Evaluation of Active Principles of Ethanol Leaf Extracts of Laportea Aestuans for Anti-Inflammatory Property in Animal Models

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Abstract

Inflammation underlies many pathological conditions and remains a major therapeutic target. Despite the efficacy of synthetic anti-inflammatory drugs, their side effects have prompted a renewed interest in medicinal plants with traditional uses. *Laportea aestuans* has been used in African ethnomedicine for treating inflammation-related disorders. This study identified the active principles and evaluated the anti-inflammatory property of ethanol leaf extracts of *Laportea aestuans* in animal models. Leaves were extracted using 90% ethanol in maceration method. The phytochemical screening was performed using Trease and Evans method (2002). The acute toxicity studies was carried out with Lorke's method (1983). Acute inflammation was induced in Swiss mice using carrageenan-induced paw edema and formalin-induced inflammation models. The extract was administered at 100, 200, and 400 mg/kg orally, and compared to standard drug; aspirin (10 mg/kg). The paw volumes were measured at 0, 1, 2, 3, and 4 hours post-induction. Results were analyzed using one-way ANOVA, mean and standard deviation taking $P<0.05$ to be significant. The ethanol leaf extracts of *Laportea aestuans* contained phenolic compound, alkaloids, flavonoids, tannins, and saponins, terpenoids and steroids. It significantly ($p<0.05$) inhibited paw edema in a dose-dependent manner in both models. Maximum inhibition was observed at 400 mg/kg, comparable to the standard drug; aspirin. Ethanol leaf extracts of *L. aestuans* possesses significant anti-inflammatory property, orchestrated by phytochemicals such as flavonoids and alkaloids, and therefore, validated its traditional use and suggested novel anti-inflammatory agent for development.

Keywords: *Laportea aestuans*, anti-inflammatory property, carrageenan, formalin, ethanol leaf extracts, phytochemicals.

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Introduction

Medicinal plants in the treatment of inflammatory conditions have been in place over the years in Africa in which human have been in continuous interactions with the environment. Plants have been a major constituents in preparing traditional medicine in the treatment of chronic and acute pains, arthritis, acne and allergies (Akomas, and Ijioma, 2015). *Laportea aestuans* (Urticaceae), also known as white nettle, is traditionally used in Africa and the Caribbean to treat pain, inflammation, and infections. However, scientific evaluation of its anti-inflammatory efficacy is still very few.

Laportea aestuans is rich in bioactive principles with minimized adverse effects. Therapeutic properties such as anti-inflammatory property have been reported in other plants (Adama, 2021). People in many cases; have claimed the effectiveness of L. *aestuans* in traditional medicine and herbal products (Alka *et al*., 2023) and therefore, it become necessary to validate the effectiveness of the bioactive principle of this plant scientifically. From the researcher's investigations, few scientific evaluations have been carried out in L. *aestuans* with limited knowledge on the activities of the bioactive principles for anti-inflammatory properties on animal models and therefore, the need for this research. And among the reporton L. *aestuans*, very few researches have been performed on the leave extracts. Inflammation is the body's natural response to injury or infection, characterized by pain, swelling, redness, heat, and loss of function (Ani *et al*., 2023). While inflammation is protective, chronic inflammation contributes to disease conditions such as arthritis, cardiovascular disorders, and cancer (Ferrero *et al*., 2017). Non-steroidal anti-inflammatory drugs (NSAIDs) are effective but associated with gastrointestinal, renal, and cardiovascular side effects (Kothari *et al*., 2020). As a result, the search for safer, plant-derived anti-inflammatory agents has gained momentum. There have been many scientific research to ensure that synthetic anti-inflammatory drugs pose no harmful effects to human health but in spite of these, they still exhibit adverse effects. And therefore, the need to evaluate *Laportea aestuans* for possible anti-inflammatory properties hence, they may have no or less adverse effect than synthetic drugs.

Though, inflammation is regarded as protective response to tissue damage and organ dysfunction which gives rise to series of events facilitated by a number of inflammatory mediators such as prostaglandins, prostacyclin, leukoterines, pro-inflammatory enzymes (COX-2, :LOX), NF-kB, and MAPK pathways and pro-inflammatory cytokines (TNF-α, IL-1β) ( ). These can activate and pose many inflammatory associated disorders such as autoimmune diseases (Rheumatoid arthritis, Type 1 diabetes, and multiple sclerosis), inflammatory bowel disease (IBD), stroke, and cancer and neurodegenerative diseases (Philk *et al*., 2017). The quest for alternative therapy with less side effects in the treatment of inflammatory conditions will be a major breakthrough. This study investigated the anti-inflammatory property of ethanol leaf extracts of *L. aestuans* using established acute inflammation models in Swiss mice and identified the active principles.

