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Phytochemical Constituent and Toxicity Assessment of Methanol Leaf Extract and Fractions of *Ficus exasperata Vahl* in Rats

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Ficus exasperata Vahl is widely used in traditional medicine across Africa for the management of diabetes mellitus, hypertension, and other conditions. However, its safety profile remains inadequately characterized. This study evaluated the phytochemical composition as well as the acute and sub-acute oral toxicity of the crude methanol extract and fractions of *F. exasperata* leaves in Wistar rats. Phytochemicals, including total phenolics, flavonoids, alkaloids, saponins, and tannins, were quantified using standard spectrophotometric methods. Acute toxicity was assessed following a single oral administration of 2000 - 5000 mg/kg extract per animal according to OECD guidelines, while sub-acute toxicity was evaluated by daily oral administration of 300 mg/kg crude extract and fractions for 21 days. Biochemical indices of liver and kidney function (alanine aminotransferase, aspartate aminotransferase, urea, and creatinine) were measured. The extract and fractions contained appreciable levels of phenolics (0.55–2.51 mg gallic acid equivalents/g dry weight), flavonoids (33.98–57.03 mg quercetin equivalents/g dry weight), alkaloids (32.04%), saponins (8.10%), and tannins (4.04%). No mortality or overt signs of toxicity were observed at 5000 mg/kg. However, repeated dosing for 21 days produced significant ($p < 0.05$) elevations in plasma transaminases, while urea and creatinine levels remained unchanged ($p > 0.05$). These findings suggest that *F. exasperata* leaf extracts are acutely safe but may exert hepatotoxic effects with prolonged use.

Keywords: *Ficus exasperata*, phytochemical composition, acute toxicity, sub-acute toxicity, hepatotoxicity.

1. Introduction

Medicinal plants are used for the prevention and treatment of various diseases worldwide. They are a rich source of diverse bioactive compounds that provide unlimited opportunities for the discovery of novel drugs and herbal remedies (Nasim et al., 2022; Yuet Ping et al., 2013). The use of herbal medicines is globally increasing (Welz et al., 2018). A survey by the World Health Organization (WHO) reports that roughly 70%–80% of the people in developing nations rely on conventional medicine for their primary healthcare (Oyebode et al., 2016). The selection of medicinal plants for the discovery of new drugs and herbal remedies has mainly been facilitated by the wealth of knowledge stored in traditional medical systems around the world. Although a plethora of compounds from medicinal plants have been isolated and their pharmacological properties reported, only a small percentage of these have gone through drug development processes to provide therapeutic drugs for clinical use (Domingo-Fernández et al., 2024; Ekanayake et al., 2019). Since safety continues to be a major issue with the use of medicinal plants (Nair & Sasidharan, 2021), it is important to conduct toxicity studies on them to ascertain their safety profile (Soren & Yadav, 2024). Therefore, evaluating the toxicological effects of any medicinal plant extract is an important aspect of its assessment for potential use in animals or humans.

Among the medicinal plants that are widely consumed and known for therapeutic properties is the *Ficus exasperata.* It is commonly known as sandpaper tree ("Ewe ipin" in Yoruba) and is widely spread in West Africa and other tropical climates (Adebayo et al., 2009; Roy et al., 2025). It belongs to the family Moraceae. The plant is a terrestrial afro-tropical shrub or small tree with scabrous, ovate leaves that grows up to 20 m and prefers evergreen and secondary forest habitats (Ahmed et al., 2012). The leaves are used industrially in wood polishing (Lawal et al., 2012). It has been ethnobotanically reported to have diverse medicinal uses. Different parts of *F. exasperata Vahl* (Moraceae) are used in traditional medicine as diuretic, analgesic, antiarthritic, antiparasitic, wound healing, vermifuge, ecbolics, abortifacient, and for treating venereal

