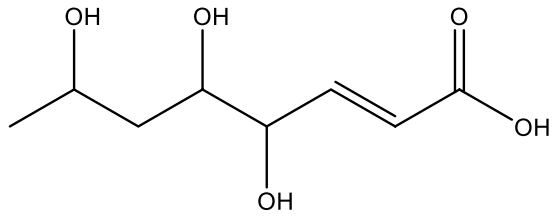
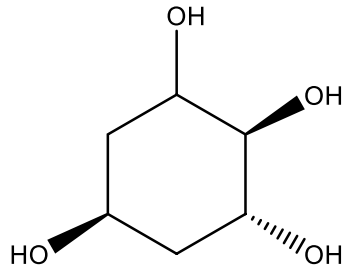
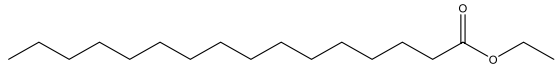
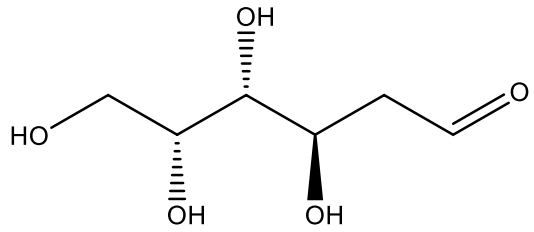
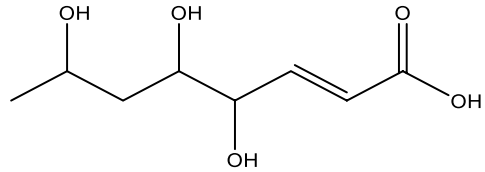
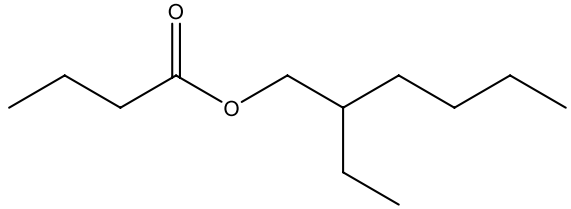
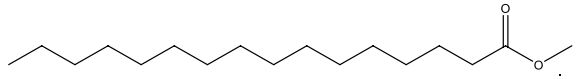
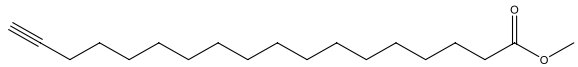
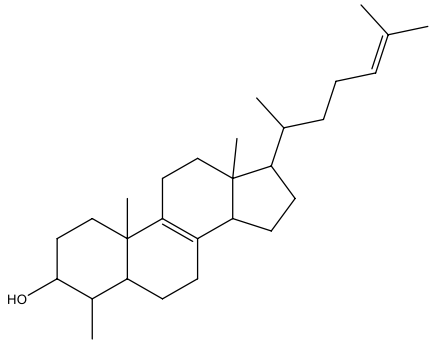
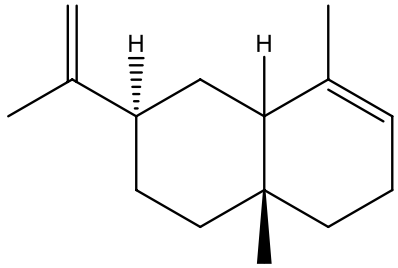
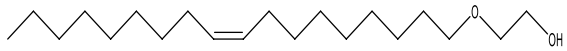
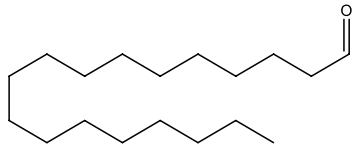
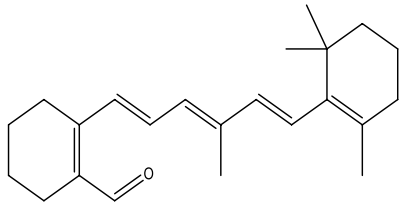
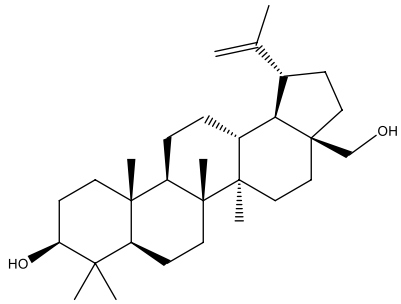
S/N	Phytochemical compound	RT (min)	Formula	Molecular weight (g/mol)	Exact mass (g/mol)	Chemical structure	Abundance (%)
Ethanolic extract							
1	2-Propyl-tetrahydro-pyran-3-ol	12.8	$C_8H_{16}O_2$	144.21	144.12		15.24
2	2-Octenoic acid, 4,5,7-trhydroxy	16.5	$C_8H_{14}O_5$	190.19	190.08		8.27
3	1,2,3,5-Cyclohex-anetetrol, (1α,2β,3α,5β)-	20.8	$C_6H_{12}O_4$	148.16	148.16		41.9
4	Hexadecanoic acid, ethyl ester	21.9	$C_{18}H_{36}O_2$	284.5	284.27		6.15
Methanolic fraction							
5	2-Deoxy-D-galactose	14.9	$C_6H_{12}O_5$	164.16	164.07		12.69

Table 2 (continued)

S/N	Phytochemical compound	RT (min)	Formula	Molecular weight (g/mol)	Exact mass (g/mol)	Chemical structure	Abundance (%)
6	2-Octenoic acid, 4,5,7-trhydroxy	20	$C_8H_{14}O_5$	190.19	190.08		6.92
7	n-Butyric acid 2-ethylhexyl ester	25.6	$C_{12}H_{24}O_2$	200.32	200.18		48.34
8	Hexadecanoic acid, methyl ester	29.8	$C_{17}H_{34}O_2$	270.46	270.26		9.28
9	17-octadedecynoic acid, methyl ester	59.4	$C_{19}H_{34}O_2$	294.47	294.47		5.52
10	Cholesta-8,24-dien-3-ol, 4-methyl-, (3β,4α)-	65.2	$C_{28}H_{46}O$	398.68	398.36		9.62

Ethyl acetate fraction

Table 2 (continued)

S/N	Phytochemical compound	RT (min)	Formula	Molecular weight (g/mol)	Exact mass (g/mol)	Chemical structure	Abundance (%)
11	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2α,4aα,8aβ)]	19.9	$C_{15}H_{24}$	204.36	204.187		8.49
12	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	56					12.99
13	Octadecanal	58.5	$C_{18}H_{36}O$	268.49	268.28		42.75
14	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl) hexa-1,3,5-trienyl] cyclohex-1-en-1-carboxaldehyde	65.1	$C_{23}H_{32}O$	324.51	324.25		9.62
15	Betulin	68.7	$C_{30}H_{50}O_2$	442.73	442.38		5.07

