



## PHYTOCHEMICAL CONSTITUENT, ACUTE AND SUBACUTE TOXICITY STUDIES OF METHANOL EXTRACT OF LAPORTEA AESTUANS LEAF

\*<sup>1,2</sup>Adeosun A. M., <sup>1</sup>Akamo A. J., <sup>1</sup>Akinloye O. A., <sup>1</sup>Shotuyo A. L. A.

<sup>1</sup>Department of Biochemistry, College of Biosciences, Federal University of Agriculture, Abeokuta, Nigeria

<sup>2</sup>Department of Biochemistry, Faculty of Basic Medical and Applied Sciences, Lead City University, Ibadan, Nigeria

\*Corresponding authors' email: [adeosun.amuhammad@lcu.edu.ng](mailto:adeosun.amuhammad@lcu.edu.ng) Phone: +2348069499550

### ABSTRACT

*Laportea aestuans* is an African herbaceous weed. This work assessed the bioactive compound content of methanol extract of *Laportea aestuans* leaf (LALME) and its acute and subacute toxicity effects in Male Wistar rats. According to qualitative and quantitative phytochemical analysis, LALME possesses a significant amount of phenolics, flavonoids, alkaloids, tannin, carotenoids, and anthraquinones. LALME is regarded as safe because it did not cause death in rats when given doses of up to 2,000 mg/kg body weight. LALME at 200 and 400 mg/kg markedly increased ( $p < 0.05$ ) serum activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT). There was a decrease in liver LDH activity in rats administered LALME, but no significant alteration was noticed in the serum of the rats. The total bilirubin concentration in the serum of rats given 200 mg/kg LALME and in the liver of rats administered 200 and 400 mg/kg LALME was significantly higher ( $p < 0.05$ ) than that in the serum and liver of control rats. The serum of rats given LALME had a nearly threefold increase in direct bilirubin compared to control. However, rats administered 200 mg/kg of LALME had an increased serum urea concentration compared to control. When comparing photomicrographs of the liver and kidney of rats given LALME to those of control rats, the liver and kidney of rats given LALME showed minor congestion and renal dissemination, respectively. In conclusion, LALME was found to be safe in acute toxicity; nevertheless, subacute administration may cause variations in liver function and renal function markers, necessitating careful use of this plant extract.

Keywords: Bioactive, Phytochemicals, *Laportea aestuans*, Toxicity Study.

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### INTRODUCTION

Medicinal plants, which include vegetables, herbs, and spices, are used in a variety of ways in traditional medicine, and they contain chemical entities that can be used to generate new pharmaceuticals (Zengin *et al.*, 2011). In addition, they contain multitudes of naturally occurring chemical compounds (Ighodaro and Akinloye, 2017). Medicinal plants have received much attention in managing several ailments because they are cheap and easy to obtain from their surroundings. Nevertheless, a medicinal plant may exhibit undesired adverse outcomes as its usage does not often have a known dosage of a standardized concentration of an active component. Less than 10% of commercialized herbal products worldwide have standardized active components (Ramulondi *et al.*, 2019).

*Laportea aestuans* (Linn.) Chew (Family: Urticaceae) is an annual herbaceous weed native to Africa. It is one of the medicinal species with active functions. The plant has an erect, angular stem, covered with pilose hairs or stinging hairs, and is green in colour (Chew, 1969). In Gabon, the cooked leaves of *L. aestuans* are eaten as a remedy for stomachaches. They are also cooked with peanuts, which are given to pregnant women. According to Lans (2007), an infusion prepared of *L. aestuans* by soaking leaves in water is taken to deliver the placenta after childbirth, and the root infusion boiled in water is taken to prevent excessive menstrual bleeding. Some use the plant as a vegetable when preparing food for babies (Essiett *et al.*, 2011). The leaves are used as a diuretic for blennorrhoea and chest problems. An extract from the leaves of *L. aestuans* can be used to treat arthritis, anaemia, hay fever, kidney problems, and pain. The extract contains active compounds that reduce pro-

inflammatory cytokines. *L. aestuans* leaves, roots, and whole plants are used to cure internal ulcers, diabetes, bronchitis, and filariasis (Focho *et al.*, 2009). *L. aestuans* as a pot herb vegetables in soups, and the leaves are air-dried, finely shredded, taken as tea per se and assuage asthma attack (Essiett *et al.*, 2011). In Nigeria, *L. aestuans* are used alone or in decoction with other herbs to manage urinary problems, stroke, rheumatism, swelling, and diabetes, which are comorbidities and complications of hypertension (Fabricant and Farnsworth, 2001). Regarding the wide ethnomedicinal utilization of *L. aestuans*, it is incumbent on us to determine the content of bioactive components and potential toxicity associated with the use of this plant, hence the reason for this study.

