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Hepato-Renal Effects of *Laportea aestuans* (L.) Chew (Urticaceae) Methanol Leaf Extract and Its Phytoconstituents

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Abstract

Many plants have been used as medicines without recourse to their adverse effects, and this study aimed to investigate their phytochemical constituents and acute and subacute toxicities in the liver and kidney of rats. Gas Chromatography/Mass Spectrometry (GC/MS) and high-performance liquid chromatography (HPLC) were used to identify phytochemicals. The subacute toxicity profile was determined by administering 200, 400, and 800 mg/kg to three groups of Wistar rats for 28 days. Alterations in hepatic biomarkers (aspartate transaminase (AST), Alkaline Phosphatase (ALP), Alanine Transaminase (ALT), total protein, total bilirubin, and conjugated bilirubin) and renal biomarkers (creatinine, urea, albumin, sodium, potassium, bicarbonate, and chloride) were also evaluated. Twenty-seven compounds were identified by the GC/MS analysis with 3,7,11,15-tetramethyl-2hexadecen-1-ol (4.01 %), thiazole tetrahydro (5.08 %), N-[3,5-dinitropyridin-2-yl], proline (5.13 %), hydrazinecarbothioamide, N-(2-ethoxyethyl) (5.51 %), hexadecanoic acid methyl ester (5.83 %), methy pyrrole2-carboxylate (8.228 %), Neophytadiene (10.79 %) and benzoic acid, methyl ester (17.99 %) as the prominent compounds. Kaempferol (5.29 g/mL), epicatechin (8.23 %), naringin (15.25 %), narigenin (2.64 %), flavan-3-ol (10.55 %), flavone (3.57 %), flavonones (8.21 %), rutin (7.92 %) and resveratrol (7.78 %) were quantified by HPLC technique. Serum tests showed that aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea, total bilirubin, chloride, and sodium levels were significantly lowered ($P < 0.05$). This study indicates a rich source of flavonoids and polyphenolic compounds in the leaves of *Laportea aestuans,* which could be responsible for its protective effects in the liver and kidney.

Keywords: *Laportea aestuans*, Aspartate transaminase. Alanine transaminase; Phytochemical, Toxicity.

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Introduction

The liver and kidney are vital organs involved in the metabolism and excretion of exogenous and endogenous substances, waste, and water. Diseases of these organs could result in the retention of water, salts, minerals, and waste in the body, as well as the non-breakdown of toxic substances. The diagnosis of kidney disease involves monitoring kidney function by determining the serum creatinine, urea, protein, and albumin levels (KDIGO, 2012). On the other hand, liver function tests provide information on the health status of the liver and include enzymes evaluation, proteins, albumin and bilirubin. Elevation of these parameters could indicate diseases in these organs.

The annual number of deaths due to liver disease is estimated to be 2 million, representing 4 % of all deaths globally (Gan *et al*., 2025). Presently, liver disease is the 11th most common cause of mortality, although it is undervalued (Devarbhavi *et al*., 2023), owing to its asymptomatic nature in affected individuals. Liver disease includes cirrhosis, viral hepatitis, and cancer. In Nigerian tertiary healthcare facilities, liver cancer and cirrhosis account for twothirds of most hospitalized cases of liver diseases, with one of the main putative risk factors being medicines (Nwokediuko *et al*., 2012). Chronic kidney disease (CKD) is an emerging condition that affects more than 10 % of the world population. It has become a serious burden on low-income countries, such as Nigeria, which are least equipped to deal with the consequences (Kovesdy, 2011). Many herbs, including Laportea aestuans, have been used to treat liver and kidney disorders (Hong et al., 2015). *Laportea aestuans* (L.) Chew (Urticaceae) is an annual herbaceous plant that is thought to have originated from tropical Africa, especially the western coastal regions, but is widely distributed in other parts of the globe. It is commonly called West Indian wood nettle plant; in Southeastern part of
Nigeria, it is known as ile nkita (Adetunji *et al*., 2021) and synonyms include *Fleurya aestuans* (Gaud), *Fleurya aestuans* (Linn.) Miq. and *Urtica aestuans* (Oloyede and Oyelola 2013). In Nigeria, this plant is used as food by edible snails, while still acting as a reservoir for the cassava mosaic virus, which affects the cassava plant. It grows mostly on fallow land during the wet season (Olufunke *et al*., 2008).