Materials and Methods

Plant Collection and Authentication

The fresh leaves of *Laportea aestuans* was collected from Abakaliki and identified by an ethno-botanist from the Department of Medicinal Plant and Traditional Medicine,

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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 performed.

Preparation of Ethanol Leaf Extracts

The leaves of *Laportea aestuans* (English; white nettle or tropical nettle weed, Igbo; Ire nkita, Hausa; Bulsum fage, Yoruba; Fiyafiya or Ofuefue) were washed and air-driedfor 14 days at ambient temperature in the laboratory. The dried samples were reduced in size to fine powder using electric blender and sieved. Eight hundred grams (800g) powder was cold macerated in 6.5L of 90 % ethanol for 48 hours with occasional shaking. The filtrate was then concentrated to dryness by gentle heating over a water bath set at a temperature (40^0C) to obtain a yield. The extracts was kept in a refrigerator and used for the experiment.

Phytochemical Screening

Phytochemical screening was performed on the lyophilized ethanol leaf extracts of *Laportea aestuans* to test for tannins, alkaloids, phenols, saponins, glycosides and flavonoids according to the standard qualitative procedures as described by Trease and Evans methods (2002) (Kumar, *et al*., 2010). Different phytochemical constituents have functional groups or structural features that selectively react with specific reagents, producing a qualitative visible changes such as colour changes, precipitate formation, or frothing which indicate their presence. Trease and Evans phytochemical screening method works by exposing ethanol leaf extracts to specific reagents that interact chemically with targeted classes of phytochemicals.

Selectionof the Experimental Animals

A total number of thirteen (13) Wistar rats (7 males and 6 females) (180-200g) and sixty (60) Swiss mice (35 males and 25 females) (16-18g) of both sexes obtained from Animal house of the Department of Physiology, Nnamdi Azikiwe University, Okofia campus were used throughout the experiment. The animals were housed in clean cages according to their sexes in a well-ventilated room with suitable temperature and relative humidity. They were allowed to acclimatize with the new environment for 7 days, fed with pelletized animal mash (Premier Feed Mills, Nigeria), allowed access to clean water *ad libitum* and fasted overnight before the experiment commenced. Organization for Economic Cooperation and Development (OECD), (2011), 425 guidelines for experimental animal along with Nnamdi Azikiwe University, Animal Research Ethics Committee (NAU-AREC) was followed.

Acute Toxicity Study

This test was performed following the Lorke's method (1983) as described by Kumar, *et al*., (2010). The study was conducted in two phases using 13 Wistar rats of both male and female, weighing 180-200 gram. In the first phase, 3 groups of 3 rats in each cage were administered with 10mg/kg, 100mg/kg and 1000mg/kg of the ethanol leaf extracts of *Laportea aestuans* orally. The rats were observed for signs of toxicity such as hyper activity, salivation, paw-licking, writhing, muscle paralysis, respiratory distress and mortality within the first 4 h and after 24 h. When no lethality was observed, phase 2 was then introduced. In the second phase, 4 groups of one rat in each cage were intra-gastrically administered with ethanol leaf extracts using orogastric tube in geometrically increasing doses of 1600mg/kg, 2900mg/kg, and 5000mg/kg respectively. They were kept

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under similar conditions and observed for signs of toxicity and mortality at first 4 hour, 24 hours and then 72 hours respectively for late toxicity. The two phases were atoxic to the animals.

Evaluationof Anti-inflammatory Property Carrageenan-induced Paw Edema

Acute inflammation was induced by injecting 0.1 mL of 1% carrageenan into the sub-plantar region of the right hind paw of Swiss mice. The Swiss mice were divided into five groups (n = 6):

- Group I: Negative control (distilledwater, 10 mL/kg)
- Group II: Positive Control; standard drug (Aspirin, 10 mg/kg)
- Group III–V: Ethanol leaf extracts of *L. aestuans* (100, 200, 400 mg/kg)

Paw measurements was taken thus: before (initial paw volume) and after the injection of carrageenan at different time range; 0.5, 1, 2, 3, 4, 5 and 24 hours using a plethysmometer. The results was expressed as percentage inhibition in relation to the control groups.

Percentage inhibition = (1-Vt / Vc) × 100

Where;

Vt and Vc represent the mean change in paw size of the treated mice and control group respectively.