Hexane fraction

Table 2 (continued)

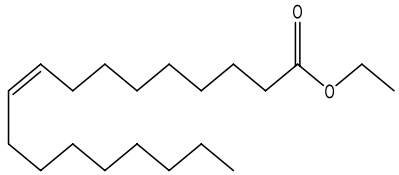
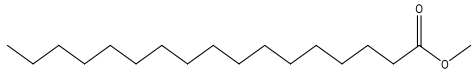
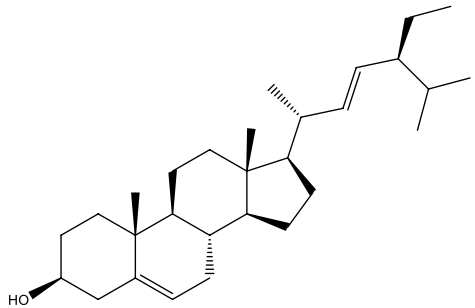
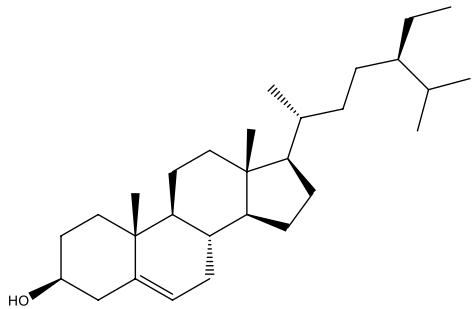
S/N	Phytochemical compound	RT (min)	Formula	Molecular weight (g/mol)	Exact mass (g/mol)	Chemical structure	Abundance (%)
16	Ethyl oleate	35.2	$C_{20}H_{38}O_2$	310.5	310.52		5.08
17	Heptadecenoic acid methyl ester	36.4	$C_{18}H_{36}O_2$	284.48	284.5		6.13
18	Stigmasterol	62.7	$C_{29}H_{48}O$	412.7	412.7		11.27
19	β-sitosterol	63.7	$C_{29}H_{50}O$	414.7	414.7		0.46

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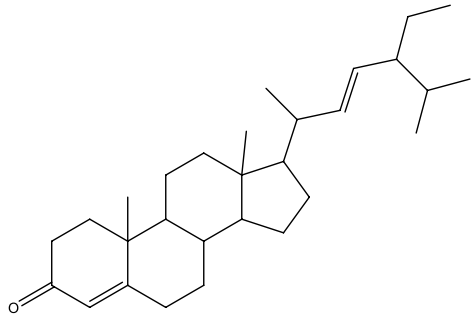
Table 2 (continued)

S/N	Phytochemical compound	RT (min)	Formula	Molecular weight (g/mol)	Exact mass (g/mol)	Chemical structure	Abundance (%)
20	4,22-Stigmastadiene-3-one	64.4	$C_{29}H_{46}O$	410.7	410.7		5.08

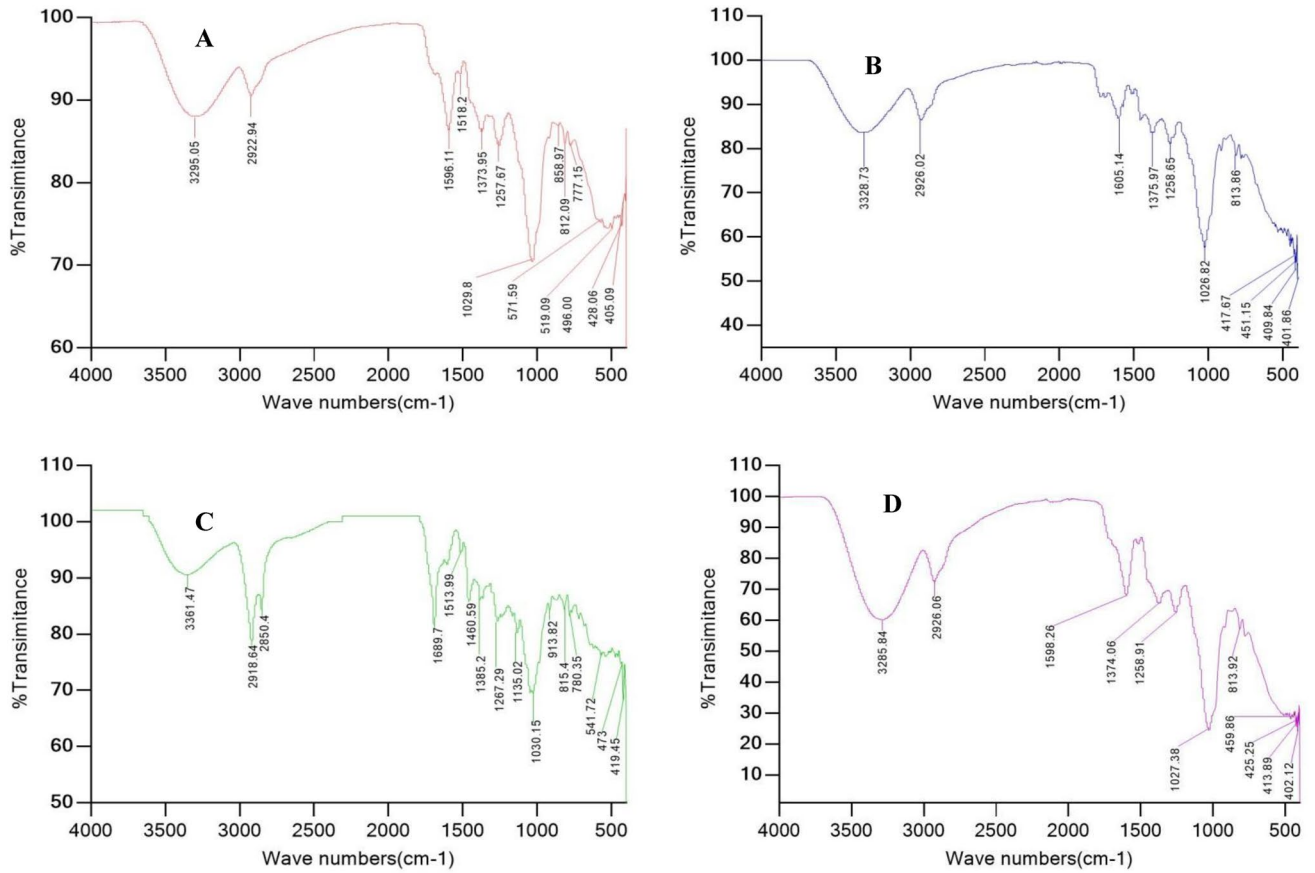


Fig. 2 FTIR spectra of the different solvent extracts of *Polyscias fulva* stem bark, **a** PFE, **b** PFME, **c** PFEA, **d** PFHE

3.7 Sub-acute toxicity effects of *Polyscias fulva* ethanolic extracts on food consumption, body and organ weights

After 28 days of repeated PFE administration to Wistar rats, significant differences ($p < 0.05$) in food consumption were observed in both the male and female treatment groups as compared to the controls. Similarly, significant differences in body weight were noted in both the male ($p = 0.0474$) and female ($p = 0.0001$) treatment animals receiving 800 mg/kg PFE compared to their respective control groups at day 28. Furthermore, a dose-dependent decrease in body weight was noted amongst the treatment groups compared to the control group in both male and female rats. Despite the changes in body weight, no significant differences were observed in the macroscopic histomorphological appearance and weights of the liver, kidneys, pancreas, lungs, heart, stomach, uterus, and testes among all test groups compared to their respective controls ($p > 0.05$) (Fig. 4).

