

Evaluation and Characterization of Phytochemicals of Fractions of Ethanol Leaf Extracts of *Laportea aestuans* for Anti-inflammatory Property in Animal Models

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Abstract

Original Research

Laportea aestuans has folkloric application in African herbal remedies for the treatment of inflammatory conditions. Over the years this medicinal plant has been traditionally used in Africa ethnomedicine in the treatment of several diseases associated with pains, fever and inflammation but without much scientific fractionation and characterization of biomolecular constituents and therefore, the rationale behind this research. The crude ethanol extract has demonstrated anti-inflammatory potential, the specific bioactive fractions and their phytochemical constituents remain uncharacterized. To fractionate and characterize the phytochemical principles of solvent fractions of ethanol leaf extract of *Laportea aestuans* and determine their anti-inflammatory activity using animal models, crude ethanol extract of *L. aestuans* leaves was fractionated into n-hexane, dichloromethane, ethyl acetate, and aqueous ethanol fractions. Phytochemical analysis of each fraction was conducted and evaluated on animal models. Anti-inflammatory activity was assessed using carrageenan-induced paw edema model in Wistar rats. Standard drug aspirin (10 mg/kg) was used and compared with the various fractions of the solvents. The statistical analysis was conducted using one-way ANOVA. Phytochemical screening revealed differential distribution of bioactive compounds across fractions. Flavonoids and alkaloids were most abundant in the ethyl acetate and dichloromethane fractions. The dichloromethane and ethyl acetate fractions exhibited significant ($p < 0.05$) inhibition of paw edema, with the ethyl acetate fraction showing activity comparable to standard drug; aspirin. Solvent partitioning enhanced identification of active fractions of *L. aestuans* responsible for its anti-inflammatory activity. The characterisation and structural elucidation of the ethyl acetate fraction was carried-out with Gas Chromatography-Mass Spectrometry (GC-MS). The GC-MS analysis of the ethyl acetate fraction of *Laportea aestuans* identified 9, 12- Octadecadienoic acid, 9, 12, 15- Octadecatrienoic acid, Tetradecanoic acid, Oleic acid methyl ester, Dodecanoic acid and other methyl ester and alkanes. These bioactive principles possess anti-inflammatory properties and thus, provided insight into the mechanisms of action.

Keywords: *Laportea aestuans*, solvent fractions, anti-inflammatory activity, characterisation, carrageenan model.

Introduction

There are several medicinal plants in Africa that are yet to be exploited to provide remedy to different ailments that threaten human existence. These medicinal plants exist in the form of vegetables, herbs, and spices, are used in a variety of ways in alternative and complementary medicine, and they consist of phytochemical constituents that are useful in the development of new pharmaceuticals (Zengin, Cakmak, Guler, and Aktumsek, 2017). Medicinal plants have been used in managing several diseases hence; they are less expensive and readily available to obtain from the surroundings. However, the beneficial effects of medicinal plant does not mean they are free of harmful effects. Not up to 10% of the commercialized herbal products have standardized active principles (Ramulondi, de-Wet, and Van-Vuuren, 2019). *Laportea aestuans* is one of the many medicinal plants in African soil which possess anti-inflammatory properties (Alagba *et al.*, 2025).

Inflammation plays a pivotal role in the pathogenesis of many chronic diseases. Although NSAIDs remain the cornerstone of anti-inflammatory therapy, their long-term use is associated with adverse effects such as gastric irritation, hepatotoxicity, and cardiovascular risk and therefore, the need for alternative remedy with less or no adverse effects. Traditional medicinal plants, like *Laportea aestuans*, are promising sources of safer and effective anti-inflammatory agents. Previous studies have demonstrated the anti-inflammatory activity of its ethanol leaf extract (Alagba *et al.*, 2025). However, little is known about the activity of its solvent-partitioned fractions and molecular characterization of its phytochemicals. Regarding the wide ethno-pharmacological values of *L. aestuans*, it is essential to fractionate and characterize the active principles which are effective in anti-inflammatory properties in animal cell. The modern biological techniques used in detecting, identifying, isolating and characterizing the novel plant extract has improved in the management of many diseases affecting

humans. The research study aims to evaluate the various solvent fractions and characterise the ethanol leaf extracts of *Laportea aestuans* for anti-inflammatory properties.