Formalin-induced Inflammation Models

In a separate experiment, inflammation was induced using 0.1 mL of 2% v/v formalin solution into the sub-planter of the left hind paw of the mice. The ethanol leafextracts of *Laportea aestuans* was assayed at 100, 200, 400mg/kg on group 3-5 from day 1 to day 7. Group 1 wasadministered with10ml/kg of distilledwater orallyas negative control and group 2 was administered with aspirin (10mg/kg) orally as positive control. Measurement of paw volume by water displacement was carried out on daily basis. Formalin induces inflammation in both peripheral and central nervous system (from 5-15 minutes after injection is peripheral while from 25-60 minutes after injection is CNS).

Group 1: Distilled water; 10ml/kg p.o (Negative control)

Group 2: Aspirin; 10mg/kg p.o (Positive control)

Group 3: EtOH Ext; 100mg/kg p.o for 7 days

Group 4: EtOH Ext, 200mg/kg p.o for 7 days

Group 5: EtOH; 400mg/kg p.o for 7 days

Daily changes in inflammation reactions was evaluated by measuring the volume of water displacedby the inflamed left hind paw once daily. Mean increase in the paw volume of each group over 7 days period was calculated and compared with the control. The mean percent inhibition of inflammation was calculated using the relation:

Inhibition of inflammation (%) = Vc-Vt/Vc x 100

Where;

Vt = Paw volume of test group

Vc = Paw volume of the control group.

Statistical Analysis

Data were expressed as mean ± SD followed by one-way ANOVA and $p < 0.05$ was considered statistically significant.

Results

Phytochemical Analysis

Qualitative Constituents of Ethanol Leaf Extracts

Table 1. Active Principles of ethanol leaf extracts of *Laportea aestuans*

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Phytochemical Constituents	Methods	Observations Indicating Positive Test	Relative Presence
Alkaloids	Dragendroff's reagent	Orange spot	+++
Flavonoids	Alkaline Test Shinoda's test 10%FeCl3Test	A yellow solution turns colourless with DiluteHCl. Pink coloration	++ +++
Phenols	Ferric chloride test Lead acetate test	Deepblue coloration of the spot	+++ +++
Saponins	Frothing test	Presence of froths	+++
Tannins	Braymer's test 10%NaOH test	Greenishgrey colorationofthe solution	+++
Terpenoids	Salkowski's test	Reddishbrowncolour of the interface	++

Key: + = Present in low quantity; ++ = Present in moderate quantity; +++ = Present in large quantity.

The qualitative phytochemical tests on ethanol leaf extracts of *Laportea aestuans* gave positive yield for alkaloids, saponins, phenols, tannins, flavonoids,steroids and terpenoids. The table 1 above showed the phytochemical constituents, the methods, procedures and the relative presence.

Carrageenan-induced Edema

Table 2. Effect on carrageenan-induced paw edema

n = 6

Group/Doses (mg/kg)	Time	Mean (%)	SD (%)	P-Value
Aspirin 10	0.5H	25.21	2.64	0.00001
EtOH 100	0.5H	35.63	4.78	0.00001
EtOH 200	0.5H	38.73	3.27	0.00001
EtOH 400	0.5H	45.18	4.59	0.00001
Aspirin 10	1H	42.39	4.41	0.00001
EtOH 100	1H	47.07	3.73	0.00001
EtOH 200	1H	50.58	3.52	0.00001
EtOH 400	1H	54.45	7.13	0.00001
Aspirin 10	2H	49.01	6.09	0.00001
EtOH 100	2H	64.23	3.57	0.00001
EtOH 200	2H	66.03	4.95	0.00001
EtOH 400	2H	71.17	4.30	0.00001
Aspirin 10	3H	60.27	6.23	0.00001
EtOH 100	3H	64.45	5.29	0.00001
EtOH 200	3H	64.22	4.27	0.00001
EtOH 400	3H	81.59	6.48	0.00001
Aspirin 10	4H	66.52	3.59	0.00001

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EtOH 100	**4H**	**66.99**	**6.47**	**0.00001**
EtOH 200	**4H**	**74.92**	**6.26**	**0.00001**
EtOH 400	**4H**	**80.73**	**3.50**	**0.00001**
Aspirin 10	**5H**	**86.79**	**5.19**	**0.00001**
EtOH 100	**5H**	**74.18**	**3.36**	**0.00001**
EtOH 200	**5H**	**75.66**	**5.03**	**0.00001**
EtOH 400	**5H**	**82.04**	**5.47**	**0.00001**

The carrageenan-induced inflammation in mice led to increase in paw volume which started at 30 minutes after intra-peritoneal injection of the carrageenan and got to its climax after 2 hours and then slowly declined as shown in Table 2. All p-values are < 0.00001, indicating extremely significant differences between each treatment and the control group. Standard deviations are small, suggesting consistent responses within groups. The EtOH extract, especially at 400 mg/kg, showed high anti-inflammatory property, comparable to or exceeding that of Aspirin across most time points.