3.8 Effect of the PFE on biochemical parameters

There was no significant increase in the serum levels of the all the biochemical parameters in both the female and male rats compared to the control group ($p > 0.05$) (Table 7). In the male rats, although there were no significant differences in liver enzyme levels, a dose-dependent increase in the serum levels of SGPT and ASAT was noted among the treatment groups.

Table 3 FT-IR peak values of the different solvent extracts of *Polyscias fulva* stem bark

S/n	Peak (Wave number cm – 1)(%intensity)				Bond responsible	Functional group assignment	Group frequency
	PFE	PFMF	PFEF	PFHF			
1	428.06(74.7)	401.86(50.563)	419.45(69.8)	402.12(60)	Not mentioned	Cyclohexanes, cis-alkenes, alkynes, aromatic aliphatic nitrile, azide, phenols, aromatics, aliphatic ethers, primary aromatic amines, secondary aliphatic amines, saturated aliphatic chlorides, aliphatic chloroformate, amide, benzene, 4-substituted pyrimidines	432–671
2	451.3(75)	409.84(52.813)	473(74.6)	413.89(23.8)			
3	496(74.6)	451.15(54.13)	541.72(80)	425.25(26.7)			
4	519.09(74.4)	417.67(55.7)		459.86(27.9)			
5	571.59(76.6)						
6	777.15(84.7)	813.86(78.9)	780.35(84)	813.92(57.4)		Alkene	713–991
7	812.09(85.1)		815.4(84.2)		C = C		
8	858.97(87)		913.82(84.3)				
9	1029.8(70.8)	1026.82(58.1)	1030.15(69.5)	1027.38(22.8)	C–H	Alkene	1020–1275
10	1257.67(84.8)	1258.65(81.3)	1135.02(80.75)	1258.91(62.3)			
11			1267.29(82.5)				
12	1373.94(86.3)	1375.97(83.4)	1385.2(86.15)	1374.06(65.6)	C = C stretching vibration	Alkene	1342–1661
13	1518.2(93.4)		1460.59(85.9)	1598.26(68.1)	NH deformation vibrations	Polyglycines	1518
13	1596.11(86.5)	1605.14(87.2)	1513.99(94.7)		C = N stretching vibration, NH deformation vibrations, NH bending vibrations, NH3 + deformation vibrations, CHN	Imine, quinone oximes, polyglycines, aliphatic azoxy compounds, sec amines, amino acids, beta diketones (metal chelates), amides, sec urethanes	1454–1661
14			1689.7(81.2)				
16	2922.94(90.7)	2926.02(86.4)	2850.4(83.6)	2926.06(72)	OH stretching vibration, CH stretching vibrations and deformations Overtones	Quinone oximes, chelated OH, intramolecular bonded aromatic, cyclic, and acyclic compounds and aldehydes	2853–3316
18			2918.64(78.2)				
19	3295.05(88)	3328.73(83.4)	3361.47(90.35)	3285.84(57.68)	OH	Carboxylic acid	3098

Table 4 in silico toxicity risk predictions of the most abundant compounds in *Polyscias fulva* stem bark fractions

S/N	Phytochemical compound	hERG Blockers	H-HT	DILI	AMES Toxicity (mutagenicity)	Rat Oral Acute Toxicity	Skin Sensitization	Carcinogenicity	Eye Corrosion	Eye Irritation	Respiratory Toxicity
1	2-Propyl-tetrahydropyran-3-ol	ND	ND	ND	ND	ND	++	ND	ND	+++	ND
2	2-Octenoic acid, 4,5,7-trhydroxy	ND	+	+	ND	ND	ND	ND	ND	+	ND
3	1,2,3,5-Cyclohexanetetrol, (1α,2β,3α,5β)-	ND	ND	ND	ND	ND	ND	ND	ND	+++	ND
4	Hexadecanoic acid, ethyl ester	ND	ND	ND	ND	ND	+++	ND	+++	+++	+++
5	2-Deoxy-D-galactose	ND	ND	ND	ND	ND	+	ND	ND	+++	ND
6	n-Butyric acid 2-ethylhexyl ester	ND	ND	ND	ND	ND	+++	ND	+++	+++	+++
7	Hexadecanoic acid, methyl ester	ND	ND	ND	ND	ND	+++	ND	+++	+++	+++
8	17-octadedecynoic acid, methyl ester	ND	ND	ND	ND	ND	+++	ND	+++	+++	+++
9	Cholesta-8,24-dien-3-ol, 4-methyl-, (3β,4α)-	+	ND	ND	ND	ND	++	ND	ND	ND	+
10	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2α,4aα,8aβ)]	ND	ND	ND	ND	ND	+	ND	+++	+++	ND
11	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	ND	ND	ND	ND	ND	ND	ND	+++	+++	ND
12	Octadecanal	ND	ND	ND	ND	ND	+++	ND	+++	+++	+++
13	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	+	ND	ND	ND	ND	+++	ND	ND	ND	+++
14	Betulin	ND	ND	ND	ND	ND	+	+	+++	ND	+++
15	Ethyl oleate	ND	ND	ND	ND	ND	+++	ND	+++	+++	+++
16	Heptadecenoic acid methyl ester	ND	ND	ND	ND	ND	+++	ND	+++	+++	+++
17	Stigmasterol	+	ND	ND	ND	+++	+++	+	ND	ND	+++
18	β-sitosterol	+++	ND	+	ND	ND	+++	ND	+++	+++	ND
19	4,22-Stigmastadiene-3-one	ND	ND	+	ND	ND	+++	ND	ND	ND	+++

hERG; human Ether-à-go-go-Related Gene, H-HT; Human hepatotoxicity, DILI; Drug Induced Liver Injury, scale of toxicity risk; low (+), medium (+ +), high (+ ++), no risk detected (ND)

Table 5 ProTox 3.0 in silico toxicity risk predictions of the most abundant compounds in *Polyscias fulva* stem bark fractions