diseases and haemorrhoids. The plant parts are also used as animal fodder (Ahmed et al., 2012; Roy et al., 2025). The leaf extract is reported to have diverse medicinal uses such as treating hypertension (Ajeigbe et al., 2021). The Yoruba-speaking people of Western Nigeria often employ decoctions and infusions of *F. exasperata* leaves traditionally for the management, control, and/or treatment of an array of human diseases, including diabetes mellitus, hypertension, and some cardiovascular dysfunctions (Adewole et al., 2011). Phytochemical analyses of the leaf and stem extracts of *F. exasperata* have revealed the presence of flavonoids, tannins, saponins, alkaloids, and cyanogenic glycosides (Irene & Iheanacho, 2007; Shemishere et al., 2020).

Despite the great phytotherapeutic importance of *F. exasperata*, the assessment of its safety or toxicity has shown conflicting results from different researchers (Salau et al., 2012; Shemishere et al., 2020; Umeh et al., 2014). This study was carried out to determine the quantitative phytochemical analysis and toxicity profile through acute and subacute toxicity tests of the crude extract and fractions of *F. exasperata* leaves in a rat model.

2. Material and Methods

2.1 Ethical Consideration

Experimental procedures were approved by the Babcock University Health Research and Ethics Committee (BUHREC, Approval No:195/20) and conducted in accordance with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines and the Organization for Economic Co-operation and Development (OECD) standards.

2.2 Collection and identification of Plant Material

Leaves of *F. exasperata Vahl* were collected in November 2022 from Babcock University, Ilishan-Remo (6.8924° N, 3.7183° E). The plant was identified and authenticated at the University of Lagos Herbarium (LUH), Lagos State, Nigeria, and a voucher number LUH:8387 was obtained.

2.3 Extraction and fractionation of Leaves

The leaves of *F. exasperata* were plucked off the plant, washed, and oven-dried at 40℃ for 72 hours. The dried leaves were then pulverized using a mechanical grinder and stored in an airtight container. Pulverized samples (100 g) were macerated in 800 ml of 70% methanol (1:8 w/v) for 72 h with intermittent shaking (every 6 h). Extract was filtered through Whatman No. 1 paper and concentrated under reduced pressure using a rotary evaporator at 40 °C. The crude methanolic extract was fractionated sequentially with n-hexane, ethyl acetate, butanol and water using a separatory funnel. Fractions were concentrated and refrigerated at 4 °C for further studies. Percentage yield was calculated as:

$$\%yield = \frac{weight\ of\ fraction}{weight\ of\ extract} \times 100$$

2.4 Quantitative phytochemical test

2.4.1 Quantitation of total phenolic contents

The total phenolic content of the different fractions of *F. exasperata* leaves was determined using the method described by Singleton et al., (1999). The reaction mixture was made by mixing 0.5 ml methanol solution of the sample (containing 100 µg/ml of the extract or fraction), 2.5 ml of 10% aqueous solution of Folin-Ciocalteu reagent, and 2.5 ml of 7.5% $NaHCO_3$ solution. The blank was concomitantly prepared by mixing 0.5 ml of methanol, 2.5 ml of 10% aqueous solution of Folin-Ciocalteu reagent and 2.5 ml of $NaHCO_3$ solution. The sample and blank were incubated in a water bath at 45 °C for 45 min, and thereafter the absorbance was measured on a UV-VISIBLE spectrophotometer at 765 nm. Standard solutions of gallic acid were prepared through the same procedure, and the absorbance values obtained were used to construct a standard calibration ($y = 74.35x - 2.6077$, $R^2 = 0.9613$). The measured absorbance of the sample was used to extrapolate its phenolic content from the standard calibration curve. The phenolic content was expressed as milligram gallic acid equivalents (GAE) per gram dry weight. Each sample was analysed in triplicate (n = 3).