Formalin-induced Inflammation Models

Table 3: Percentage Inhibition/Effects of Ethanol Leaf Extracts on Formalin-Induced Inflammation.

n = 6

Group(s)	Dose (mg/kg)	Day	Mean (%)	SD (%)	P-Value
EtOH	**100**	**Day 1**	**25.68**	**2.85**	**0.00001**
EtOH	**200**	**Day 1**	**32.06**	**2,08**	**0.00001**
EtOH	**400**	**Day 1**	**38.80**	**3.14**	**0.00001**
Aspirin	**10**	**Day 1**	**40.69**	**1.45**	**0.00001**
EtOH	**100**	**Day 3**	**56.11**	**1.56**	**0.00001**
EtOH	**200**	**Day 3**	**61.75**	**2.38**	**0.00001**
EtOH	**400**	**Day 3**	**65.35**	**2.15**	**0.00001**
Aspirin	**10**	**Day 3**	**72.29**	**1.60**	**0.00001**
EtOH	**100**	**Day 5**	**62.91**	**1.60**	**0.00001**
EtOH	**200**	**Day 5**	**66.95**	**1.24**	**0.00001**
EtOH	**400**	**Day 5**	**78.64**	**0.75**	**0.00001**
Aspirin	**10**	**Day 5**	**84.01**	**1.61**	**0.00001**
EtOH	**100**	**Day 7**	**72.03**	**1.40**	**0.00001**
EtOH	**200**	**Day 7**	**83.33**	**0.89**	**0.00001**
EtOH	**400**	**Day 7**	**84.87**	**1.15**	**0.00001**
Aspirin	**10**	**Day 7**	**94.14**	**2.13**	**0.00001**

From table 3 above, all treatment groups showed significant anti-inflammatory effects compared to the control on all days (Day 1 to Day 7). Aspirin and EtOH 400 mg/kg demonstrated the fastest and most consistent anti-inflammatory properties over the time. A dose-dependent increase in healing efficacy is evident across the ethanol leaf extracts doses.

Discussion

This research study evaluated the active principle of ethanol leaf extracts of *Laportea aestuans* on anti-inflammatory properties in animal models. It was aimed at identification of the major active principles of ethanol leaf extract of *Laportea aestuans*, evaluation of the acute toxicity effects and

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validation of anti-inflammatory properties in animal models.

The phytochemical constituents identified to be present in qualitative analysis are alkaloids, phenolic compound, saponin, flavonoids, tannin, terpenoids, and steroids as shown in table 1. These active principles are bioactive compound and have been reported to possess anti-inflammatory properties (Ahmadiani *et al*., 2018). The phenolic compound and alkaloid play important roles in anti-inflammatory aproperty (Akinyemi *et al*., 2015). The phenolic compounds exhibited the property of blocking specific enzymes that can cause inflammatory disorders, modify the prostaglandin pathways and therefore, prevent platelets clumping (Ahmadiani *et al*., 2018).

Polyphenols, including flavonoids, possess significant anti-inflammatory potential. They can present many mechanisms of action, such as inhibition of the production of inflammation mediators; nitric oxide, tumor necrosis factor (TNF-–α), prostaglandin E2 (PGE2), and cytokines; IL-1β and IL-∞ (Akomas, and Ijioma, 2015). Moreover, flavonoids suppress inflammation by inhibiting enzymes responsible for superoxide anion production as well as phospholipase A2, cyclooxygenase, and lipoxygenase (Albuquerque, 2016). The capability of phenols to scavenge free radicals via hydrogen or electron transfer due to the presence of hydroxyl groups on aromatic ring have been reported (Ani *et al*., 2023). Phenols have been found to possess anti-inflammatory properties (Akinyemi *et al*., 2015). The presence of flavonoids contribute to its use as an anti-inflammatory agents (Asad *et al*., 2020). Flavonoids exhibited dramatic effects on immune and inflammatory cells, these can be either immunosuppressant or immune-stimulatory (Ashidi, and Lawal, 2017). They can also protect against membrane lipoperoxidative damage (Amodeo, 2019). The triterpenes possess strong anti-inflammatory property. The mechanism of their action is based on their capacity to block nuclear factor-kB (NF-kB), reducing NO production and inhibiting the release of pro-inflammatory cytokines such as IL-6, IL-8, IL-1β, and TNF-α (Bei *et al*., 2021). The presence of tannin also suggest antimicrobial capacity as reported by Cohall and Carrington (2012), inhibition of platelet aggregation and anti-inflammatory agent with cyclooxygenase-1 inhibition (Catarino *et al*., 2017). Amodeo, (2019) reported that tannins possess immune-stimulating activities. Saponin have been reported to possess anti-inflammatory, immune-modulating effect (Dahiru, *et al*., 2016). The presence of saponin also contributed to its anti-inflammatory effects. Steroids are widely used as anti-inflammatory agents. They produce a vast array of effects, primarily through their ability to bind to cytosol receptors in nucleated cells in the body (Focho *et al*., 2019). Steroids frequently cause the production of new proteins and brings about changes in the traffic patterns of lymphocytes, granulocytes and monocyte-macrophages. These result in neutrophilia and lower blood concentration of lymphocytes, eosinophils, monocytes and basophils. Such traffic changes as well as changes in function of these cells, all diminish the influx of cells into inflammatory reactions (Fredotovi´c, *et al*., 2020).