Laportea aestuans are used in veterinary medicine in Trinidad and Tobago to treat urinary tract infections in farm ruminants, shorten infant delivery, and facilitate placental removal (Lan, 2006, 2007). Extracts from this plant have been prescribed by traditional healers for the treatment of liver-related illnesses (Ganiyu *et al*., 2023), as painkillers, to relieve stomach ache, abortifacient, laxative, and for the treatment of diarrhea and dysentery (Chew, 1969; Friis, 1993; Nadine, 2001). Other uses include the treatment of swelling and ulcers and management of diabetes (Essiett *et al*. 2001). Qualitatively, the leaves of *Laportea aestuans* contain alkaloids, flavonoids, steroids, phlobatinin, tannins, cardiac glycosides, and terpenoids (Adeosun *et al*. 2022). Reported pharmacological activities include antacid (Christensen *et al*., 2015), antimicrobial, antioxidant (Oloyede and Ayanbadejo, 2013), and antimalarial potential (Faloye *et al.*, 2024). Compounds isolated from *Laportea aestuans* include hexadecanoic acid methyl ester, hexadecanoic acid butyl ester, heptadecanoic acid, octadecane, (4E)-3,6dimethylhep-4-en-3-ol; 1,2,3,4,4a,4b,5,6,6a,7,8,9,10,10a,10b,11hexadecahydro-1,1,6a,10b-tetramethyl-7-((E)-4,7dimethyloct-5-enyl)chrysen-2-ol (Oguntimehin *et al*., 2019; Oloyede and Oyelola, 2013). Alot of studies have been carried out on the leaves of *Laportea aestuans* (Adetunji *et al*., 2021; Odo *et al.,* 2022; Ganiyu *et al.,* 2023; Odo *et al*., 2024;). However, little information is available on the quantification of the methanol leaf extract of *Laportea aestuans* (MLELA) using HPLC, and the effect of MLELA on sodium, potassium, bicarbonate, and chloride in the kidney. Thus, this study aimed to quantify the phytochemicals in MLELA and evaluate its acute and subchronic toxicities

Materials and Methods

Collection, Identification and Extraction

Whole plants of *Laportea aestuans* were collected around the male hostel of the Elele Campus of Madonna University in October. It was identified by Mr. Felix Nwafor of the University of Nigeria Herbarium (UNN/11804), and the specimen was stored in the herbarium for future reference. The leaves were carefully plucked-off and air-dried for 21 days away from direct sunlight. They were pulverized using an electric milling machine at the Department of Pharmacognosy of the same university. Powdered leaves (500 g) were macerated with methanol (2.5 L) for 3 days after initial agitation every 30 min for 2 h. On the fourth day, the solvent was carefully decanted, placed through a filter paper

(size 1), and concentrated *under vacuum* using a rotary evaporator at 50 °C. The concentrated methanol leaf extract (MLELA) was stored at 4 °C until use.

Gas Chromatography/Mass Spectrometry *(GC/MS) of Methanol leaves Extract of Laportea aestuans*

Analysis of MLELA (2.00 g) was performed using hyphenated GC/MS with a triple-axis detector and a 10 µL auto injector syringe. The gas chromatography system was an Agilent model 7890A (USA), while the mass spectrometer was an inert MSD 5675C model. A capillary column (19091-433HP-5Ms) measuring 30 m in length; 0.2 µm internal diameter, and 250 µm treated with phenyl methyl silox was used for the separation process. The mobile phase was an inert gas (He), the ion source temperature was set at 250 °C, the injector temperature was 280 °C, and the interface temperature was 300 °C. The pressure was maintained at 16.2 psia with a split ratio of 1:50. The manipulation of the GC/MS to achieve the retention time, percentage area, molecular weight and fragmentation pattern of the compounds was as described by Odion and co-workers (2024)

High Performance Liquid Chromatography of the Methanol Leaves Extract of Laportea aestuans

MLELA (1.00 g) was analyzed for phytochemicals by using HPLC (LC-10AD Shimadzu Japan) with twofold pump, CTO-10AS column oven and prominence UV-Visible dectector (SPD-20A). A C12 column with a thickness of 5.00 µm x length of 200.00 mm x internal diameter of 4.80 mm. The constitutive solvents (A and B) with a flow rate of 0.8 mL/min were acetic acid-acidified deionized water (pH 2.8 and acetonitrile. The column was equilibrated with 5 % solvent B after sample injection. The temperature of the column used for the analysis was set to 38 °C, the injection volume was maintained at 20 µL, and the wavelength was 280 nm. Standards were used to quantify the phytochemicals in MLELA as described by Odion *et al*. (2024).