### Sample Preparation

The plant sample was obtained from the Botanical Garden of the Federal University of Agriculture, Abeokuta, authenticated and deposited in the herbarium with registration number FUNAABH-0071 by Dr Oyelakin S. The leaves of the plant were collected, rinsed, and dried. The powdered leaf was soaked in absolute methanol at 1 g to 8 mL for 48 hours with intermittent stirring. The mixture was then filtered, and methanol was evaporated from the filtrate to obtain methanol extract in the form of a paste.

### Qualitative Phytochemical Analysis

A qualitative phytochemical analysis was performed on a crude methanol extract of the plant using the method described by Sofowara (1996) and Trease and Evans (1984).

**Quantitative Phytochemical Analysis**

The total flavonoid content in the crude methanol extract of the plant was measured using the colourimetric assay developed by Zhishen et al. (1999). Quercetin was used as a standard; the total flavonoid content was expressed as quercetin equivalent. The total phenolic compound in the crude methanol extract of the plant was determined using a spectrophotometric Folin-Ciocalteu method (Singleton et al., 1999). The phenolic compound content was expressed as gallic acid equivalent. The method described by Soladoye and Chukwuma (2012) was used to determine anthraquinone in the methanol extract of the plant. Anthraquinone was expressed as alizarin equivalent. The determination of the alkaloid content of the plant sample was performed spectrophotometrically using the method described by Ghate et al. (2013). The alkaloid content was extrapolated from the standard curve of the atropine standard and was expressed as atropine equivalent. Total carotenoid content was determined using the method described by de Carvalho et al. (2012).

**Acute toxicity test (oral lethal dose (LD50) determination) of *Larportea aestuans* leaf methanol extract**

Male rats were administered a single oral dose of *Larportea aestuans*, 1000, 2000, 3000, 4000, and 5000 mg/kg for acute toxicity (OECD, 2001). The rats were observed for changes in the skin, fur, eyes, mucous membrane (nasal), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, and defecation), and central nervous system (drowsiness, gait, tremors, and convulsions) changes. Mortality, if any, was determined over 24 hours and further monitored for seven days.

**Subacute toxicity status of *Larportea aestuans* leaf methanol extract**

Eighteen *Wistar* rats were randomized into three groups of control and treatments (n = 6/group). The control group was given distilled water (vehicle), while the treated groups were given 200 and 400 mg kg<sup>-1</sup> body weight of crude methanol extracts of *Larportea aestuans* orally daily for 28 days.

**Determination of tissue weight**

The tissues excised were washed in 1.15% KCl, blotted, and their relative weight was recorded as weight/100 g rat using the formula below:

$$Relative\ organ\ weight = \frac{Tissue\ weight}{Animal\ weight} \times 100$$

**Evaluation of biochemical parameters**

Blood was collected, and serum was separated from whole blood by centrifuging in plain tubes at 4000 rpm for 10 minutes. The liver and kidney were harvested, rinsed in 1.15% KCl, homogenized in 10 mM phosphate buffer solution, pH 7.0, centrifuged at 12500 rpm for 15 minutes to obtain post mitochondria fraction ((PMF), and kept at -4°C for biochemical analyses. The liver function markers such as alkaline phosphatase activity (Englehardt et al., 1970), gamma-glutamyl aminotransferase GGT activity (Orlowsky and Meister, 1963), aspartate aminotransferase (AST) activity (Reitman and Frankel, 1957), and alanine aminotransferase (ALT) activity (Reitman and Frankel, 1957), total and direct bilirubin concentrations were determined in the serum and PMF of the liver. In addition, creatinine concentration (Bartels et al., 1972) and urea (Fawcett and Scott, 1960) were determined in the serum and PMF of the kidney.

**Histology**

The heart, liver, and kidney were analyzed for the presence or absence of macroscopic abnormalities. The organs were fixed with 10% formalin for 24 h, dehydrated, and fixed into paraffin blocks. Histological sections were made and stained with hematoxylin and eosin (H & E) and examined under a light microscope.