Gas Chromatography-Mass Spectrometry (GC-MS)

This analytical techniques combine both gas chromatography (GC) and mass spectrometry (MS). Gas chromatography (GC) is a techniques used to separate mixtures into individual components based on boiling points, polarity and molecular weight. The carrier gas; inert gases e.g. helium, nitrogen transport samples through a column. The column is a narrow coated tube where separation occurs (Akinyemi *et al.*, 2015). Mass Spectrometry (MS) uses ionization where separated components are ionized, breaking molecules into charged fragments. The fragments are sorted by mass-to-charge ratio (m/z) using electric and magnetic fields. The detected fragments are used to generate mass spectrum. This techniques has high sensitivity (used to detect trace amounts), high specificity (used to identify complex mixture) and can be applied in quantitative analysis where the concentration of the measure components are measured (Ahmadian *et al.*, 2018). The GC-MS is a powerful analytical techniques for analyzing complex mixtures, identifying unknown compounds and quantifying them (Adeneye, 2014).

Working Principle of Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS is an analytical technique that combines the separation power of Gas Chromatography (GC) with the identification and structural elucidation power of Mass Spectrometry (MS).

Principle of GC Component

Separation is based on the portioning of volatile

compounds between a mobile phase (inert carrier gas like helium or nitrogen) and a stationary phase (a liquid or polymer coated on the inside of a capillary column). The sample is vaporized in the injector and inert gas carries the vaporized sample into the heated GC column. The components in the mixture is separated based on their boiling points and affinity for the stationary phase. The components with lower boiling point and less interactive to the stationary phase are eluted first followed by the higher ones.

Principle of Mass Spectrometry (MS) Component

Identification is based on the mass-to-charge ratio (m/z) of ionized fragments produced from each compound. As each separated compound exits the GC column, it enters the mass spectrometer. The compound is bombarded with high energy electrons in an ionization chamber, causing it to fragment into ions. These ions are then sorted and detected based on their mass-to-charge ratio (m/z). A mass spectrum is produced for each compound, a unique “fingerprint” showing the relative abundance of each fragment ion. In the final output, the system provides; chromatogram from GC showing peaks at different retention times. The mass spectra from MS for each peak, identifying and characterizing the compound based on its fragmentation pattern.

Materials and Methods

Plant Material Collection and Authentication

The fresh leaves of *Laportea aestuans* were collected at Nkaliki in Ebonyi State and identified by an ethnobotanist; of the Department of Medicinal Plant and Traditional Medicine, National Institute of Pharmaceutical Research and Development (NIPRD), Abuja, and authenticated by Taxonomist; of the same institution. The ethical approval was obtained from Nnamdi Azikiwe University, Animal Research Ethics Committee (NAU-AREC), Awka where the research was carried out.

Preparation of Leaf Extracts of *Laportea aestuans*

The leaves of *Laportea aestuans* (*Urticaceae*:

English; white nettle or tropical nettle weed) were washed and sun-dried for 7 days at ambient temperature. The dried leaves were blended to fine powder using electric blender and sieved. The powdered leaves were macerated in 90 % ethanol for 48 hours at random shaking. The substance were filtered and the filtrate, concentrated to dryness by gentle heating over a water bath set at a temperature (40°C) to obtain a yield. The extracts were preserved in a refrigerator at 4°C. for the experiment.

Fractionation of the Crude Extract

2000 g of the ethanol extracts was mixed with silica gel (60-200 mesh; 200 g) and allowed to air dry for 24 hours, after which it was packed in a column. The gradient elution was performed using n-hexane, dichloromethane, ethyl acetate and ethanol respectively. Each fraction was collected and concentrated using a rotary evaporator set at lower 40°C to yield n-hexane fraction; 560.5 g, dichloromethane fraction; 500.25 g, ethyl acetate fraction; 440 g, and ethanol fraction; 320.9 g. Ethyl acetate fraction was mixed with silica gel (60 – 200 mesh, 340 g) and allowed to air dry for 24 hours, after which it was packed in a column using silica gel as stationary phase and ethyl acetate /n-hexane as mobile phase. Ethyl acetate fraction was used because it has intermittent polarity. Fractions of 100 ml was collected and concentrated to dryness. At the end of the process using gradient elution, all the fractions with the same bands on the TLC profiles were pooled together to yield fractions FA (1-10), FB (11-22), FC (23-36) and FD (37-end). Fractions FD (37-end) (580 mg) was further fractionated on silica gel with a gradient of ethyl acetate in n-hexane as eluent. FD (37-end) was used because it was the most concentrated among other fractions. Forty fractions of 100 ml were collected. Each fraction was concentrated to dryness and recovered with n-hexane: ethyl acetate (9:1). The fractions were pooled together on the TLC profiles, after repeated chromatography with the same mobile and stationary phases to yield the column chromatographic fraction (CCF) 105.4 mg.