Experimental Animal

Mature, healthy, inbred Wistar rat (91-139 g) and mice (15-30 g) of both sexes were procured from the Pharmacology and Toxicology Animal House of Madonna University, Elele. The Animals were kept in cages (iron) with bedding that was constantly changed (daily). Access to commercial rodent pellet feeding (Topfeed®) and water (nipple water device) *ad libitum* was allowed, a 12 hr light and dark cycle was maintained. The procedures in this study were adapted to the guiding principles for animal research as endorsed by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (2002). Ethical Approval (PHARM1012013279) was granted by the Madonna University Ethical Committee following the request of the authors.

Acute Toxicity Study

Lorke's (1983) method was used to evaluate the acute oral toxicity of MLELA. The extract was freshly prepared in Tween-80. The method used in this study has been previously described (Osigwe *et al*., 2025). Briefly, 12 mice were used for the test; nine for the first phase and three for the second phase. In the first phase, nine mice were divided into three groups, with three mice in each group receiving 10, 100, or 1000 mg/kg extract. In the second phase, three mice were assigned to three different groups and received 1600, 2900, or 5000 mg/kg of the extract, respectively. The mice were observed for behavioral changes and death for 24 h.

Sub-chronic Toxicity Study

Sixteen rats of both sexes were randomly distributed into four groups (A–D), with four rats per group. Group A, control, were administered with 10 ml/kg of distilled water for 28 days
Group B was administered 200 mg/kg of MLELA for 28 days. Group C had 400 mg/kg body weight of MLELA for 28 days, while Group D had 800 mg/kg body weight of MLELA for 28 days
The animals were monitored for changes in behavior and mortality, and weekly changes in body weight for the duration of the study. Each animal was euthanized on the 29th day with diethyl ether, and 5 mL of blood was collected by cardiac puncture into plain (sterile) bottles for biochemical analysis. The weights of the internal organs (the liver and kidneys) were recorded after harvesting.

Biochemical Analysis

Blood samples obtained from the cardiac puncture were centrifuged at 3500 rpm for 5 min, and the serum was subjected to the following assays: Aspartate Transaminase (AST), Alanine
Transaminase (ALT), Alkaline Phosphatase (ALP), total and conjugated bilirubin, albumin, and total protein. Kidney functions, including creatinine, urea, sodium, chloride, potassium, and bicarbonate levels, were also assessed. (Asefaw *et al*., 2020;
Adetunji *et al*., 2021)
Statistical analysis

Data are presented as mean ± standard deviation (n=5). Differences between means were evaluated using one-way analysis of variance, followed by Tukey's post-hoc test using GraphPad Prism software version 5.01.

Results

The GC/MS analysis of MLELA revealed twentyseven compounds (Table 1) with benzoic acid, methyl ester (17.99 %); Neophytadiene (10.79 %); methyl pyrrole-2-carboxylate (8.228 %); hexadecanoic acid methyl ester (5.83 %); hydrazinecarbothioamide, N-(2-ethoxyethyl) (5.51 %); N-[3,5-dinitropyridin-2-yl]proline (5.13 %); thiazole, tetrahydro (5.08 %) and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (4.01 %) as the prominent compounds. The identified compounds can be grouped into phenolics, esters, hydrazines, saturated and unsaturated fatty alcohols and acids, diterpenes, alkaloids, flavonoids, and steroids. Twenty groups of compounds were quantified and identified by HPLC assay of MLELA (Table 2): phytate (18.8296 µg/mL), steroids (17.1678 µg/mL), naringin (15.2487 µg/mL), ephedrine (13.5202 µg/mL), oxalate (11.0380 µg/mL), and flavan-3-ol (10.5455 µg/mL). These compounds include alkaloids, anthocyanins, flavonoids, glycosides, saponins, steroids, and antinutrients.

Effect of the extract on liver enzyme markers

All doses of MLELA (200, 400, and 800 mg/kg) significantly ($P < 0.001$) decreased aspartate transaminase (AST) levels, although the decrease was greater in the 200 mg/kg group than in the 400 mg/kg group (Figure 1). A significant decrease in the level of alanine transaminase (ALT) was observed only in the treated groups compared to that in the control group ($P < 0.05$). Alanine phosphatase was not affected by the administered doses of MLELA compared with the control (distilled water).

Effect of the extract on kidney function

There was a significant decrease ($P < 0.05$, $P < 0.01$)) in the creatinine levels at all doses. A similar decrease was observed for urea (Figure 2). There was also a significant decrease in the serum total bilirubin level (Figure 2)

Effect of the extract on serum electrolytes

Sodium and chloride ions were significantly ($P < 0.05$) reduced compared with the control (Figure 3). Reduction in sodium ion levels occurred across the treatments, but a significant reduction was observed only at the 800 mg/kg dose. A significant reduction in chloride ion concentration was observed at 400 and 800 mg/kg.