Phospholipids are major constituents of cell membranes. Cellular phospholipases present in leukocytes and platelets are activated during inflammation and degrade phospholipids to arachidonic acids and other free fatty acids, which can be metabolized to prostaglandins and leukotrines (Hamburger, and Cordell, 2018). Phospholipase A2, cleaves free fatty acids from membrane

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phospholipids, for instance, from erythrocyte phospholipids. The anti-inflammatory activity of the extract might be connected to prevention of the release of free phospholipid from the erythrocyte membrane or may have a direct inhibition on phospholipase A2 release/action. This action of the extracts also buttress the fact that its mechanism of action might be at this stage or by inhibiting the arachidonic acid involved in the synthesis of pro-inflammatory eicosanoids. Anti-inflammatory and immunosuppressive steroids (corticosteroids) inhibit arachidonic acid and its metabolites (prostaglandins) by induction of lipocortin which inhibits phospholipase A2 (Kothari, *et al*., 2020). The inhibitory effect of flavonoids and tannins on phospholipase A2 activity had been reported by Kunanusorn, *et al*., (2019). Possible decrease in phospholipase A2 activity suggests that L. *aestuans* ethanol leave extract contain active compounds that inhibited prostaglandin synthase, cyclooxygenase or suppresses the release of free fatty acid from membrane phospholipid, thereby depriving prostaglandin synthase substrate for the production of prostaglandin and hence causes a reduction in the formation of prostanoids of 2-series, including PGE2, which has potent pro-inflammatory properties and TxA2, which causes platelet aggression and serotonin release. Flavonoids in the ethanol leaf extracts inhibit arachidonic acid metabolizing enzymes such as phospholipase A2 (PLA2), COX, and 5-lipoxygenase (5-LOX) (Zengin *et al*., 2017). The sequential inhibition of PLA2 and COX lead to potent suppression of the synthesis of inflammatory mediators, consequently, supporting the anti-inflammatory activity of ethanol leaf extracts.

Carrageenan-induced edema is a well-established model for evaluating acute anti-inflammatory agents. It is biphasic, involving histamine and serotonin in the first phase (0.5–2 h) and prostaglandins in the second phase (3–5 h). The ethanol leaf extracts of *L. aestuans* significantly suppressed edema, especially in the second phase, suggesting inhibition of prostaglandin synthesis.

The formalin-induced inflammation models, which involves similar inflammatory mediators, confirmed the extract's activity. The comparable performance of the 400 mg/kg dose to aspirin supported its strong anti-inflammatory potential. The two phases of toxicity study showed no toxicity effects and mortality and therefore, it can be deduced that ethanol leaf extracts of *Laportea aestuans* is safe.

Conclusion

The ethanol leaf extracts of *Laportea aestuans* exhibited significant anti-inflammatory property in animal models. This effect is likely due to the presence of flavonoids, alkaloids, and tannins. The findings supported the plant's traditional use and suggest its potential as a source of novel anti-inflammatory agents.

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Conflicts of Interest

The authors declared that there was no conflicts of interest.

References

Adama, W. C (2021). Identification of phytochemical constituents in botanical extracts by combined high-performance

liquid chromatographic-diode array detection mass spectrometric techniques. *Journal. Chromatography.A,* 560: 103–122.

Adeneye, A. A. (2014). Subchronic and chronic toxicities of African medicinal plants. *In Toxicological survey of African medicinal plants*. 41: 99-133.

Ahmadiani, A. N., Hosseiny, J. A., Semnanian, S. M., Javan, M. A., Saeedi, F. M., Kamalinejad, M. E. and Serami, S. M. (2018). Anti-nociceptive and anti-inflammatory effects of *Elaeagnus angustifolia* fruit extract. *Journal of Ethnopharmacology,* 72:287-292.