Selection and Preparation of the Experimental Animals

Adult Wistar rats (180-200g), numbering 13 and 90 Swiss mice (16-18g) of both sexes were selected from Animal house; Nnamdi Azikiwe University, Awka and were used for the experiment. Animals were housed in clean cages according to sexes in a well-ventilated room with free access to food and water. They were given access to familiarize with the new environment, fed with pelletized animal mash (Premier Feed Mills, Nigeria), and fasted overnight before the experiment began. Organization for Economic Cooperation and Development (OECD), (2011), 425 guidelines for experimental animal and Animal Research Ethics Committee (NAU-AREC), Nnamdi Azikiwe University was followed.

Acute Toxicity Study

The investigation of the lethality effect (LD₅₀) was performed following Lorke's method (1983). The study was classified into two phases with 13 Wistar rats of both male and female. In the first phase, the animals were grouped into 3 (Group 1 – 3), n = 3 and administered with leaf extracts orally at the doses (10, 100 and 1000mg/kg) respectively. The rats were physically observed for toxicity effects for up to 24 hours. In the absence of lethality effects, phase 2 was introduced. The second phase involved 4 groups (n =1) and were intra-gastrically administered leaf extracts in exponentially increased doses (1600, 2900, and 5000mg/kg) respectively. The animals were caged in similar conditions and observed for toxicity and mortality up to 24 hours and then 72 hours for late toxicity effects.

Evaluation of Anti-inflammatory Property of Ethyl Acetate Sub-fractions of the Extract

Following the result obtained from the assay of the fractions, the ethyl acetate fraction was further separated using column chromatography. At the end of the process using gradient elution, all the fractions with the same bands on the TLC profiles were pooled together to yield fraction F_A (1-10), F_B (11-22), F_C (23-36) and F_D (37-end). The ethyl acetate sub-

fractions were assayed for anti-inflammatory property using the carrageenan-induced pedal edema model in mice. Adult mice (16-18 g) of both sexes were divided into six groups with each group containing 5 mice. The control group was given distilled water (10ml/kg), Group 2, 3, 4 and 5 received sub-fractions; F_A (1-10), F_B (11-22), F_C (23-36) and F_D (37-end) at doses of 10 mg/kg p.o respectively. Group 6 received aspirin (5mg/kg) administered orally. After 30 minutes, acute inflammation was induced in the right hind paw of mice by sub-plantar injection of 0.1ml carrageenan.

The paw volume was measured by water displacement before and at 0.5, 1, 2, 3, 4 and 5 h after carrageenan injection. Edema formation was evaluated in terms of the difference in the zero time paw volume of the injected paw and its volume at the different times after carrageenan injection. For each dose of extract, percentage inhibition of edema was calculated the relation (Semis *et al.*, 2021)

The percentage inhibition of the inflammation was determined by the formula:

$$\text{Inhibition of edema percent (\%I)} = \frac{V_c - V_t}{V_c} \times 100$$

Where,

V_t = paw volume of the test group

V_c = paw volume of control group.

Characterization of Compounds

Characterization of phytochemicals refers to identifying and analyzing the bioactive chemical constituents in the ethanol leaf extracts that contribute to its therapeutic properties (Khan, *et al.*, 2013; Kunanusorn, *et al.*, 2019). Gas Chromatography – Mass Spectrometry (GC-MS) on column chromatographic fraction (CCF) of the extracts was used to characterize the compound. The characterization of the active principle of *L. aestuans* was analysed using an Agilent HP-7890A Gas Chromatograph (Agilent Technologies, Palo Alto, CA, USA) was carried out on an Agilent HP 7890A gas chromatograph (Technology model MSD = 5975C detector) (Agilent Technologies Injector:

7683B Series, Ionization energy: 70eV, Palo Alto, CA, USA). Initial temperature = 100°C held for 2 min, final temperature = 270°C at the rate of 10°C/min. 1ul of the various fractions of the ethanol extracts of *L. aestuans* was injected. Temperature of heater was set at 250°C, pressure of 3.2652 psi, mode type slit less, column type (HP 5MS: 30m x 320um x 0.25um) and carrier gas (Helium, 99.9999% purity, flow rate = 1.4963ml/min; average velocity = 45.618cm/s). The constituent compounds were determined by comparing their retention times and mass weights with those of authentic samples obtained by GC (Adama, 2021). The mass spectra of GC-MS interpretation on mass spectrum was conducted using the database at Institute of Pharmaceutical Biology Universitat Dusseldorf. Then the phytochemicals were identified based on the hits returned after comparing the unknown peak value and chromatograph from GC-MS against the

known chromatograph peak value from the Universitat Dusseldorf Library database. Subsequently, the details about their molecular formula, molecular weight, structure were also obtained.