Effect of the extract on body weight and relative organ weight

A significant increase in the relative organ weight of the liver was observed at 800 mg/kg. However, there was no significant change ($P > 0.05$) in the body weight of the MLELA-treated groups compared with that of the control group (Figure 4).

Table 1: Compounds from GC-MS analysis of the methanol leaves extract of *Laportea aestuans*

S/N	Retention time [min]	Molecular weight	Compound	Molecular formula	Percentage Area
1	3.373	250.25	Methyl pyrrole-2-carboxylate	$C_{12}H_{14}N_2O_4$	8.28
2	4.827	136.15	Benzoic acid, methyl ester	$C_8H_8O_2$	17.99
3	6.200	89.16	Thiazole, tetrahydro-	C_3H_7NS	5.08
4	6.841	170.59	Benzoic acid 4-chloro, methyl ester	(C_8H_7ClO	1.17
5	7.585	284.48	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	0.75
6	8.157	163.24	Hydrazinecarbothioamide, N-(2-ethoxyethyl)-	$C_5H_{13}N_3OS$	5.51
7	9.771	214.34	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	2.26
8	9.868	282.06	N-[3,5-Dinitropyridin-2-yl] proline	$C_{10}H_{14}N_4O_6$	5.13
9	10.148	207.27	1H-Indole, 5-methyl-2-phenyl-	$C_{15}H_{13}N$	0.83
10	11.728	242.40	Methyl tetradecanoate	$C_{15}H_{30}O_2$	1.60
11	12.746	278.50	Neophytadiene	$C_{20}H_{38}$	10.79
12	12.952	268.5	Z-2-Octadecen-1-ol	$C_{18}H_{36}O$	1.51
13	13.112	296.5	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	4.01
14	13.307	208.39	(2Z,4E)-3,7,11-Trimethyl-2,4-dodecadiene	$C_{15}H_{28}$	0.56
15	13.496	270.45	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	5.83
16	13.759	334.45	1,2-Benzenedicarboxylic acid, butyl 2-$C_{20}H_{30}O$	2.20 ethylhexyl ester	
17	14.016	110.10	Catechol	$C_6H_6O_2$	1.00
18	14.909	282.46	6-Octadecenoic acid	$C_{18}H_{34}O_2$	2.12
19	14.995	410.80	Eicosyl octyl ether	$C_{28}C_{58}O$	1.04
20	15.109	298.49	Methyl stearate	$C_{19}H_{38}O_2$	2.29
21	15.229	282.47	Oleic acid	$C_{18}H_{34}O_2$	1.40
22	15.378	207.27	2-Ethylacridine	$C_{15}H_{13}N$	0.69
23	15.481	206.20	N3- (4-fluorophenyl)-N5-methyl-4H-1,2,4-	$C_9H_{10}N_5F$ 1.05 triazole-3,5-diamine	
24	15.681	207.27	1H-Indole, 5-methyl-2-phenyl-	$C_{15}H_{13}N$	1.18
25	15.870	282.47	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	0.62
26	16.134	207.27	Hexahydropyridine, 1-methyl-4- [4,5-$C_{12}H_{17}NO_2$	0.90 dihydroxyphenyl]-	
27	16.340	207.27	Indolizine, 2-(4-methylphenyl)-	$C_{15}H_{13}N$	0.83

Table 2: HPLC quantification of the methanol leaves extract of *Laportea aestuans*

S/N	Compounds	Retention Time	Area (m^2)	Concentration (µg/ml)
1	Proanthocyanin	0.080	232.8066	0.2183
2	Lunamarin	0.280	3400.3980	4.9786
3	Naringin	2.390	12252.811	15.2487
4	Cardiac glycoside	4.120	6344.548	3.9370
5	Flavan-3-ol	6.016	18154.069	10.5455
6	Anthocyanin	7.470	8442.984	7.2410
7	Ribalinidine	10.366	19598.067	8.4040
8	Naringenin	12.970	6238.326	2.6361
9	Spartein	15.460	4967.564	8.9024
10	Rutin	20.313	12756.484	7.9159
11	Flavonones	22.730	9573.141	8.2102
12	Steroids	25.650	10008.818	17.1678
13	Kaempferol	27.536	11458.010	5.2899
14	Epicatechin	29.860	5478.441	8.2188
15	Phytate	32.996	14009.1853	18.8296
16	Flavone	34.600	5756.4320	3.5721
17	Oxalate	36.876	6988.5601	11.0380
18	Resveratrol	39.200	10234.602	7.7771
19	Sapogenin	42.276	3473.142	5.7077
20	Ephedrine	44.170	10509.676	13.5202