2.4.2 Determination of flavonoid content

The total flavonoid content of the different fractions of *F. exasperata* leaves was determined using the method described by Dewanto et al., (2002). Quercetin served as the standard substance. 1 ml of the sample (containing 100 µg/ml of extract or fraction) was prepared in methanol and mixed with distilled water (4 ml) in a 10 ml volumetric flask, followed by the addition of 0.3 ml 5 % $NaNO_2$ solution. After 5 minutes, 10% $AlCl_3$ (0.3 ml) was added, and at the 6th minute, 1.0 M NaOH (2 ml) was added. Distilled water (2.4 ml) was added to the reaction flask, and the mixture was thoroughly shaken. Absorbance of the resulting reaction mixture was taken at 510 nm on a spectrophotometer. Quercetin was used as a standard to obtain a calibration curve ($y = 662x - 24.039$, $R^2 = 0.9577$). The total flavonoid content was expressed as milligram quercetin equivalents (QE) per gram dry weight using the equation obtained from the calibration curve. Each sample was analysed in triplicate (n =3).

2.4.3 Determination of tannin content of crude extract

The tannin content of the crude extract of *F. exasperata* was evaluated using the modified method as described by Mohammed et al. (2011). Ten

millilitres of 1% HCl in methanol was mixed with 0.2 g of the sample in a conical flask. The conical flask was covered and shaken for 20 minutes. The mixture was centrifuged at 2500 rpm for 5 minutes. 1 ml of the supernatant was pipetted into a test tube containing 5 ml of vanillin-HCl reagent. Absorbance was measured at 450 nm after 20 minutes of incubation at 30°C. A standard curve was prepared with tannic acid and the result expressed as percentage tannin content. Each sample was analysed in triplicate (n =3).

$$\%Tannin = \left\{\frac{c \times 10}{200}\right\} \times 100$$

Where C = concentration corresponding to the optical density; 10=volume of the extract (mL); 200=sample weight (mg)

2.4.4 Determination of alkaloid content in crude extract

The alkaloid content of *F. exasperata* leaf extract was evaluated using the method described by Onyilagha & Islam (2009). Each sample was analysed in triplicate (n =3). 5 g of the sample was weighed into a beaker and 200 ml of 20% acetic acid was added, covered, and allowed to stand for 4 hours. The mixture was filtered and concentrated using a water bath at 100 °C to one-quarter of the original volume. Ammonium hydroxide was added drop-wise into the mixture to form a precipitate. The solution was allowed to settle, and the precipitate was washed with dilute ammonium hydroxide and filtered. The filtrate was dried and weighed. The percentage alkaloid content was determined using this formula:

$$\%Alkaloid = \frac{Final\ weight\ of\ sample}{Initial\ weight\ of\ the\ sample} \times\ 100$$

2.4.5 Determination of saponin content of crude extract

The saponin content of *F. exasperata* leaves was evaluated using the method described by Okwu & Josiah (2006). Each sample was analysed in triplicate (n =3). 5 g of the sample was added to 50 ml of 20 % v/v ethanol in a conical flask. Heat was applied to the suspension using a hot water bath for 1 hour with constant stirring at 55 °C. The suspension was filtered, and 50 ml of 20% ethanol was used to re-extract the residue. The collective extract was concentrated to 20 ml over a hot water bath at about 90 °C. The concentrated solution obtained was shaken vigorously with 10 ml of diethyl ether. The aqueous layer was poured into a beaker while the ether layer was discarded. 20 ml of but-1-ol was added to the filtrate and washed three times with 10 ml of 5% w/v sodium chloride. The mixture was heated to evaporation on a hot water bath and oven-dried to a constant weight. The percentage saponin content was calculated using the formula:

$$\%Saponin = \frac{final\ weight\ of\ sample}{Initial\ weight\ of\ the\ sample} \times 100\%$$

2.5 Determination of acute toxicity of crude *F. exasperata* leaf extract

Acute toxicity was evaluated following the Organization for Economic Co-operation and Development (OECD) Test Guideline 425 (Up-and-Down Procedure, 2008 revision) (OECD 2022) using male albino rats (80–140 g). Graded doses of the extract (2000 - 5000 mg/kg) was administered to four rats following overnight fasting, each sequentially dosed at intervals of 48 h. Animals were observed individually at 30 min, 1 h, 2 h, 4 h, and then daily for 14 days for behavioural changes and mortality.