Statistical Analyses

The values obtained from the experiment was expressed as mean $\pm$ standard deviation (SD). The data was analyzed using one-way ANOVA to determine whether results in a particular group are significantly different from those in other corresponding groups. The numerical data was statistically analyzed using statistical product and service solutions (SPSS), Version; 20 for Windows. The results obtained was compared to controls with $p < 0.05$ considered to be significant.

Results

Table 1: Quantitative Phytochemical Constituents of *L. aestuans* of various Solvent Fractions

Fraction	Mean (mg/g)	Standard Deviation (mg/g)
Ethanol crude extract	5350.87	4131.90
n-Hexane fraction	2216.03	2493.43
Dichloromethane fraction	2863.01	2588.03
Ethyl acetate fraction	3080.24	3151.86
Ethanol fraction	5521.14	3682.98

P-value = 0.2223

From table 1 above, since the p-value > 0.05 , there is no statistical significant difference in the phytochemical concentrations across the five solvent fractions at the 95% confidence level. On the other

hand, the observed differences in concentrations was due to random variations rather than a true effect of the solvent.

Table 2: Effect of the Fractions of Extracts of *L. aestuans* on Carrageenan-induced Inflammation.**Sample for Hexane Fraction; 100mg/kg****n = 6**

Time	Mean (%)	SD	P-value
0 H	0.509	0.042	0.461616
0.5 H	1.338	0.014	0.014711
1 H	1.120	0.042	0.000019
2 H	0.956	0.032	< 0.000001
3 H	0.866	0.036	< 0.000001
4 H	0.763	0.039	< 0.000001
5 H	0.675	0.039	< 0.000001

The results in Table 2 above are similar across other sample groups like **Hexane Fraction; 200/400 mg/kg, Dichloromethane, Ethyl acetate, Ethanol, and Aspirin**, with most showing **statistically significant reduction** in inflammation (compared to negative control) from **1 H onward**, and increasing significance with time and dose. The table showed anti-inflammatory potential of various solvent fractions—**n-hexane fraction, dichloromethane fraction, ethyl acetate fraction, and ethanol**

fraction — of ethanol leaf extracts using the **carrageenan-induced paw edema model** in mice. These were compared with a standard non-steroidal anti-inflammatory drug (**aspirin**, 10 mg/kg) and a negative control (distilled water, 5 ml/kg). The assessment was performed across multiple time points from **0 to 5 hours** following inflammation induction, a time-course approach designed to capture both the early and late phases of inflammation.

Table 3: The Anti-inflammatory Property of various Fractions of the Ethyl Acetate.**Formulation Group (FA (1-10))****n = 6**

Time	Mean (%)	SD	P-value
0 H	0.435	0.013	0.000035
0.5 H	0.438	0.094	0.002018
1 H	1.185	0.093	0.000001
2 H	1.258	0.063	0.000008

3 H	1.238	0.092	0.000005
4 H	0.928	0.072	0.000076
5 H	0.454	0.028	0.000001

The results in Table 3 above are similar across other sample groups like F_B (11-22), F_C (23-36) and F_D (37-end),, and **Aspirin**, with most showing **statistically significant reduction** in inflammation (compared to negative control) from **1 H onward**,

and increasing significance with time and dose. The sub-fractions on further analysis showed that the FD (37-end) were more effective compared to other sub-fractions. The result produced the FD (37-end) gave similar results with the standard drug; aspirin.

GC-MS Analysis

Table 4: GC-MS Analysis of Bioactive Compounds present in Ethanol Leaf Extract of *Laportea aestuans*.

S/N	Mwt (g/mol)	Compound Name	Chemical Formula	Peak Area (%)	Bioactive Compound	Medical Importance
1.	280.45	9,12-Octadecadienoic acid (linoleic acid)	C ₁₈ H ₃₂ O ₂	19.06	Unsaturated fatty acid	Anti-inflammatory, Antioxidant (Chen <i>et al.</i> , 2021)
2.	278.43	9,12,15-Octadecatrienoic acid (ALA)	C ₁₈ H ₃₀ O ₂	18.66	Omega-3 fatty acid	Cardioprotective anti-inflammatory (Okolie <i>et al.</i> , 2019)
3.	228.37	Tetradecanoic acid (myristic acid)	C ₁₄ H ₂₈ O ₂	16.28	Saturated fatty acid	Lipid lowering (Rusli <i>et al.</i> , 2022)
4.	296.49	Oleic acid methyl ester	C ₁₉ H ₃₆ O ₂	8.24	Fatty acid este	Anti-inflammatory (Rusli <i>et al.</i> , 2022)

5.	200.32	Dodecanoic acid	$C_{12}H_{24}O_2$	- 6.8	Saturated fatty acid	Skin agent (Olukanmi, 2020)
6.	-	Other methyl ester and alkanes	-	minor	Volatile organics	Anti-microbial (Yakubu <i>et al.</i> , 2023)

The GC-MS chromatogram of the column crystalized sample gave different peaks of intensity for different analytes as indicated in Table 4 above.