S/N	Phytochemical compound	Mol wgt	Predicted LD50 (mg/kg)	Predicted toxicity class	Average similairy (%)	Prediction accuracy (%)	Toxicity targets
1	2-Propyl-tetrahydropyran-3-ol	144.21	3100	5	85.19	70.79	None
2	2-Octenoic acid, 4,5,7-trhydroxy	190.19	3000	5	65.37	68.07	None
3	1,2,3,5-Cyclohexanetetrol, (1α,2β,3α,5β)-	148.16	10,000	6	100	100	None
4	Hexadecanoic acid, ethyl ester	284.48	5000	5	100	100	
5	2-Deoxy-D-galactose	164.46	20,000	6	89.64	70.97	None
6	n-Butyric acid 2-ethylhexyl ester	200.32	5000	5	100	100	None
7	Hexadecanoic acid, methyl ester	270.45	5000	5	100	100	none
8	17-octadedecynoic acid, methyl ester	294,47	5000	5	79.81	69.26	none
9	Cholesta-8,24-dien-3-ol, 4-methyl-, (3β,4α)-	398.66	2000	4	100	100	None
10	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2α,4aα,8aβ)]	204.35	5000	5	88.13	70.97	None
11	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	312.53	3460	5	81.66	70.97	none
12	Octadecanal	268.8	5000	5	100	100	none
13	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	324.5	10,000	6	97.5	72.9	none
14	Betulin	442.72	2000	4	95.24	72.9	None
15	Ethyl oleate	320.52	5000	5	100	100	None
16	Heptadecenoic acid methyl ester	284.48	5000	5	100	100	None
17	Stigmasterol	412.9	890	4	89.38	70.97	none
18	β-sitosterol	414.71	890	4	89.38	70.97	none
19	4,22-Stigmastadiene-3-one	410.68	2300	5	90.75	72.9	None

Table 6 Acute toxicity effects of *Polyscias fulva* ethanolic extracts on Wistar rats

Acute effects	2000 mg/kg	5000 mg/kg
Decreased activity	3/3	3/3
Increased activity	0/3	0/3
Salivation	0/3	0/3
Lacrimation	0/3	0/3
Diarrhoea	0/3	3/3
Lethargy	3/3	3/3
Pilo erection	3/3	3/3
Loss of a righting reflex	0/3	0/3
Unusual vocalization	2/3	2/3
Sedation and Somnolence	0/3	0/3
Convulsions	0/3	0/3
Death	0/3	0/3

3.9 Effect of the *Polyscias fulva* ethanolic extract on hematological parameters

The sub-acute administration of PFE had no significant effects on the hematological parameters of both female and male rats ($p > 0.05$). However, a dose-dependent increase in hematocrit and hemoglobin levels was noted in the PFE-treated groups of both female and male rats. No significant changes were observed in the other hematological parameters among the groups (Table 8).

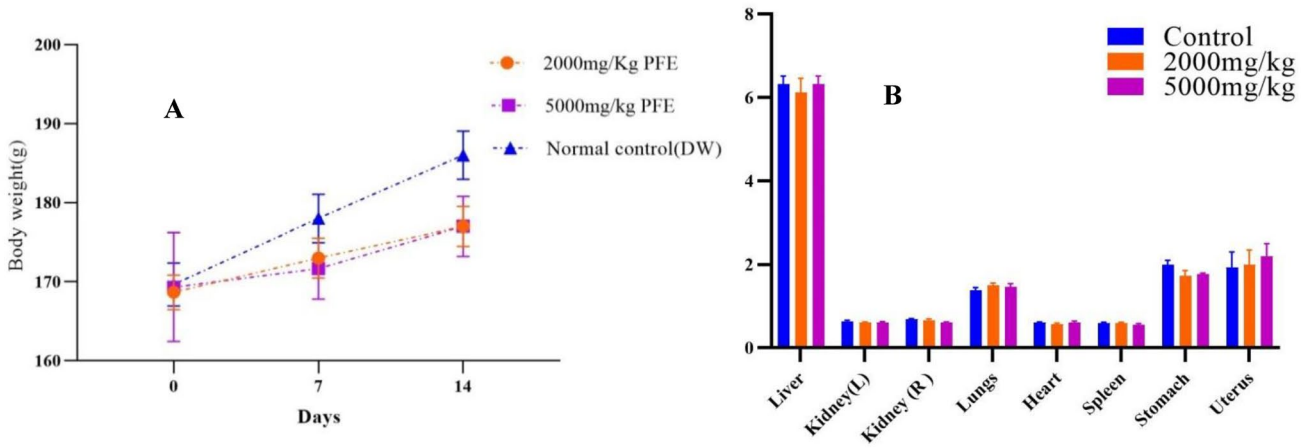


Fig. 3 Acute toxicity effects of PFE on; **a**: Body weight; **b**: Weights of vital organs