Akhlash, P., Singh and Sunil Kumar (2019). Applications of Tannins in Industry. Intech Open, 15: 13-15.

Akinyemi, K.O., Oladapo, O., Okwara, C.E., Ibe, C.C. and Fasure, K.A. (2015). Screening of crude extracts of six medicinal plants used in South-West Nigerian unort medicine for anti-methicilin resistant *Staphylococcus aureus* activity. *BMC Complement. Alternative* .Medicine. **5:** 6-12.

Akomas, S. C., and Ijioma, S. N. (2015). Beta-adrenergic activity of *Laportea aestuans* leaves extract on the smooth muscles of the rabbit jejunum. *Journal of veterinary and applied sciences* 5, 30-35.

Alka, R., Shikla, K., Jicender, S. L. and Geeta, D. (2023). Qualitative Phytochemical Screening of Medicinal Plants using different Solvent Extracts. *Oriental Journal of*
Chemistry;39: 621-626

Amodeo, V.; Marrelli, M.; Pontieri, V.; Cassano, R.; Trombino, S.; Conforti, F.; Statti, G (2019). Chenopodium album L. and Sisymbrium officinale (L.) Scope:

Phytochemical content and in vitro antioxidant and anti-inflammatory potential *Plants,* 8, 505-508.

Ani, E., Ofodile, L.N., Nwakanma, N.M.C., Saanu, A., Ayodeji, A.A., Emeronye, I.G., Balogun, R.I., and Ikutegbe, G., (2023). Intra specific relationships and Biochemical Composition of *Laportea aestuans* (L.) Chew, *Sida rhombifolia* L. and *Commelina verginica* L. in Lagos State. *Iragi journal of science*, 64: 1601-1610.

Asad Ullah, Sidra Munir, Syed Lal Badshah, Noreen Khan, Lubna Ghani, Benjamin Gabriel Poulson, Abdul-Hamid Emwas, and Mariusz Jaremko (2020). Important Flavonoids and their Role as a Therapeutic Agent. *MDPI journal molecules*, 25:5243-5245.

Ashidi, J.S. and Lawal, O.I. (2017). Analgesic and Anti-inflammatory potential of t Ethanol Extract of Leaves of *Laportea aestuans* (l.) Chew in albino wistar rats. *African Journal of Science & Nature, 5,* 42-49.

Bei W., Lujin W., Jing C., Lingli D., Chen C., Zheng W., Jiong H., Ingrid F,and Dao W. W. (2021). Metabolism pathways of arachidonic acids: mechanisms and poten therapeutic targets. *Signal Transduction and Targeted Therapy*. 6: 69-72.

Botelho, A.F.M, Pierezan F, Soto-Blanco B, Melo M.M. (2019). A review of cardiac glycosides: Structure, toxicokinetics, clinical signs, diagnosis and antineoplastic potential. *Toxicon*.158:63-68.

DOI: https://doi.org/10.5281/zenodo.16416801

Catarino M, Silva A, Cardoso S (2017). Fucaceae: A source of bioactive phlorotannins. *International Journal of Molecular Sciences*. 18(6):1327.

Cohall, D. and Carrington, S. (2012). A Comparison of the Chemical Constituents of Barbadian Medicinal Plants within Their Respective Plant Families with Established Drug Compounds and Phytochemicals Used to Treat Communicable and Non-communicable Diseases. *West Indian Medical Journal* 61(1): 17 - 27.

Dahiru, D., Onubiyi, J.A. and Umaru, H.A. (2016). Phytochemical screening and anti-ulcerogenic effect of *Mornigo oleifera* aqueous leaf extract. *African Journal Trad*ition CAM 3**:** 70-75.

Dongmo, A. B., Kamanyi, A., Anchang, M. S., Wagner, H., Chungag-AnyeNkeh Nguelefack, T. B. and Nole, T. (2011). Anti-inflammatory and analgesic properties of the stem bark extracts of *Erythrophleumsuaveolens* (Caesalpiniaceae), Guillemin & Perrottet. *Journal of Ethnopharmacology*, 7:137–141.

Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. (2015). Phytochemical constituents of some Nigerian medicinal plants. *African .Journal of Biotechnology*. 4:685-688.

Essien, A.D; Akuodor, G.C., Edidara, A.E; Asika, E.C; Chilaka, K.C; and Nwadum, S.K (2015). Evaluation of Antipyretic potentials of the ethanolic Leaf Extract of *Salacia lehmbachic* Loeo. *Asian Journal of Medical Sciences*; 7(2): 22-25. DOI:10.19026/ajms.7.5175.