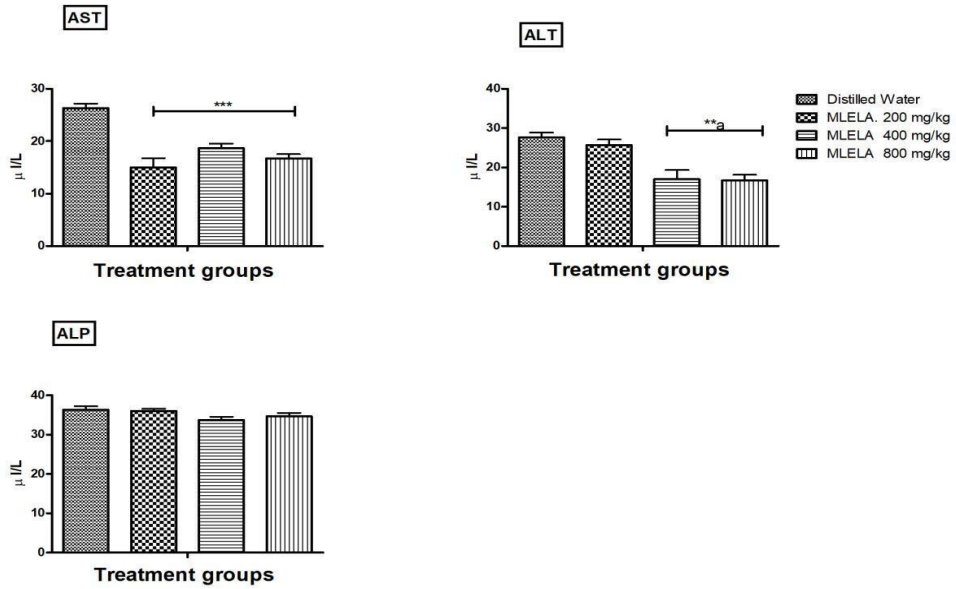

Figure 1: Effect of subacute administration of *Laportea aestuans* leaf methanol extract on liver enzymes. **Significantly different ($P < 0.01$) from control (distilled water); ***Significantly different ($P < 0.001$) from control; [a] Significantly different ($P < 0.05$) from 200 mg/kg treatment group; AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alanine phosphatase