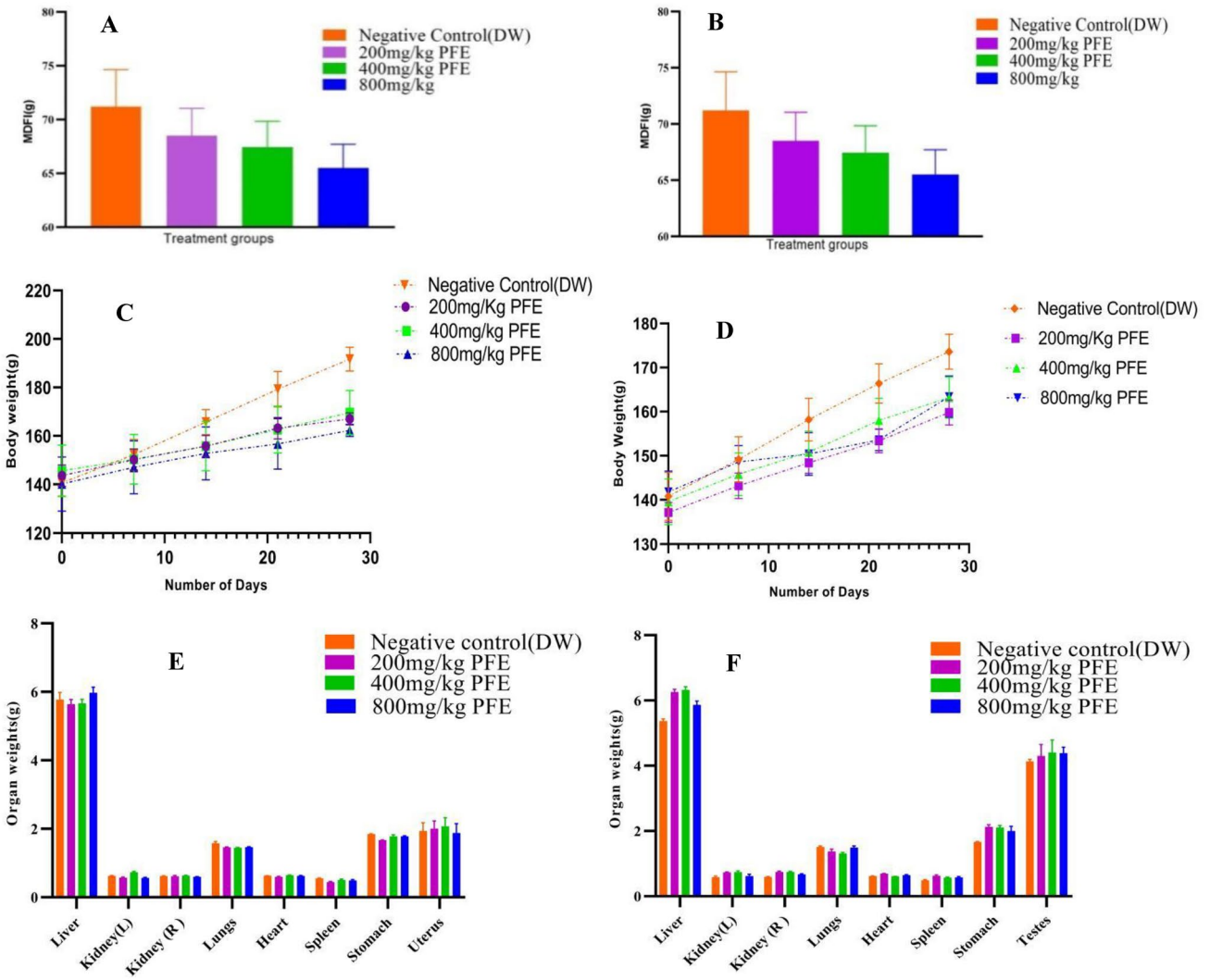


Fig. 4 Sub-acute toxicity effects of *Polyscias fulva* ethanolic extracts: Food consumption **a** male rats, **b** female rats, **c** Body weights of female rats, **d** Body weights of male rats, **e** Organ weights of female rats, **f** Organ weights of male rats

Table 7 Effect of the *Polyscias fulva* ethanolic extract on Biochemical parameters of Wistar albino rats

	Sex	200 mg/kg PFE	400 mg/kg	800 mg/kg	Control (DW)
Urea (mg/dl)	F	43.6 ± 1.99	43.6 ± 1.36	46 ± 1.82	46.2 ± 1.88
	M	43.8 ± 1.16	45.4 ± 1.08	44.8 ± 1.77	46.2 ± 0.8
Creatinine (mg/dl)	F	0.644 ± 0.02	0.576 ± 0.03	0.598 ± 0.02	0.6 ± 0.05
	M	0.72 ± 0.05	0.574 ± 0.06	0.632 ± 0.02	0.536 ± 0.02
Bilirubin Total (mg/dl)	F	0.168 ± 0.01	0.174 ± 0.01	0.158 ± 0.01	0.153 ± 0.01
	M	0.17 ± 0.003	0.872 ± 0.44	0.172 ± 0.02	0.166 ± 0.02
Bilirubin Direct (mg/dl)	F	0.11 ± 0.01	0.058 ± 0.01	0.086 ± 0.02	0.058 ± 0.01
	M	0.062 ± 0.007	0.09 ± 0.01	0.078 ± 0.02	0.1 ± 0.03
Bilirubin Indirect (mg/dl)	F	0.146 ± 0.07	0.094 ± 0.02	0.09 ± 0.01	0.076 ± 0.01
	M	0.078 ± 0.012	0.12 ± 0.01	0.116 ± 0.01	0.116 ± 0.02
SGPT (U/L)	F	56.4 ± 7.10	61.6 ± 8.31	71.14 ± 7.14	59.4 ± 4.7
	M	66.4 ± 8.4	66.2 ± 4.26	64 ± 1.14	55.8 ± 0.58
ASAT/GOT (U/L)	F	477.6 ± 49.48	548.2 ± 11.12	597.2 ± 80.38	491.2 ± 43.42
	M	548.2 ± 11.12	559 ± 18.47	575.2 ± 29.18	544 ± 14.38
Sodium (mEq/L)	F	137.24 ± 3.08	135.82 ± 3.01	130.72 ± 2.97	129.38 ± 2.49
	M	142.44 ± 2.05	142.76 ± 3.51	139.4 ± 2.06	135.06 ± 3.68
Potassium (mEq/L)	F	4.52 ± 0.17	4.18 ± 0.12	4.182 ± 0.11	4.32 ± 0.18
	M	4.32 ± 0.31	4 ± 0.34	4.48 ± 0.34	4.16 ± 0.15
Chloride (mEq/L)	F	102.38 ± 1.36	102.1 ± 1.86	104.34 ± 1.67	105.78 ± 0.9
	M	101.92 ± 1.73	103.92 ± 2.55	106.02 ± 1.69	106.34 ± 1.04
Bicarbonates (mEq/L)	F	23.76 ± 0.4	24.74 ± 0.93	24.4 ± 0.39	25.64 ± 0.69
	M	23.38 ± 1.36	23.98 ± 1.4	26.04 ± 1.11	24.02 ± 1.07

Table 8 Effect of the *Polyscias fulva* ethanolic extracts on hematological Parameters after 28 days oral administration on Wistar albino rats

Parameter	Sex	200 mg/kg PFE	400 mg/kg PFE	800 mg/kg PFE	Normal control (DW)
HEAMOGLOBIN	F	13.6 ± 3.3	15.32 ± 0.64	15.94 ± 0.49	13.08 ± 2.23
	M	15.28 ± 0.63	15.58 ± 0.34	17.4 ± 1.02	15.76 ± 0.37
HEMATOCRIT	F	48.8 ± 2.08	51 ± 1.38	54.2 ± 3.21	44.14 ± 0.63
	M	50.8 ± 3.26	54.2 ± 1.16	54.8 ± 0.96	50.52 ± 2.85
RBCS	F	10.51 ± 1.28	8.598 ± 0.42	7.57 ± 1.61	7.912 ± 0.5
	M	9.55 ± 0.53	10.564 ± 0.59	9.738 ± 0.71	10.96 ± 0.45
MCV	F	55.2 ± 3.97	57.2 ± 1.91	56.8 ± 2.74	58.24 ± 3.49
	M	50.1 ± 2.64	52.02 ± 3.5	55.6 ± 1.31	55.48 ± 3.44
MCH	F	18.2 ± 0.73	17.42 ± 0.65	16.82 ± 0.78	19.8 ± 0.89
	M	15.7 ± 0.56	16.5 ± 0.7	17.14 ± 1.09	17.28 ± 0.7
MCHC	F	34 ± 1.65	33.74 ± 2.79	27.42 ± 3.87	33.86 ± 1.04
	M	37.58 ± 2.89	33.5 ± 0.66	33.16 ± 1.06	34.18 ± 1.3
TLC	F	5.66 ± 1.07	8.88 ± 1.63	9.34 ± 1.56	9.74 ± 0.99
	M	11 ± 0.86	11.86 ± 1.16	11.76 ± 0.94	8.22 ± 0.77
PLATELET	F	913.8 ± 154.9	1007.2 ± 23.54	780.6 ± 174.5	924 ± 94.03
	M	842.2 ± 36.64	905.4 ± 40.01	951.4 ± 32.74	859.4 ± 22.15