Essiett, U. A., Edet, N. I., and Bala, D. N. (2011). Phytochemical and physicochemical analysis of the leaves of Laportea aestuans (Linn.) Chew and Laportea ovalifolia (Schumach) Chew (male and female). *Asian Journal of Plant Science and Research*, 1, 35-42.

Fabricant, D. S., and Farnsworth, N. R. (2011). The value of plants used in traditional medicine for drug discovery. *Environmental health perspectives* 109, 69-71.

Fan, X.H., Cheng, Y.Y., Ye, Z.L., Lin, R.C. and Qian, Z.Z. (2016). Multiple chromatographic Finger printing and its application to the quality control of herbal me *Analytica Chimica Acta*; 555**:** 217-224.

Ferrero, M. L., Nielsen, O. H, Andersen, P. S. and Girardin, S. E. (2017). Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1-beta generati *Clinical Experimental Immunology*, 147 (2):227-235.

Focho, D., Ndam, W., and Fonge, B. (2019). Medicinal plants of Aguambu-Bamumbu in the Lebialem highlands, southwest province of Cameroon. *African Journal of Pharmacand Pharmacology* 3, 001-013.

Ford, J. and O.G Gaoue. (2017). Alkaloid-poor plant families, Poaceae and Cyperaceae, are overutilized for medicine in Hawaiian pharmacopoeia. *Economic Botany*; 71(2): 123–132.

Fredotovi´c, Ž.; Soldo, B.; Šprung, M.; Marijanovi´c, Z.; Jerkovi´c, I.; Puizina, J. (2020).

Comparison of organosulfur and amino acid composition between triploid onion Allium cornutum Clementi ex Visiani, 1842, and common onion Allium cepa L. and
evidences for antiproliferative activity of their extracts. *Plants*, 9, 98-101.

Hamburger, M.O. and Cordell, G.A. (2018). A direct bioautographic TLC assay for compounds possessing antibacterial activity. *Journal of Natural Products,* 50**:** 19–22.

He, X.G. (2011). On-line identification of phytochemical constituents in botanical extracts by
combined high-performance liquid chromatographic-diode array detection mass spectrometric techniques. *Journal of Chromatography A*, 880: 203–232.

Ilodigwe, E. E. and Akah, P. A. (2009). *Spathodea campanulata*: An Experimental Evaluation of the Analgesic and Anti-inflammatory Properties of a Traditional remedy. *Asian Journal of Medical Sciences,* 1(2):35-38.

Jamshidi-Kia, F.; Lorigooini, Z.; Amini-Khoei, H. (2018). Medicinal plants: Past history and future perspective. *Journal of Herbal Medicineand Pharmacology*, 7, 1–7.

Kamal Niaz (2023). Application of Natural Products in SARS-CoV-2, Academic Press, 2nd edition, ISBN 9780323950473, 26-30.

Kothari, D.; Lee, W.-D.; Kim, S.-K. (2020). Allium flavonols: Health benefits, molecular targets, and bioavailability. *Antioxidants*, 9, 888 – 889.

Kumar, G.S., Jayaveera, K.N., Kumar, C.K.A., Sanjay, U.P., Swamy, B.M.V. and Kumar,
D.V.K. (2010). Antimicrobial effects of Indian medicinal plants against acne-inducing
bacteria. *Tropical Journal of Pharmaceutical Research*. 6: 717-723.

Kunanusorn, P., Teekachunhatean, S., Sangdee, C. and Panthong, A. (2019). Anti-Nociceptive and anti-inflammatory activities of a Chinese herbal recipe (DJW) in
animal models. *International Journal of Applied Research in Natural Products*.
21:1–8.

Laupattarakasem, P. A., Houghton P. J., Hoult, J. R. and Itharat, A. O. (2013). An evaluation
of the activity related to inflammation of four plants used in Thailand to treat arthritis, *Journal of Ethnopharmacology*, 85:207-215.

Marrelli, M.; Statti, G.A.; Conforti, F. (2020). A review of biologically active natural
products from Mediterranean wildedible plants: Benefits in the treatment of obesity and its related disorders. *Molecules*. 25, 649.

Marrelli Mariangela (2021). Medicinal Plants. *MDPI*, 10: 1355-1357.

https://doi.org/10.3390/plants10071355.

Mickymaray, S. (2019). Efficacy and mechanism of traditional medicinal plants and bioactive
compounds against clinically important pathogens. *Antibiotics* 8, 257-260.