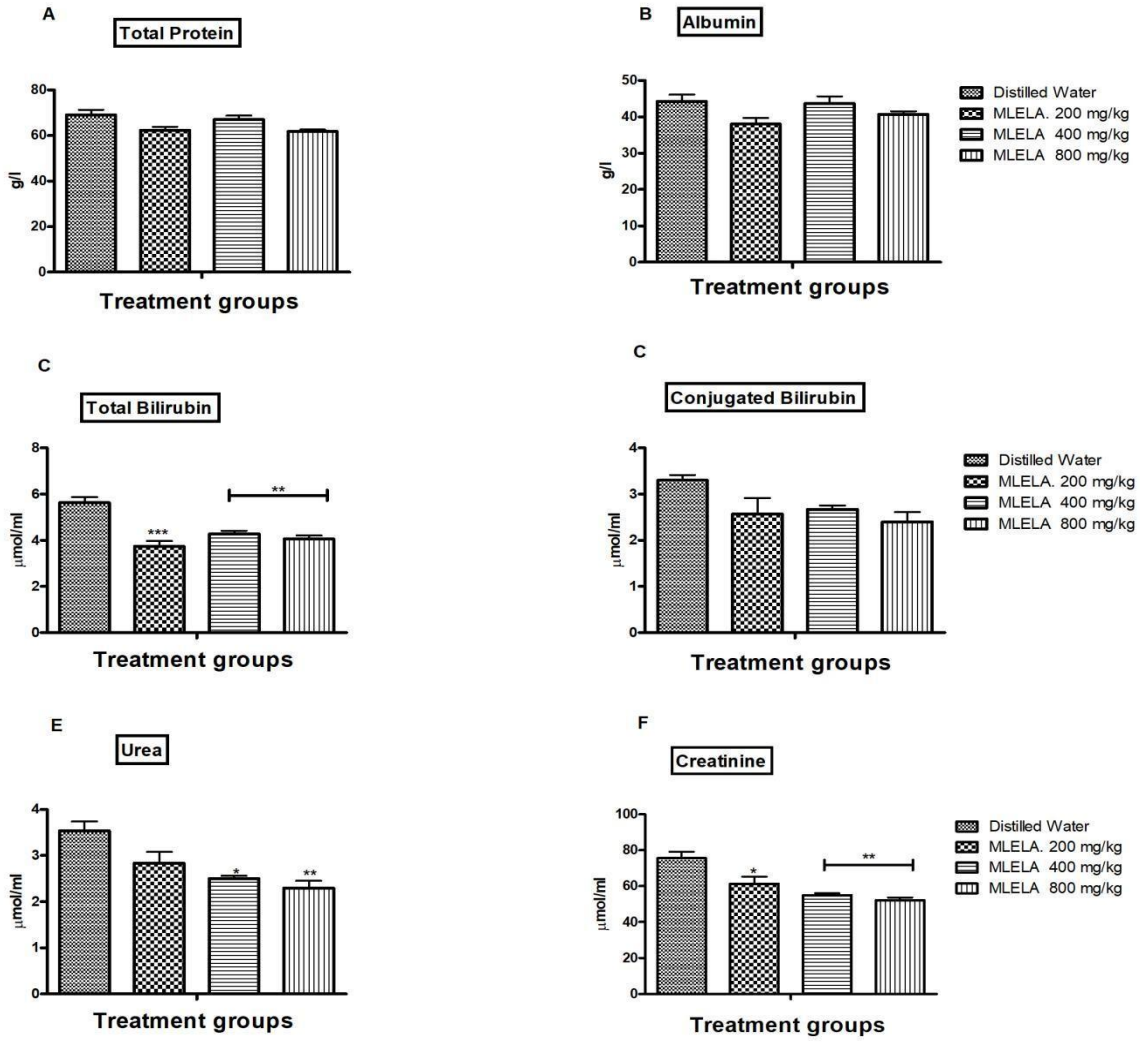

Figure 2: Effect of subacute administration of *Laportea aestuans* leaf methanol extract on total protein and albumin concentrations (A and B), total bilirubin and conjugated bilirubin concentrations (C and D), and urea and creatinine concentrations (E and F). *Significantly different ($P < 0.01$) from control (distilled water); **Significantly different ($P < 0.01$) from control; ***Significantly different ($P < 0.001$) from control