3.10 Effects of the *Polyscias fulva* ethanolic extraction gross pathology and histopathology

Generally, the histopathological analysis revealed no major cellular differences between the treatment groups as compared to the control group across the analyzed organs (Table 9). However mild periportal inflammation was noted in the liver lobules in the group that received 800 mg/kg PFE, Specifically, microscopic analysis of histopathological

Table 9 Histopathological findings after 28-day oral administration of *Polyscias fulva* ethanolic extracts to Wistar rats

n = 10/group

Organs			200 mg/kg	400 mg/kg	800 mg/kg	Normal control
	Part	Histopathological findings				
Kidney	Glomerulus	Enlargement	Unremarkable	Unremarkable	Unremarkable	Unremarkable
		Fibrosis	Nil	Nil	nil	nil
		Inflammation	Nil	Nil	nil	nil
	Interstitial tissue	Casts	Not seen	Not seen	Not seen	Not seen
		Hydropic changes	Nil	Nil	Nil	Nil
		Hemorrhage & congestion	Not seen	Not seen	Not seen	Not seen
		Inflammation	Unremarkable	Unremarkable	Unremarkable	Unremarkable
		Necrosis	Not seen	Not seen	Not seen	Not seen
Liver	Hepatocytes	Hydropic Changes	Nil	Nil	Nil	Nil
		Steatosis	Nil	Nil	Nil	Nil
		Necrosis	Nil	Nil	Nil	Nil
		Fibrosis	Nil	Nil	Nil	Nil
	Lobule	Lobular Inflammation	Scattered	Scattered	seen	Scattered
		Portal tract	Unremarkable	Unremarkable	Periportal inflammation	Unremarkable
		Sinusoids	Unremarkable	Unremarkable	Unremarkable	Unremarkable
		Central Vein	Intact/congested	Intact/congested	Intact/congested	Intact/congested
Pancreas	Architecture and capsule	Intact	intact	intact	intact	
	Acini		WNL	WNL	WNL	WNL
	Acinar ducts		Intact	Intact	Intact	Intact
	Islets of Langerhans		Intact	Intact	Intact	Intact
	Blood vessels		Intact	Intact	Intact	Intact
		Inflammation	Nil	Nil	Nil	Nil
		Necrosis	Nil	Nil	Nil	Nil
		Hemorrhage	Not seen	Not seen	Not seen	Not seen
Stomach	Mucosa		Intact	Intact	Intact	Intact
	Lamina Properia		WNL	WNL	WNL	WNL
	Muscularis		Intact	Intact	Intact	Intact
	Serosa		Intact	Intact	Intact	Intact
		Hemorrhage	WNL	WNL	WNL	WNL
		Inflammation	WNL	WNL	WNL	WNL
		Necrosis	Normal	Normal	Normal	Normal
Ileum	Mucosa		Intact	Intact	Intact	Intact
	Lamina Properia		WNL	WNL	WNL	WNL
	Muscularis		Intact	Intact	Intact	Intact

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Table 9 (continued)

n = 10/group						
Organs			200 mg/kg	400 mg/kg	800 mg/kg	Normal control
	Serosa		Intact	Intact	Intact	Intact
		Hemorrhage	WNL	WNL	WNL	WNL
		Inflammation	WNL	WNL	WNL	WNL
		Necrosis	Normal	Normal	Normal	Normal
Heart	Ventricles		NSL	NSL	NSL	NSL
	Auricles		NSL	NSL	NSL	NSL
	Great vessels		NSL	NSL	NSL	NSL
Duodenum			NSL	NSL	NSL	NSL
Heart			NSL	NSL	NSL	NSL
Testes			NSL	NSL	NSL	NSL
Uterus			NSL	NSL	NSL	NSL
Lungs			Mild Bronchitis with mononuclear cell accumulation	Marked peribronchiolar lymphoid accumulation	Marked peribronchiolar and perivascular inflammatory cell infiltration	NSL