2.6 Sub-acute Toxicity Study of Extract and Fractions of *F. exasperata*

Thirty-six (36) male albino rats (weighing 80 – 140g) used for the sub-acute toxicity study were randomly divided into six groups of 6 rats each and weighed. Rats received 300 mg/kg/day of extract or fractions for 21 days, a dose selected based on one-tenth of the LD_{50} from acute studies. Control rats received distilled water. The animals were observed for signs of toxicity and mortality throughout the experimental period. At the end of the experiment, the animals were fasted for 12 h, weighed, and and euthanized by decapitation under anaesthesia (diethyl ether). Blood was collected by retro-orbital plexus into EDTA tubes, and plasma separated by centrifugation for biochemical analysis. Organs (liver, kidneys) were excised, rinsed in saline, blotted, and weighed for relative organ weight determination:

$$Relative\ organ\ weight = \frac{organ\ weight}{body\ weight} \times 100$$

2.6.1 Biochemical assays

Plasma for biochemical assays was obtained from the blood samples following centrifugation at 3000 rpm for 5 min. Biochemical markers (AST, ALT, creatinine, urea, total protein, and albumin) were measured using commercial diagnostic kits (Randox Laboratories Ltd., Cat. Nos: AS8306, AL1200, CR8317, UR8334, TP 245 and AB8301) according to manufacturer's protocols.

2.7 Statistical Analysis

Data were expressed as mean ± SEM (n = 6). Normality was tested using Shapiro–Wilk test. Group differences were analyzed by one-way ANOVA followed by Tukey's post-hoc test using SPSS v21 (IBM Corp., Armonk, NY, USA). Statistical significance was set at $p \leq 0.05$.

3. Results and Discussion

Table 1 shows the percentage yield of the fractions following methanol crude extraction. The percentage yield of the fractions were 35.84%, 32.81%, 27.69% and 3.66% for aqueous fraction, hexane fraction, ethyl acetate fraction, and butanol fraction, respectively.

Table 1: Percentage yield for the different fractions

Fraction	Percentage yield (%)
Aqueous	35.84
Hexane	32.81
Ethyl acetate	27.69
Butanol	3.66

3.1 Phytochemical composition of extract and fractions of *F. exasperata* leaves

Table 2 shows the total phenol content of the crude extract and fractions of *F. exasperata* leaves. The values ranged from 0.55 to 2.51 mg GAE/g dry weight. The flavonoid contents of the crude extract and fractions of *F. exasperata* leaves are also shown in Table 2. The flavonoid content ranged from 33.98 to 57.03 mg QE/g dry weight. The total phenol and flavonoid contents of the crude extract were significantly higher than in the fractions ($p < 0.05$). Alkaloid, saponin, and tannin contents were measured only in the crude extract and the values obtained were 32.04 ± 2.49%, 8.1 ± 0.06% and 4.04 ± 0.30% respectively (Table 2).

Table 2: Quantitative phytochemical screening of extract and fractions of *F. exasperata* leaves

Phytochemical	Crude extract	Butanol fraction	Ethyl acetate fraction	Hexane fraction	Aqueous fraction
Total Phenol (mg GAE/g dry weight)	2.51 ± 0.10*	1.71 ± 0.02	2.05 ± 0.00	0.55 ± 0.00	1.32 ± 0.00
Flavonoid (mg QE/g dry weight)	57.03 ± 0.78*	42.88 ± 0.24	33.98 ± 0.80	41.10 ± 0.14	44.45 ± 0.47
Alkaloid (%)	32.04 ± 2.49				
Saponin (%)	8.1 ± 0.06				
Tannin (%)	4.04 ± 0.30				