**Data analyses**

Data obtained from the experiments were expressed as mean ± standard deviation. Values were compared with control by using one way analysis of variance (ANOVA) with Tukey’s posthoc comparison. Mean differences were taken to be significantly different at P< 0.05.

**RESULTS**

Qualitative determination of phytochemicals revealed the presence of tannin, saponin, phlobatinnin, flavonoid, terpenoids, steroids, cardiac glycoside, and alkaloids, while anthraquinone was not detected. Table 1 reveals the amounts of bioactive compounds found in the methanol extract of *Larportea aestuans* leaf. The extract is rich in phenolic, flavonoids, and alkaloids.

**Table 1: Content of bioactive phytochemicals in *Larportea aestuans* leaf methanol extract**

Phytochemicals	Amount (mg/100 g)
Alkaloids	803.61±1.65 AE
Anthraquinone	303.93±8.59 AZE
Total Carotenoids	101.88±0.05
Total flavonoid	1439.88±16.38 QE
Total phenolic	2145.72±46.85 GAE

Results are presented as the mean ± standard deviation of triplicate determinations. AE represents atropine equivalent, AZ represents alizarin equivalent, QE represents quercetin equivalent, and GAE represents gallic acid equivalent. Table 2 shows the effect of subacute administration of LALME on some liver enzymes. Rats administered 200 and 400 mg/kg LALME both had increased activity of alanine aminotransferase (ALT) in the serum, while the activity of the enzyme in the liver decreased in the liver when compared to control (p<0.05). There was an increase in AST activity in the serum of rats administered 200 mg/kg LALME (p<0.05), and there was a significant decrease (p<0.05) in AST activity in

the liver of rats administered 400 mg/kg LALME when compared to AST activity in the serum and liver of control rats, respectively. There was no change in ALP activity in the serum and liver of rats treated with LALME at selected doses relative to the control. GGT activity increased in the serum of rats administered 400 mg/kg LALME while it decreased in the liver of rats administered 200 mg/kg compared with GGT activity in both the serum and liver, respectively. There was no significant change in LDH activity in the serum of rats administered LALME. However, liver LDH activity was noticed to decrease significantly (p<0.05) in rats that were administered 200 and 400 mg/kg LALME.

**Table 2 Effect of subacute administration of LALME on liver enzyme activities.**

Parameters	Serum (U/L)			Liver (U/g protein)		
	Control	200mg/kg LALME	400mg/kg LALME	Control	200 mg/kg LALME	400 mg/kg LALME
ALT	5.81±0.47	4.45±0.28*	7.97±0.31*	15.85±0.78	8.85±0.62*	10.51±0.69*
AST	22.21±2.01	29.66±2.06*	25.41±2.15	18.74±1.58	17.77±1.22	11.07±0.84*
ALP	52.39±0.49	53.48±1.78	51.79±1.10	2.79±0.53	2.47±0.67	3.10±0.70
GGT	3.59±0.48	4.17±0.26	7.53±1.30*	2.02±0.31	0.99±0.14*	1.86±0.18
LDH	81.85±1.45	80.27±2.73	79.88±6.08	185.00±1.15	114.7±3.38*	83.06±2.40*

Results are expressed as mean ± standard deviation (n=6). \*indicate values are significant (p<0.05) when compared to the control group.

There was an increase in the serum concentration of total bilirubin in rats administered 200 mg/kg LALME compared to control (p<0.05). Rats administered 200 and 400 mg/kg of LALME had high total bilirubin concentrations in their liver compared to control. Direct bilirubin was raised by almost three folds in the serum of rats that were administered LALME compared to control (p<0.05), though there were no changes in the level of direct bilirubin observed in the liver of the rats. There were no marked changes in the albumin concentration in the serum of the rats administered LALME. However, the concentration was higher (p<0.05) in the liver

of rats administered LALME at selected doses when compared to control (Figure 1). LALME significantly decreased serum creatinine concentration without any changes in its concentration in the kidney. However, rats administered 200 mg/kg LALME had higher urea levels in their blood and kidney (Figure 2). Figure 3 shows the photomicrograph of the liver and kidney of rats administered LALME compared to control. The liver of rats administered LALME showed mild congestion when compared to control. Also, little dissemination of the kidney was observed in rats administered LALME.