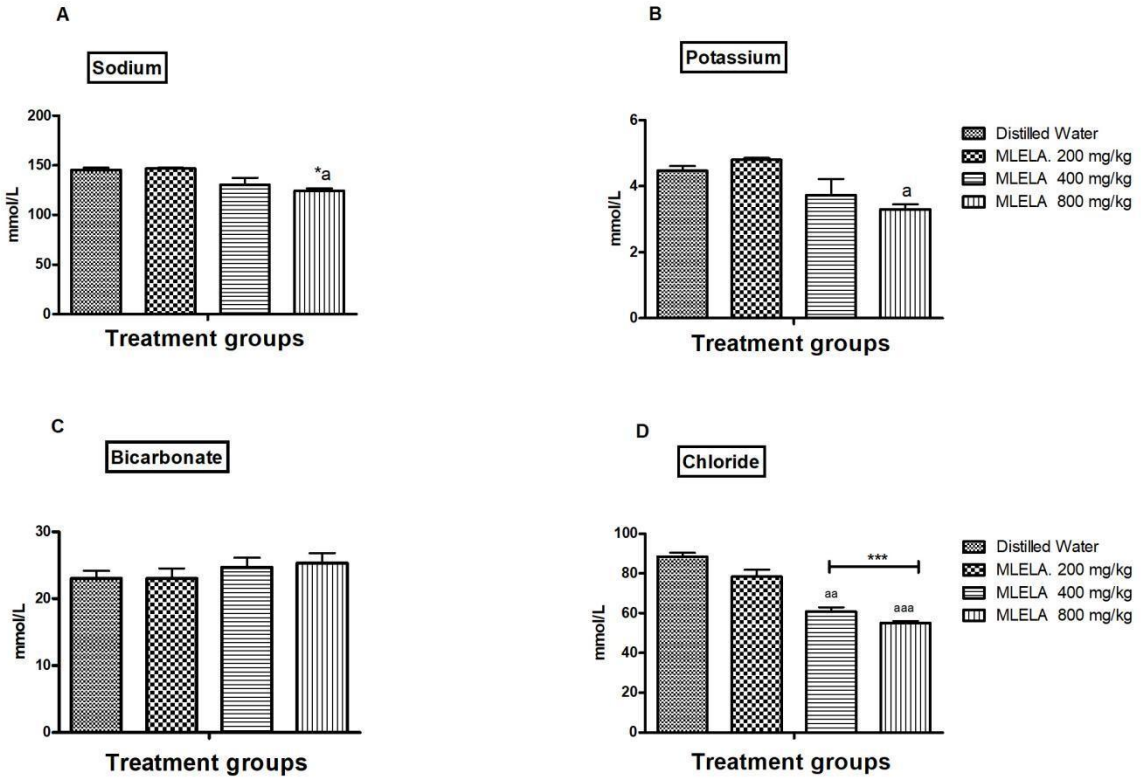

Figure 3: Effect of subacute administration of *Laportea aestuans* leaf methanol extract on sodium, potassium (A and B), Bicarbonate and Chloride (C and D) levels. *Significantly different ($P < 0.01$) from control (distilled water); ***Significantly different ($P < 0.001$) from control; [a] Significantly different ($P < 0.05$) from 200mg/kg treatment group; [si]gnificantly different ($P < 0.01$) from 200mg/kg treatment group; [ins]ignificantly different ($P < 0.01$) from 200 mg/kg treatment group.

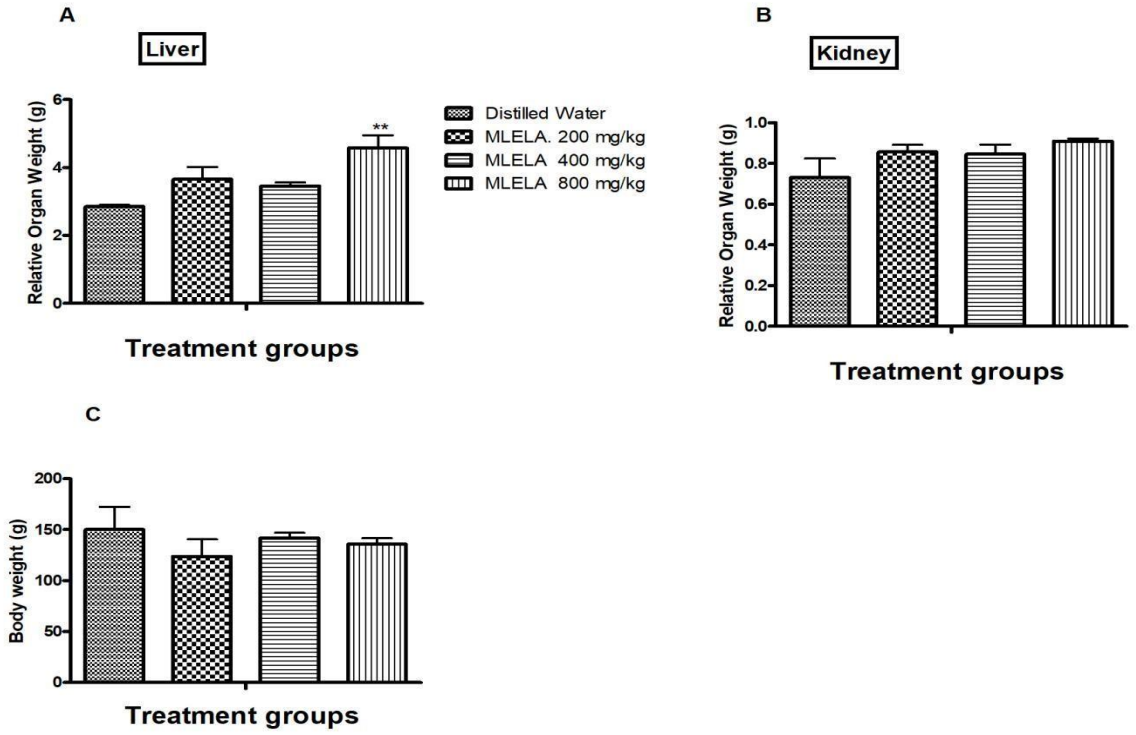

Figure 4: Effect of subacute administration of *Laportea aestuans* leaf methanol extract on Relative Organ weights of the liver (A), kidney (B), and body weight (C). **Significantly different ($P < 0.01$) from control