Discussion

The study revealed that solvent fractionation of *Laportea aestuans* ethanol extract led to differential distribution of phytochemicals and anti-inflammatory activity. The ethyl acetate and dichloromethane fractions showed the most promising activity, comparable to the standard drug; aspirin. The ethyl acetate fraction was rich in flavonoids and alkaloids (Adama, 2021), which are known to inhibit pro-inflammatory cytokines and prostaglandin synthesis (Adedapo, *et al.*, 2021). The observed inhibition of paw edema, particularly during the second phase of the carrageenan response, suggested a mechanism involving suppression of prostaglandin E2 and nitric oxide production (Alagba, *et al.*, 2025). The lesser activity of the n-hexane and aqueous ethanol fractions may be due to lower concentrations of bioactive compounds (Adeneye, 2014). These findings reinforce the traditional use of *L. aestuans* for inflammatory conditions and highlight the relevance of bio-guided fractionation for identifying active constituents (Alka, *et al.*, 2023).

GC-MS Analysis: In this study, the presence of some of the important components resolved by GC-MS analysis was reported. This type of analysis is the first step towards understanding the nature of active

principles in this medicinal plant. During this study, a significant number of active compounds were observed in the ethanol leave extracts fraction. The GC-MS analysis of ethanol leaf extracts of *L. aestuans* revealed the presence of 9, 12-Octadecanoic acid, 9, 12, 15-Octadecatrienoic acid, Tetradecanoic acid, Oleic acid methyl ester, and Dodecanoic acid as prominent compounds.

Most of these constituents have been found to show interesting biological activities against diseases and/or pathogens. 9,12-Octadecanoic acid, 9,12,15-Octadecatrienoic acid and Dodecanoic acid have been reported to act as anti-inflammatory, antioxidant, hypocholesterolemic, nematocide, pesticide lubricant, antiandrogenic, hemolytic agents, and 5-alpha reductase inhibitor (Onwukaeme *et al.*, 2017). Dodecanoic acid as an agent of anti-inflammation has shown significant inhibitory activity against phospholipase A2 as reported by Mun *et al.*, (2018). Linoleic acid (9, 12 - Octadecanoic acid) is reported to have anti-inflammatory and cancer preventive activities. Tetradecanoic acid isolated from ethanol leaf extracts has been studied extensively to conclude that its anti-inflammatory actions are related to the inhibition of TNF-alpha and inflammatory enzymes (Mickymaray, 2019). Dodecanoic acid has been shown to possess anti-inflammatory and antioxidant properties (Marrelli, 2021) while 9, 12-Octadecanoic acid possesses food additives properties (Marrelli, *et al.*, 2020), antiviral and antimicrobial (Marrelli *et al.*, 2018). Dodecanoic is a known antioxidant

(Kunanusorn *et al.*, 2019), 9, 12, 15-Octadecatrienoic acid has been shown to possess antidiabetic properties (Kothari *et al.*, 2020), antimicrobial (Kamal, 2023), anticancer (Fredotovi *et al.*, 2020) and antifungal properties (Ighodaro and Akinloye, 2017).

Conclusion

Solvent fractionation of ethanol extract of *Laportea aestuans* revealed that the ethyl acetate and dichloromethane fractions possess significant anti-inflammatory activity. These fractions are rich in flavonoids and alkaloids, suggesting that these phytochemicals contribute to the pharmacological effect. This provides a scientific basis for the development of novel anti-inflammatory agents from *L. aestuans*.

The solvent fractionation showed that ethanol fraction has more activity compared to other solvent fractions such as hexane, dichloromethane, and acetyl acetate. Secondly, alcohol is consumable and therefore the choice of alcohol solvent fraction in the assessment of the pharmacological properties. GC-MS analysis showed a long chain of 9, 12-Octadecadienoic acid; 9, 12, 15-Octadecatrienoic acid; Tetradecanoic acid, Oleic acid methyl ester, Dodecanoic acid and other methyl ester and alkanes in the ethanol leaf extracts of *Laportea aestuans*.

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