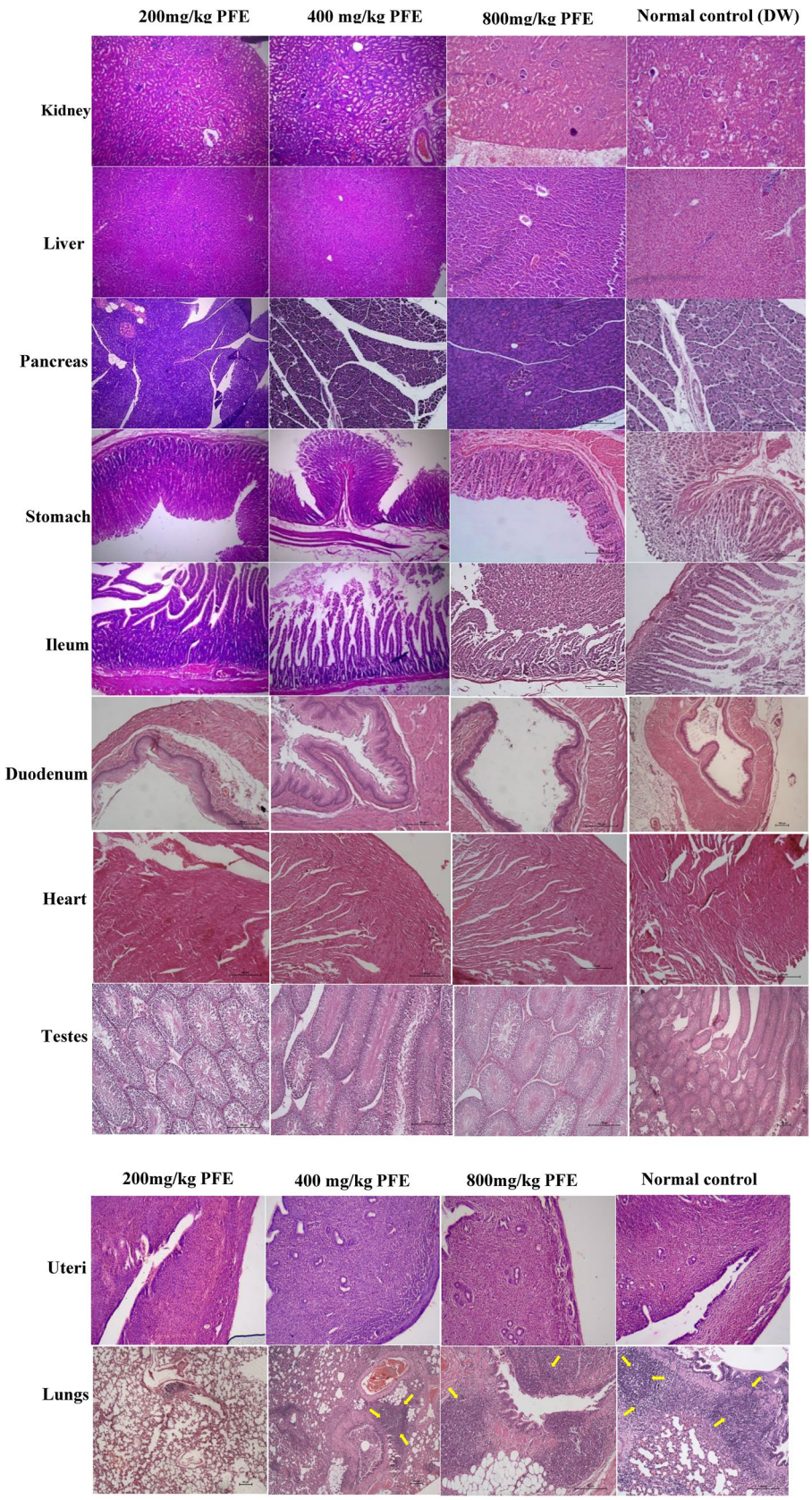

Fig. 5 Histopathology of selected organs after 28 days of oral administration of *Polyscias fulva* ethanolic extracts to Wistar albino rats. No significant lesions were detected in most of the organs except the dose dependent toxicity exhibited in the in the lung tissue where Mild Bronchitis with mononuclear cell accumulation was noted in the 200 and 400 mg/kg PFE whereas marked peribronchiolar and perivascular inflammatory cell infiltration noted in the 800 mg/kg PFE

slides indicated no evidence of toxicity in the organs, including the liver, kidney, and heart, among the groups treated with the extract (Fig. 5).

NSL-No significant lesions detected, WNL-Within normal limits.

4 Discussion

The study profiled the phytochemical composition and evaluated the acute and sub-acute toxicity effects of *Polyscias fulva* ethanolic extract in Wistar albino rats. Quantitative phytochemical analysis of the PFE confirmed the presence of well-known phytochemical classes, including flavonoids, steroids, alkaloids, saponins and anthraquinones compounds from which numerous pharmacologically active agents have been discovered, these findings are consistent with previous studies, that have also identified these compounds in crude extracts of ethyl acetate, n-butanol, and hexane fractions [21]. The GC–MS analysis of various solvent extracts from *Polyscias fulva* stem bark identified several pharmacologically active compounds. Compound 1 from the ethanolic extract has demonstrated anti-inflammatory, anti-apoptotic [22] and anti-infective properties [23]. Another compound 4 has exhibited antimicrobial activity [24]. The methanolic extract revealed the presence of compound 5 whose close congeners 2-deoxy-D glucose has demonstrated potential antitumor activity in in hypoxic and normoxic tumor cells [25]. The ethyl acetate extract revealed the presence of compound 14 with reported antioxidant properties [26], additionally, compound 13, identified in the same fraction has exhibited potential antiviral [27], anti-inflammatory [28] and antineoplastic properties [29]. FTIR spectroscopy revealed the presence of functional groups in such as the alkenes, polyglycines, carboxylic acids and quinones in the different fractions of *Polyscias fulva* stem bark. These functional groups play a critical role in a compounds' biological activity by influencing molecular interactions and mechanisms of action [30]. For instance, compounds containing alkene groups have been shown to exhibit antioxidant properties [31], antibacterial, antifungal, and antiproliferative activity [32]. Carboxylic acid groups have demonstrated notable antimicrobial and anticancer properties [33]. Quinones on the other hand, have been associated with a wide range of pharmacological effects including antitumor [34], antiproliferative, cytotoxic, antiphlogistic, anti-rheumatic and anti-inflammatory properties [35], However, quinones also present toxicological concerns, with studies indicating potential acute cytotoxicity, immunotoxicity, and carcinogenicity reactions [36]. The medicinal properties of *Polyscias fulva* can be attributed to the presence of these bioactive compounds whose pharmacological properties have been widely reported. This evidence further supports its traditional use in East Africa in the management of cancer and uterine fibroids.

Acute toxicity assessment is essential for determining the lethality of a compound and understanding its safety profile [37]. In this study, no mortalities were observed at oral doses of 2000 mg/kg and 5000 mg/kg indicating that the LD_{50} of *Polyscias fulva* ethanolic extract in Wistar albino rats exceeds the recommended maximum test dose of 5000 mg/kg, classifying it as non-toxic [20]. Previous studies on *Polyscias fulva* have reported its safety in dermal toxicity assessments in Wistar rats. Single doses (0.5, 4.25 and 8 g/kg body weight) and repeated dermal administrations (13, 256.5 and 500 mg/kg bw) of the oil-moistened dichloromethane-methanol (1:1 v/v) stem bark extract of *Polyscias fulva* caused no significant effects on the animal behaviors, organ morphology or histology. Furthermore, no significant changes were observed in hematological or biochemical parameters in guinea pigs and the LD_{50} was predicted to be higher than 8000 mg/kg [38].

In contrast, the in silico toxicity predictions in our study suggest that some phytocompounds in *Polyscias fulv*a may possess potential skin sensitization properties. This discrepancy could be attributed to the use of a crude, solvent-specific extract, in the in vivo study, which may not have contained the sensitizing compounds detected in the in silico analysis of pure constituents. These findings underscore the need for more advanced and targeted dermal toxicity studies on the plant's stem bark extracts to comprehensively assess its safety. Overall, the results provide additional support for the traditional use of the crude *Polyscias fulva* extracts in East and West African communities for the management of the various ailments, highlighting its reported efficacy and favorable safety profile.

Body weight loss is a key indicator of potential toxicity following administration of compounds [39]. In this study, a significant decrease in the body weight was observed in both the male and female rats at a dose of 800 mg/kg compared to the control group. Additionally, a general dose-dependent reduction in body weight across other treatment groups, suggests that PFE may affect food consumption, metabolism, overall body weight regulation. *Polyscias fulva* has previously been reported to help control appetite in obese individuals and enhancing overall digestive health [12]. These plausible anorectic effects may be partly attributable to the phytochemicals present in the plant's stem bark.