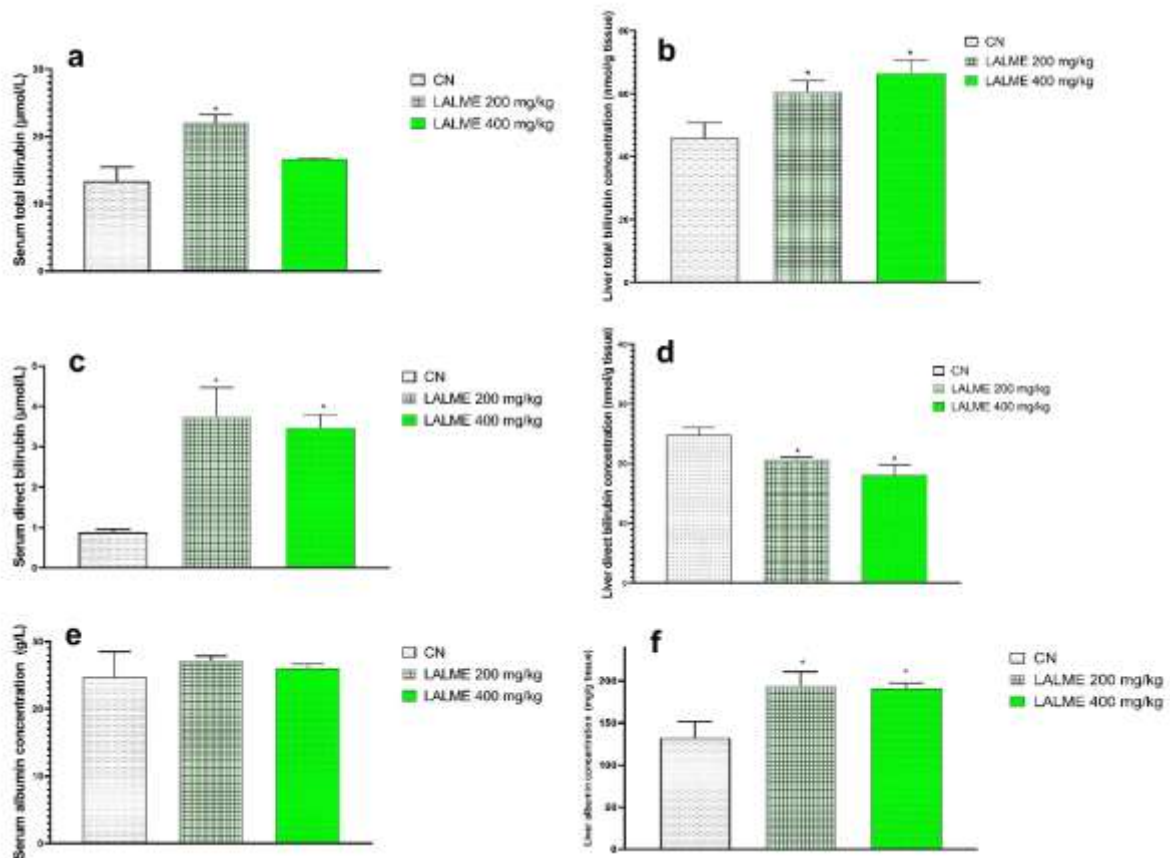


Figure 1: The effect of acute administration of *Laportea aestuans* leaf methanol extract on serum and liver total bilirubin concentration (a and b), serum and liver direct bilirubin concentration (c and d), and serum and liver albumin concentration (e and f). Bars represent the mean ± standard deviation of each parameter (n=6). \* represent value significantly different when compared with control (P < 0.05)