GAE: Gallic acid equivalent; QE: Quercetin equivalent.
*= Values are significantly different at $p < 0.05$ (One-way ANOVA with Tukey's post hoc test)
Values are expressed as mean ± SEM; (n=3)

3.2 Acute toxicity test result for crude extract of *F. exasperata.*

The acute toxic effect of the crude methanol extract of *F. exasperata* was determined using the Up and Down protocol of the OECD guideline 425 at an oral limit dose of 2000, 3000, 4000 and 5000 mg/kg. No treatment-related toxic symptoms such as tiredness, weakness, convulsion, hyper activeness, dullness, diarrhoea, and diuresis were observed after oral administration of a single dose of the tested plant extract. No drug-related changes in behaviour, breathing, skin effects, water consumption, or impairment in food intake were observed in the extract-treated animals through the short and long-term observation period, even at a high dose of 5000 mg/kg over the 14-day toxicity test. Based on this finding, the LD_{50} of *F. exasperata* crude extract may be >5000 mg/kg.

3.3 Sub-acute toxicity of crude extract and fractions of *F. exasperata* leaves.

3.3.1 Body weight following oral administration of extract and fractions of F. exasperata leaves.

Results in Table 3 show the weight, weight gain, and percentage body weight gain following oral administration of the extract and fractions of *F. exasperata.* Animals treated with aqueous fraction had the highest weight and percentage body weight gains (37.17 ± 6.12 g and 27.85 ± 7.16% respectively) among the treatment animals. Animals treated with the extract and hexane fraction had the least weight gain which were statistically different from the control ($p < 0.05$).

Table 3: Changes in body weight following repeated oral administration of extract and fractions of *F. exasperata*

	Weight (Day 0) (g)	Weight (Day 22) (g)	Weight gain (g)	% Weight gain
Control	86.50 ± 6.28	117.83 ± 5.80	30.18 ± 0.17	35.33 ± 2.54
Normal Saline	87.15 ± 1.75	126.0 ± 1.81	39.47 ± 0.03	45.53 ± 0.97
Crude	115.46 ± 4.95	135.37 ± 9.99	19.91 ± 5.94	17.15 ± 8.19*
Hexane	118.35 ± 3.31	137.10 ± 6.06	18.74 ± 3.67	15.77 ± 2.83*
Ethyl acetate	120.38 ± 0.49	148.99 ± 3.39	28.61 ± 3.02	23.75 ± 2.44
Aqueous	132.78 ± 3.34	169.95 ± 8.91	37.17 ± 6.12	27.85 ± 7.16

Groups: Control; Normal Saline; Crude- *F. exasperata* crude extract; Hexane- *F. exasperata* hexane leaf fraction; Ethyl acetate– *F. exasperata* ethyl acetate leaf fraction; Aqueous – *F. exasperata* aqueous leaf fraction.
Values are expressed as mean ± SEM, (n=6)

* indicate values are significantly different from control at $p < 0.05$ (One-way ANOVA with Tukey's post hoc test)

3.3.2 Organ weight following oral administration of crude extract and fractions of F. exasperata leaves.

Results in Table 4 show the liver and kidney weights following oral administration of crude extract and fractions of *F. exasperata* at 300 mg/kg body weight. Among the treatment animals, those treated with ethyl acetate fraction had the highest relative liver (3.22 ± 0.22 % liver weight/body weight) and kidney (0.67 ± 0.04 % kidney weight/ body weight) weights. There was no significant difference in liver and kidney weights of animals across the treatment groups compared to the control group ($p > 0.05$).