The liver, being the primary organ involved in the metabolism of xenobiotics, plays a central role in toxicity assessments [41]. Serum levels of hepatic enzymes, particularly AST and ALT, are commonly used to detect potential liver injury. In

our study, administration of PFE did not result in any significant changes in serum SGPT and ASAT levels in either male or female rats when compared to the control group. However, findings from a previous study demonstrated that the ethanolic extract of *Polyscias fulva* exhibited hepatoprotective effect against STZ-induced diabetes in Wistar rats. Specifically a dose of 200 mg/kg dose improved hepatic architecture, although mild bile duct inflammation was noted, while a dose of 400 mg/kg dose completely reversed the diabetes-induced hepatic damage, restoring normal histoarchitecture [12]. These findings suggest that PFE may confer hepatoprotective effects against liver damage in pathological conditions, while having minimal impact on liver enzymes in healthy animals, such as those used in our study.

Changes in biochemical parameters following repeated exposure to test compounds are important indicators of sub-acute, sub-chronic, and chronic toxicity effects [40]. The kidney, due to its high perfusion rate, is particularly vulnerable to the toxic effects of drugs and other exogenous compounds. Biological markers such as serum creatinine and urea are essential in assessing the effect of compounds on the nephrons. Altered levels of these parameters indicate compromised nephron functionality [41]. Our study reports no significant effect on the above listed renal function tests following repeated administration of PFE for 28 days at increasing dose levels. Furthermore, no significant effects were noted on the serum levels of various electrolytes. These findings suggest that PFE does not compromise nephron integrity.

The observed dose-dependent increase in hemoglobin and hematocrit in our study suggests an effect of PFE on the hematopoietic system. Possibly the phytochemical compounds present in the crude extracts of this plant may stimulate and enhance blood cell production. The hematological effects observed may also be attributed to the demonstrated antioxidant properties of *Polyscias fulva*, particularly its ability to inhibit lipid peroxidation and increase glutathione levels in both the liver and blood [10]. Elevated glutathione content is known to provide enhanced tissue protection against oxidative stress, which could guard against hemolysis and or further contribute to improved hematopoiesis [42–44]. These findings suggest that *Polyscias fulva* may have therapeutic potential in the management of anemic conditions, supporting its traditional use as a blood tonic agent.

Significant changes in the relative weight of vital organs is a dependable marker that can be utilized in toxicological studies to evaluate the drug induced toxicity [29]. In our study, we observed no significant changes in the absolute weights and no lesions detected in most vital organs (liver, kidneys, heart, stomach, ileum, duodenum, testes, and ovaries) across treatment groups compared to the control group. However, the dose-dependent inflammatory effects observed in the lungs suggest potential toxicity from PFE. This lung toxicity may result from direct cellular damage due to the accumulation of PFE phytocompounds in lung tissues or from immune-mediated reactions triggering lung cell damage. The toxicity was dose-dependent, with lower doses causing mild bronchitis and higher doses leading to pronounced peribronchiolar and perivascular lymphoid cell accumulation. The computational toxicity risk predictions further support these findings, indicating that compounds 15. 16, 17, 19 and 19 may pose a risk of respiratory toxicity. Additionally, many of the identified compounds demonstrated a potential risk for skin sensitization and eye corrosion. Based on these findings, while *Polyscias fulva* shows promise as a medicinal plant, it is not entirely devoid of toxicity, particularly concerning its potential sub-acute effects on the respiratory system. Further studies are warranted to assess long-term safety profiles and optimize its therapeutic applications.

5 Conclusion

In conclusion, GC–MS analysis identified 19 major phytocompounds (Table 2) in the various solvent extracts of *Polyscias fulva*. Several of these compounds have demonstrated significant biological activities. The pharmacological activities of *Polyscias fulva* can be attributed to the presence of these compounds. *Polyscias fulva* ethanolic extract was safe in acute toxicity tests, with an LD50 exceeding 5000 mg/kg, and did no observable acute toxic effects were noted in the experimental animals. Furthermore, sub-acute administration revealed no significant effects on the biochemical and hematological parameters. However, in silico toxicity risk predictions revealed skin and eye sensitivity reactions and potential respiratory toxicity whereas, histopathological analysis of the lung tissues revealed potential toxic effects in the lung tissue at all the tested doses with the highest dose 800 mg/kg exhibiting marked lung toxic effects. In this study we utilized the GC–MS for phytochemical analysis that primarily detects volatile and thermally stable compounds, while non-volatile or thermally labile compounds, such as large polyphenols, alkaloids, and glycosides, may be missed. This could result into an incomplete phytochemical profile, missing key bioactive constituents that contribute to the plant's therapeutic potential. This calls for further studies using more advanced techniques like Liquid Chromatography-Mass Spectrometry (LC–MS) or Nuclear Magnetic Resonance (NMR) spectroscopy for a more comprehensive analysis of the plant's chemical composition. In regard to the toxicity studies, we used in silico and in vivo animal models hence this

limits the direct extrapolation of our findings to humans. Hence this calls for further advanced mechanistic preclinical and clinical trials to evaluate the safety of *Polyscias fulva*.

Polyscias fulva's diverse applications in ethnobotany and ethnomedicine underscore its importance in traditional African societies. Its role extends beyond mere utility, contributing to cultural practices, healthcare, and agricultural sustainability. As modern science continues to explore the potential of traditional plants, *Polyscias fulva* stands out as a valuable resource with promising medicinal properties that merit further research and conservation efforts.

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Author contributions KK, YG, EMG, WLM: conceptualization, funding acquisition, methodology, data curation, supervision and manuscript review. KK, SBO, AA, MIC: Literature search, investigation, data curation, analysis and manuscript drafting. All authors read, agreed and approved the final version of the manuscript.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate The study acquired Ethical clearance from Kenyatta National Hospital/University of Nairobi-Ethics and Research Committee (KNH/UON-ERC/RR/761). Consent to participate was not applicable.

Consent for publication Not applicable.

Animals ethical statement To conduct animal experiments, approval (ICCBS-ASP-02-2024-05) Ethical approval (ICCBS-ASP-02-2024-05) was obtained from the Ethical Committee of Animal Research, Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD), International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, Pakistan. All experiments were conducted in accordance with standard laboratory animal care protocols [20].

Plants ethical statement Permission to access the field sites was granted by the local area administration, as the plant specimens were collected from individual home gardens rather than from a protected forest area. The plant material was identified by a taxonomist (Dr. Carol Kawuma), and a voucher specimen was deposited at and deposited at the Makerere University Herbarium, Department of Plant Sciences, and Microbiology & Biotechnology with ID (KK/001/2023). The collection of the plants used in the study complies with national and regional guidelines with no need for further affirmation, as previously approved as part of the ethics research committee clearance (KNH/UON-ERC/RR/761).

Competing interests The authors declare no competing interests.

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