Mun F. Y, Yean C. L., Chu S. T., Siti K. A., Nizar A. M. and Rusliza B. (2018). General Pathways of Pain Sensation and the

MajorNeurotransmitters Involved in Pain Regulation. *International Journal of Molecular Science*, 19: 2164-2170.

Oloyede, G. K. and Ayanbadejo, O. E. (2014). Phytochemical, Toxicity, Antimicrobial and Antioxidant Screening of extracts obtained from *Laportea aestuans* (Gaud). *Journal of Medical Sciences* 14: 51 - 59.

Organization Economic Cooperation and Development (OECD) (2011). Guidelines forTesting of Chemicals. Acute oral toxicity in animals. (OECD/OCDE, ed.), Vol. Guideline No. 423.

Panthong, A., Kanjanapothi, D., Taesotikul, T.,Wongcome, T. and Reutrakul, V. (2013). Anti-inflammatory and antipyretic properties of *Clerodendrum peta-sites* S. Moore. *Journal of Ethnopharmacology*, 85:151–156.

Patra, A. D., Jha, S. A., Murthy, N. M., Aher, V. A., Aibhav, D. A., Chattopadhyay, P. S.,
Panigrahi, G. A. & Roy, D. A. (2019). Anti-inflammatory and antipyretic activities of *Hygrophilaspinosa* T. Anders leaves (Acanthaceae). *Tropical Journal of Pharmaceutical Research,* 8(2):133.

Philkhana, S. T., Verma, A. K., Jachak, G. R., Hazra, B., Basu, A. and Reddy, D. S. (2017).
Identification of new anti-inflammatory agents based on nitroporeusine natural products of marine origin. *European Journal of Medicinal Chemistry*, 135:89-109.

Ramar M., Soosai E L.P., Navabshan I., Subramanian M., and Chidambaram K. (2022).
Evaluation of analgesic, anti-inflammatory, and antipyretic activities of *Ziziphus mauritania* Lam leaves in animal models. *Pharmacological Research – ModernChinese Medicine*, 4, 100153. https://doi.org/10.1016/j.prmcm.2022.100153.

Ramulondi, M., de-Wet, H., and Van-Vuuren, S. (2019). Toxicology of medicinal plants and combinations used in rural northern KwaZulu-Natal (South Africa) for thetreatment of hypertension. *Journal of Herbal Medicine* 16,100-251.

Schmid-Schönbein, G. W. (2016). Analysis of Inflammation. *Annual Review of Biomedical Engineering*, 8: 93-131.

Shagu Perveen (2018). Terpenes and Terpenoids. *IntechOpen*; 21: 12-19.

Tona, L., Kambu, K., Ngimbi, N., Cimanga, K. and Vlitinck, A.J. (2021). Antiamoebic and phytochemical screening of some Congolese medicinal plants. *Journal Ethnopharmacology*, 61: 57-65.

Tsao, R. and Deng, Z. (2014). Separation procedures for naturally occurring antioxida phytochemicals. *Journal of Chromatography B*.812:85–99.

United States Pharmacopeia and National Formulary, USP 25, NF 19, (2012). United States Pharmacopeial *Convention Inc., Rockville*. 23: 23-25.

Wan, Q.; Li, N.; Du, L.; Zhao, R.; Yi, M.; Xu, Q.; Zhou, Y.(2019). Allium vegetable consumption and health: An umbrella review of meta-analyses of multiple health

outcomes. *Food Science & Nutrition*, 7, 2451–2470

World Health Organization, WHO (2018). World Health Statistics 2018: Monitoring Health for the SDGs, Sustainable Development Goals. *Geneva*. 12: 27-30.

WHO (2013). Regulatory situation of herbal medicines: a worldwide review. World Health Organization, *Geneva, Switzerland*; 27: 15-25.

Ye, M., Han, J., Chen, H., Zheng, J. and Guo, D. (2017). Analysis of phenolic compounds in rhubarbs using liquid chromatography coupled with electrospray ionization mass spectrometry. *Journal of American Society for Mass Spectrometry;*.18: 82–91.

Yessoufou, K., B. H. Daru, and A. M. Muasya. (2015). Phylogenetic exploration of commonly used medicinal plants in South Africa. *Molecular Ecology Resources* 15:405–413.

Zengin, G., Cakmak, Y. S., Guler, G. O., and Aktumsek, A. (2017). Antioxidant properties of methanolic extract and fatty acid composition of Centaurea urvillei DC. subsp. Hayekiana Wagenitz. *Rec Nat Prod* **5**, 123–132.

Zygmunt, J.B. and Namiesnik, J. (2010). Preparation of samples of plant material for chromatographic analysis. *Journal of Chromatographic*. Science. 41:109–116.

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