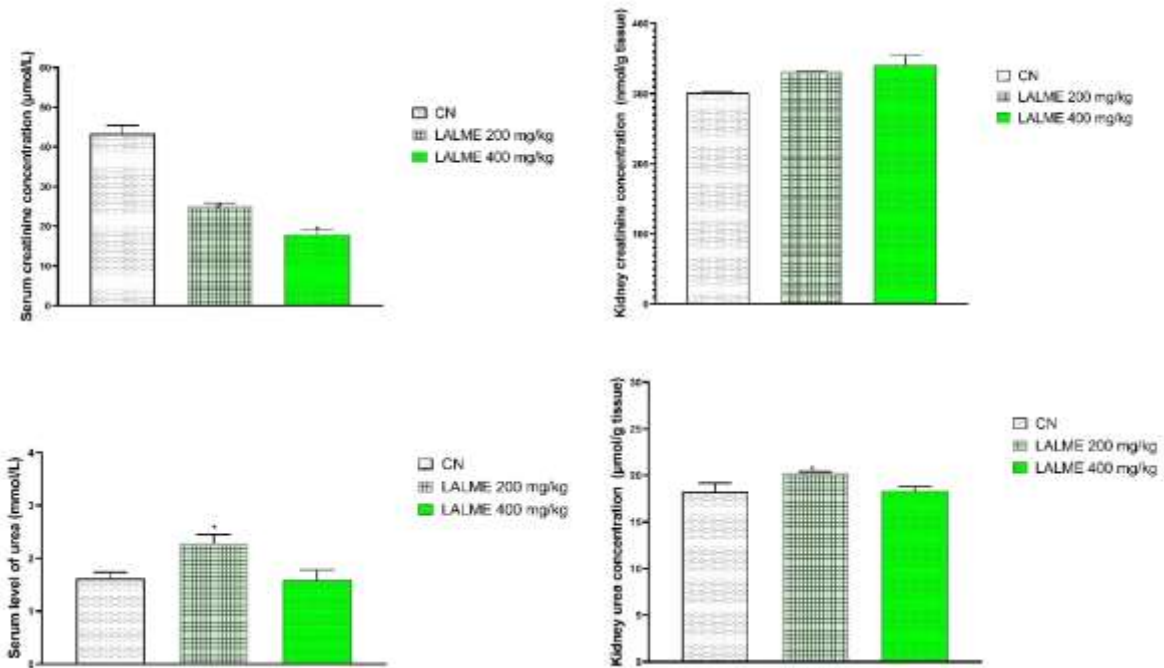


Figure 2: The effect of acute administration of *Laportea aestuans* leaf methanol extract on creatinine concentration in (a) serum, (b) kidney, and the concentration of urea in the (c) serum and (d) Kidney. Bars represent the mean ± standard deviation of each parameter (n=6)

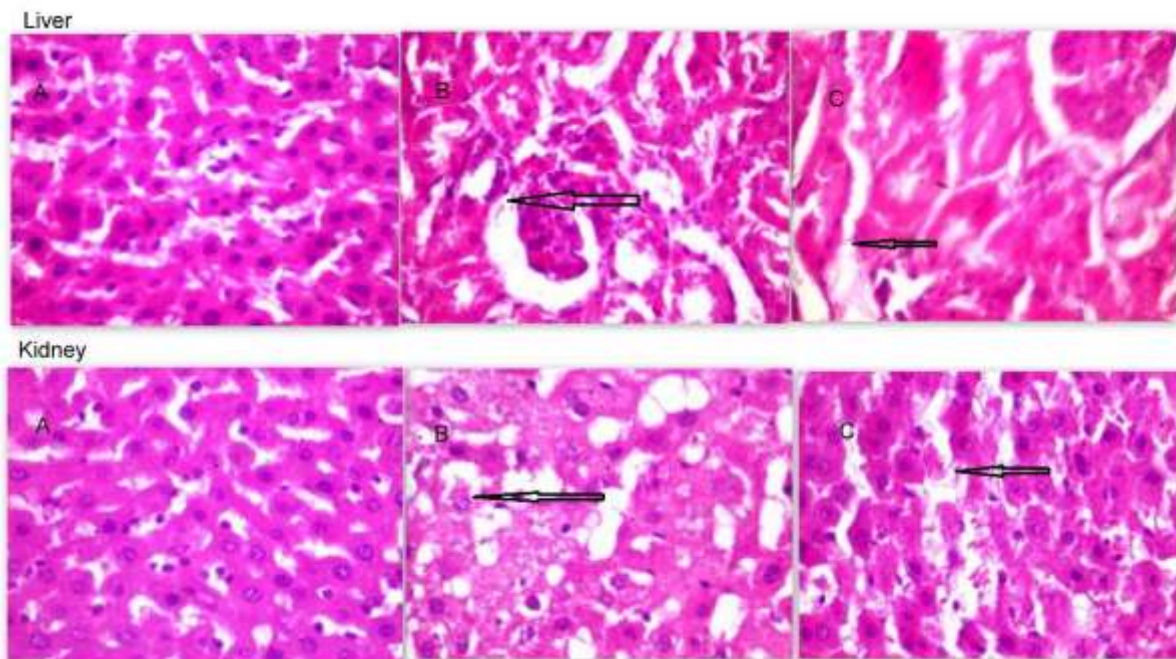


Figure 3: Photomicrographs (x 100) showing the effect of subacute administration of LALME on the architecture of the liver and kidney of rats.