Table 4: Liver and kidney weight following repeated oral administration of extract and fractions of *F. exasperata*

	Liver weight (g)	Relative Liver weight (% liver weight/body weight)	Kidney weight (g)	Relative kidney weight (% kidney weight/body weight)
Control	3.66 ± 0.09	3.09 ± 0.13	0.81 ± 0.01	0.69 ± 0.06
Normal Saline	3.41 ± 0.01	2.73 ± 0.05	0.67 ± 0.01	0.52 ± 0.02
Crude	3.28 ± 0.36	2.43 ± 0.46	0.88 ± 0.11	0.64 ± 0.09
Hexane	3.65 ± 0.11	2.66 ± 0.06	0.83 ± 0.03	0.61 ± 0.08
Ethyl acetate	4.78 ± 0.10	3.22 ± 0.22	1.00 ± 0.02	0.67 ± 0.04
Aqueous	4.64 ± 0.51	2.77 ± 0.77	1.07 ± 0.08	0.64 ± 0.13

Groups: Control; Normal Saline; Crude- *F. exasperata* crude extract; Hexane- *F. exasperata* hexane leaf fraction; Ethyl acetate– *F. exasperata* ethyl acetate leaf fraction; Aqueous – *F. exasperata* aqueous leaf fraction.
Values are expressed as mean ± SEM; (n=6)

3.3.3 Biochemical Indices

Data in Table 5 show results for AST, ALT, urea and creatinine. Animals treated with crude extract and fractions of *F. exasperata* at 300 mg/kg body weight, had significantly elevated levels of ALT and AST compared to the control group ($p < 0.05$). Significant differences were not observed between the treatment groups and the control group in the levels of urea and creatinine ($p > 0.05$).

Table 5: Serum biochemical indices following repeated oral administration of the extract and fractions of *F. exasperata*

	AST (U/L)	ALT (U/L)	Urea (mMol/L)	Creatinine (mg/dL)
Control	26.32 ± 9.20	2.59 ± 1.95	21.12 ± 6.38	0.72 ± 0.17
Normal Saline	45.70 ± 12.17	11.39 ± 1.43	19.43 ± 3.88	0.94 ± 0.24
Crude	138.49 ± 13.12*	35.18 ± 10.09*	13.52 ± 5.28	0.83 ± 0.10
Hexane	150.73 ± 1.56*	53.21 ± 4.89*	10.56 ± 1.47	0.48 ± 0.28
E. acetate	146.00 ± 1.56*	33.18 ± 7.33*	14.35 ± 1.91	0.81 ± 0.19
Aqueous	100.08 ± 5.68*	39.31 ± 4.43*	21.96 ± 5.72	0.73 ± 0.10

Groups: Control; Normal Saline; Crude- *F. exasperata* crude extract; Hexane- *F. exasperata* hexane leaf fraction; E. acetate– *F. exasperata* ethyl acetate leaf fraction; Aqueous – *F. exasperata* aqueous leaf fraction.
Values are expressed as mean ± SEM; (n=6)
* indicate values are significantly different from control at $p < 0.05$ (One-way ANOVA with Tukey's post hoc test)

3.4 Discussion

Plants play important roles in the discovery of new beneficial therapeutic agents and have received significant focus because of their bioactive substances like antioxidants, hypoglycaemic, and hypolipidemic factors. Phytochemical studies are based on exploring plants for their use in the production of novel therapeutic drugs. Phytonutrients have numerous health benefits, for example, they may have antimicrobial, anti-inflammatory, anti-diabetic, cancer preventive, and antihypertensive properties (Kasote et al., 2015; Saxena et al., 2013). Isolation of chemical compounds from plants through solvents of different polarity is frequently

practiced in phytochemistry (Arif et al., 2016). Depending on the nature of solvents, different extracts yield differently, as described by Shah et al., (2014). In this study, maximum yield was obtained in the aqueous fraction, followed by the hexane and ethyl acetate fractions. The butanol fraction had the minimum yield of the crude methanol extract. This suggests that the extract had a good mix of polar and non-polar compounds.