Discussion

Previous studies have shown that the ethanol leaf extract of *Laportea aestuans* to contain thirty-three compounds with linoleic acid ethyl ester, hexadecanoic acid ethyl ester, and diazo progesterone as the prominent compounds (Odo *et al*., 2022). In addition, hydro distilled essential oil from whole plants of *Laportan aestuans* afforded 13 compounds with methyl salicylate, fenchol, 1,4-dioctadiene, and 1,2-cyclohexanedione dioxime (Oloyede, 2011). The dichloromethane fraction of the aerial parts of *Laportea aestuans* gave twenty-three compounds with tetra decanoic acid, 9,12,15-octadecatrienoic acid (Z, Z, Z), and 9,12octadecadienoic acid (Z, Z) as conspicuous compounds (Faloye *et al*., 2024). Some closely related compounds identified in previous studies included methyl salicylate, linoleic acid, and 9hexadecanoic acid methyl ester (Faloye *et al*., 2024), which is in agreement with the results of the present study. Many of these compounds have distinct pharmacological properties. The limitation of this test is that GC/MS analysis identified volatile components, whereas non-volatile constituents were identified and quantified by HPLC analysis with the aid of standards.

The leaves of *Laportea aestuans* have been shown to contain high concentrations of saponins, phenolics, and flavonoids (Ganiyu *et al*., 2023) as well as tannins, steroids, oxalate, phytate, and flavonoids (Adetunji *et al*., 2021). These data are not in agreement with the present analysis, in which anthocyanins and cardiac glycosides were detected and quantified. This can be attributed to the high sensitivity of the HPLC technique used in this assay. In addition to the sensitivity associated with this method of analysis, phytochemicals can be analyzed in a single test, thus reducing the time lag associated with the tests when carried out on an individual basis. The polyphenols and flavonoids identified in this study are implicated in the treatment of liver diseases because of their ability to improve liver antioxidant defense enzymes by mediating the expression of Nrf-2 or cytochrome P450 2E1, which can enhance the inflammatory process (RodríguezNegrete *et al*., 2024). Similar antioxidant effects have been reported previously (Rabizadeh *et al*., 2024).

An acute toxicity study indicated that the oral LD_{50} of MLELA was above 5000 mg/kg. This is consistent with previous reports (Adeosun *et al*., 2015; Adeosun *et al*., 2022).

A decrease in AST and ALP levels has been reported in aqueous leaf extracts of *Laportea aestuans* when studying the protective effect of this plant against acetaminophen-induced liver injury (Ganiyu *et al*., 2023). Adetunji *et al* (2021) reported a significant decrease in AST and significant increase in ALP and ALT with MLELA while this present study showed no effect in serum ALP. This disparity in our observations could be due to the duration of the extract administration. In this study, MLELA was administered for four weeks, whereas Adetunji *et al*. (2021) administered the extract for two weeks. The significant decrease in transaminase enzymes following administration of increasing doses of MLELA is indicative of the protective effect of the extract on the liver.

A significant decrease in serum creatinine levels is indicative of functional glomeruli, whereas a change in urea levels indicates the extent of protein metabolism and reflects the disease state of the kidney (Abiola *et al.*, 2019). These parameters are important markers of the state of the kidney and demonstrate that MLELA has a renoprotective effect. Sodium, potassium, bicarbonate, and chloride ions are important markers of chronic kidney diseases; thus, a significant reduction is an indication of the absence of prolonged disease conditions in experimental animals. This renoprotection is further reaffirmed by the lack of effect of the extract on the relative organ weight of the kidney, which may indicate reduced hemolysis following MLELA dosing or increased hemoglobin metabolism, further reiterating the protective effect of MLELA on the liver.

Conclusion

This study showed that MLELA is rich in flavonoids and polyphenolic compounds including epicatechin, flavan-3-ol, flavone, flavonones, narigenin, naringin, kaempferol, resveratrol, and rutin. Most of these compounds are documented antioxidant which have been shown to protect the liver and kidney against exogenous and endogenous molecules by acting as free radical scavengers. Our results show that MLELA reduced the levels of hepatic enzymes, bilirubin, renal creatinine, and urea. Therefore, MLELA possesses hepatorenal protective properties in adult Wistar rats, validates its safety, and uses trado-medical management of liver and kidney perturbations.

Conflict of Interest

None

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Not Applicable

Author's Contribution

Emmanuel Eimiomodebheki Odion was involved in the conceptualization of the study, writing and editing of the manuscript and supervision of the work. Somrofeechukwu Freeson Festus was involved in sample and data collection. Theresa Chioma Umeji was involved in conceptualization, data analysis and proofreading of the manuscript. Chinyelu Clementina Osigwe and Ugonma Florence Uwaeme was involved in manuscript edition and data collection

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