**Discussion**

Plants are widely known for containing various bioactive substances with medicinal potential, but they also include specific molecules that can have unfavourable side effects. (Mickymaray, 2019). One of the most significant issues with ethnomedicinal plant use is the lack of a precise dose. Unfortunately, when medicinal plants are utilized carelessly, they can be harmful (Obidike and Salawu, 2013). This work determined the phytochemical composition and toxicity of *Laportea aestuans* leaf methanol extract. *Laportea*

*aestuans* has a high phenolic and flavonoid content. Antioxidants such as flavonoids and phenolic chemicals have anti-inflammatory, antithrombotic, and vasodilatory effects (Mbaveng et al., 2014). Alkaloid derivatives are utilized in medicine as antineoplastic, analgesic, and antibacterial medications (Kutchan, 1995), and alkaloid derivatives are also discovered in this plant. LALME is high in carotenoids, which are the precursors to vitamins, including vitamin A, vitamin E, and vitamin D in plants (Asensi-Fabado and Munné-Bosch, 2010). Beta carotene and tocopherol, for

example, are potent nutraceutical antioxidants that have been commercialized. One efficient method of assessing the toxicity of medicinal plants is to evaluate oral doses up to 5000 mg/kg BW (Osagie-Eweka *et al.*, 2021). When rats were given LALME at a 2000 mg/kg dose, no abnormalities or mortality were seen. The plant is considered safe in acute phase testing because, at an oral dosage of 5000 mg/kg, LALME did not produce any mortality nor a noticeable deformity in the rats. This finding is similar to the toxicity evaluation of the plant reported by Akomas and Ijioma (2015). Subacute or chronic toxicity testing can reveal a plant's long-term or cumulative harmful effects on drug-metabolizing organs (Adeneye, 2014). According to a subacute toxicity study, when LALME was given for 28 days, it caused changes in various hepatic and renal function markers. Foreign chemical metabolism is primarily carried out in the liver; this makes the liver vulnerable to harm from xenobiotics and necrosis or changes in the liver's membrane integrity (Nekvindova *et al.*, 2020). These events can result in the release of enzymes typically found in the liver into the bloodstream. Serum levels of liver enzymes are used to indirectly assess the integrity of the tissue (Batt and Ferrari, 1995). The increases in ALT, AST, GGT, and LDH activities seen in both the serum and liver of rats given LALME suggest that subacute or long-term usage of LALME at a specific dosage can cause liver impairment. ALT is usually located in the hepatocyte cytoplasm, whereas AST, GGT, and LDH are found in the liver and other organs such as the heart, kidney, and pancreas. Other enzymes, such as AST, GGT, and LDH, are found in the liver and other organs such as the heart, kidney, and pancreas. We also noticed an increase in the activity of these enzymes. Other tissues may have contributed to the increased activity in the serum and the liver. There is an indication that extended-term usage of LALME may increase hemolysis (the breaking down of haemoglobin) or impair haemoglobin metabolism, as it was found to cause a raised bilirubin concentration in the serum and liver of rats. Raised serum and tissue bilirubin concentrations have been associated with liver injury or bile duct obstruction (Woreta and Alqahtani, 2014).

When the body's ability to remove waste, maintain body fluid, maintain electrolyte balance, and induce a decline in the production of vital hormones is hampered, hazardous substances build up in the kidney (Schnellmann, 2008). A slight increase in serum and kidney urea concentrations in rats administered 200 mg/kg LALME. Because urea accumulates in the bloodstream when urea production exceeds the rate of urea clearance, it is a better endogenous predictor of renal function (Ghadirkhomi *et al.*, 2016). Over a subacute period, rats administered LALME had lower blood creatinine levels. Creatinine level in the blood is influenced by catabolic rate, muscle mass, and a high-protein diet and is not considered a substantial predictor of kidney disease. It is, however, noticed from a photomicrograph of the section of renal tissue that LALME-treated rats may experience kidney disintegration in long term usage.

## CONCLUSION

This finding indicates that the methanol extract of *Laportea aestuans* leaf contains a significant amount of bioactive phytochemicals. Though it is pretty safe in the short term, long-term use may compromise the functional and structural integrity of the liver and kidney, which are the body's primary metabolizing organs. As a result, the herb may not be recommended for long-term oral usage in ethnomedicine.

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