The medicinal value of a plant lies in its phytochemicals such as alkaloids, phenols, saponins, flavonoids, etc. Qualitative phytochemical analysis reported by Shemishere et al., (2020) showed the presence of saponins, alkaloids, tannins, flavonoids, and cardiac glycosides in the aqueous extract of the leaf**.** Earlier, Adebayo et al., (2009) reported that the methanol extract of the leaves, stem, bark, and roots of *F. exasperata* contained steroids, flavonoids, phlobatannins, tannins, and saponins. This finding was corroborated by Mikail et al., (2019). This study indicates *F. exasperata* leaf extract possesses these phytochemicals, which may be responsible for the pharmacological activities attributed to the leaf extract and fractions of the plant. Phenols are diverse secondary bioactive metabolites from plants that regulate human health through antioxidant, anticarcinogenic, antithrombotic, antiulcer, antiartherogenic, antiallergenic, antiinflammatory, immune-modulating, antimicrobial, cardioprotective, and analgesic agents (Hernández-Miranda et al., 2025, Zhang et al., 2022). The non-polar hexane fraction had the least amount of phenols. Flavonoids, on the other hand, were almost evenly distributed in all the fractions, suggesting that this plant is rich in different flavonoids of varying polarities. Flavonoids are essential components in nutraceutical, pharmacological, medical, and cosmetic products. Their role as anticancer, antioxidant, anti-infective, antitoxic, hepatoprotective, anti-inflammatory, antidiabetic, and antiviral agents is well documented (Shamsudin et al., 2022, Maleki et al., 2019).

Alkaloids are plant secondary metabolites that contain at least one nitrogen atom. They have been shown to have both toxic and medicinal effects on animals and human beings (Bhambhani et al., 2021). Some tannins have been shown to possess antinutrient, mutagenic and hepatotoxic effects while others have demonstrated health benefits such as antioxidative, anticarcinogenic and antimicrobial properties (Sharma et al., 2019). Saponins are non-volatile glycosidic plant secondary metabolites. They possess positive effects such as antioxidant, antitumour and anti-inflammatory properties. They are natural emulsifiers and drug-delivery agents. However, some saponins have antinutrient and toxic properties (Sharma et al., 2023). The crude extract of *F. exasperata* in this study showed an appreciable amount of alkaloids, saponins and tannins which may enhance the pharmaceutical potential of the plant or induce toxicity. Future studies to identify the classes of these secondary metabolites, their potential toxicities and dose-response curve is needed to establish the safety of this plant.

The principal aim of evaluating the safety of any medicinal plant is to identify the nature and significance of adverse effects it may induce and to establish the exposure level at which this effect is observed (Ibrahim et al., 2016). No treatment-related toxic symptoms or mortality were observed after oral administration of a single dose of the tested plant extract in this study. No drug-related changes in behaviour, breathing, skin effects, water consumption, or impairment in food intake were observed in the control and extract-treated animals on short (4 h) and long (72 h) period observation. *F. exasperata* crude extract can therefore be considered to have an LD_{50} > 5000 mg/kg. Umeh et al., (2014) and Shemishere et al., (2020) also reported an LD_{50} > 5000 mg/kg for the plant. According to OECD criteria under its Globally Harmonised Classification System (GHS) for chemical substances and mixtures, substances with LD_{50} > 5000 mg/kg are categorised as unclassified (Sensitization, Dermal & Flammability, 2004). This suggests that the plant with an oral LD_{50} greater than 5000 mg/kg may not be lethal at the doses generally consumed by people. The lack of obvious anatomical and physiological changes in the animals does not rule out potential biochemical alterations that may progress to morbidities.

Substances administered in chronic disease conditions may need repeated toxicological evaluation (sub-acute toxicity study) since daily use may result in accumulation in the body with gradual effects on tissues and organs (Bariweni et al., 2018). Sub-acute toxicity testing is useful in assessing target organ and haematological or biochemical effects of extracts since these effects are usually not observable in acute toxicity testing. It is also essential in establishing human safety, especially in the development of pharmaceuticals. Hence, in this study, the sub-acute toxicity profile of the crude extract and fractions of *F. exasperata* were evaluated in rats using measurement of body weight, organ weight, and biochemical parameters. Oral administration of the crude extract and fractions of *F. exasperata* daily for three weeks showed no mortality. Shemishere et al., (2020) and Salau et al., (2012) also reported no deaths in the sub-acute and sub-chronic assay of the plant extracts. Umeh et al., (2014), however, reported some deaths in animals administered 4000 mg/kg of the aqueous extract of the plant within 20 days, even though there were no deaths in animals given higher doses for the same period. Available evidence has shown that body weight gain and organ weights are important and sensitive indices of toxic effects. In this study, there was no significant difference in the relative liver and kidney weights compared to the control group. But

animals administered the crude extract and hexane fraction had the least percentage gain in body weight compared to the control animals, which may be due to interference with digestion, absorption and metabolism of nutrients from constituents of this extract and its fractions (Lebeloane et al., 2024; Shemishere et al., 2020).

Elevated levels of AST and ALT have been reported to be indicators of underlying hepatocellular injuries (Ugwah-Oguejiofor et al., 2019). Significant elevations in AST and ALT levels across all treatment groups compared to the control group was observed in this study ($p < 0.05$). Similar elevations in AST and ALT have been reported by other studies (Salau et al., 2012; Shemishere et al., 2020; Umeh et al., 2014). This suggests that continuous use of the extract and fractions may be harmful to the liver. However, there was no significant difference in urea and creatinine levels across all groups compared to the control group. Although this may suggest that the kidneys may be safe from the toxic effects of the plant, other tests of kidney function and histopathological analysis will be needed to make such conclusions. Contrary to our finding, Salau et al., (2012), reported decreased creatinine levels, and Umeh et al., (2014), demonstrated changes in kidney histology following administration of *ficus exasperata* aqueous leaf extract. The differences in outcome may reflect the differences in experimental design and possible seasonal and location variations in the phytocomposition of the plant. Salau et al. gave three repeated doses of the extract daily to the animals for 8 days, and Umeh et al. administered high concentrations of the extract (> 1000 mg/kg) daily to the animals for 90 days. But irrespective of the design, the liver showed signs of toxicity, which highlights the central role it plays in metabolism and detoxification. This study focused on the liver and kidney as they play important roles in detoxification and excretion. This is a limitation as evaluating the toxic effect of the plant on other tissues such as the brain, spleen and lungs, as well as other biochemical parameters will give a holistic view of the toxicity of the plant. Future studies should address these areas.

Although all fractions of the methanolic extract of *F. exasperata* showed hepatotoxicity through elevation of AST and ALT, the hexane fraction may be considered the most toxic. This fraction had the highest elevation in liver enzymes, lowest levels of creatinine and urea, as well as the least weight gain. The fraction also had the least phenol content. This suggests that the toxic substances in *F. exasperata* leaf may be predominantly non-polar. The aqueous fraction, on the other hand, may be the least toxic as it demonstrated the highest percent yield, flavonoid content and weight gain, as well as urea levels that were similar to those of control animals.

4. Conclusion

Ficus exasperata is rich in phytochemicals. The aqueous fraction which gave the highest percentage yield, flavonoid content, weight gain and urea concentration, may be considered the least toxic fraction while the hexane fraction may be the most toxic having the highest AST and ALT levels, least urea and creatinine levels, and phenol content. The extract may be non-lethal acutely, but subacute administration may be hepatotoxic, as pronounced elevations in liver enzymes were documented. Caution should be exercised when using the plant for treatment. The following recommendations are made from the study;

1. Histopathological examination of tissues to establish the extent of organ damage should be conducted in future studies.
2. Chronic toxicity studies to evaluate the impact long term use of the plant may have on tissues should be carried out.
3. Dose-response curves to determine the safest dose for optimal treatment should be conducted.
4. Exploration of polyherbal formulations that would reduce the toxicity of the plant should be undertaken.

Authors' Contributions

IE developed the concept and design of the work, supervised the data collection and analysis, wrote and revised the manuscript. IOF carried out the experiment, analysed and interpreted the data, and wrote the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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