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Evaluation of Active Principles of Ethanol Leaf Extracts of Laportea Aestuans for Analgesic Property in Animal Models

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Abstract **Original Research Article**

Pain is an unpleasant significant clinical issues globally, and there is growing interest in plant-derived remedies for safer, alternative analgesics due to hepatotoxic effects of NSAIDs. Laportea aestuans has been traditionally used in African ethnomedicine for pain relief, but its analgesic efficacy and active principles remain underexplored. The objectives of the study are to identify the active principles, assess the toxicity effects and evaluate the ethanol leaf extracts of Laportea aestuans for analgesic property in animal models. Leaves of Laportea aestuans were extracted at 90% ethanol using maceration method, followed by phytochemical screening using Trease and Evans method (2002). Acute toxicity study was assessed in mice with Lorke's method (1983) following the OECD guidelines (2011). Analgesic effects were evaluated in Swiss mice (16-18g) using thermal stimuli models and acetic acid-induced analgesia. Statistical analysis was carried out using mean ± standard deviation followed by one-way ANOVA at the P-value of <0.05 considered to be significant. The active principles identified from the ethanol leaf extracts are terpenoids, flavonoids, alkaloids, phenolic compounds, tannins, saponins and steroids. The LD_{50} at maximum dose of 5000mg/kg, was atoxic to the animal models. The ethanol leaf extracts at the doses of 100, 200, and 400mg/kg, significantly reduced hot plate-induced analgesia and writhing responses, compared to the standard; aspirin (10mg/kg) and negative control (distilled water). Laportea aestuans leaf extracts indicated analgesic property which are significantly related to the active principles. This validated its use and safety in African traditional medicine and suggested potential for development as a plant-derived analgesics.

Keywords: Laportea Aestuans, Analgesic Property, Active Principles, Animal Models, Leaf Extract.

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INTRODUCTION

Pain is a complex physiological response with significant impact on quality of life. Conventional analgesics such as opioids and NSAIDs, though effective, often cause adverse effects including gastrointestinal disturbances, tolerance, and dependence. This has driven interest in plant-based alternatives that offer efficacy with lower toxicity.

Analgesics are pain relieve that are used to treat categories of disease associated with pain ranging from headaches and injuries to arthritis. For instance, anti-inflammatory analgesics reduce the effects of pain and the way nerves perceive it (Ferrero, 2017). Some analgesics are sold as over-the-counter drug while others require prescription. Analgesics eliminate the feeling of pain that is associated with pathologic conditions. Several disease conditions require the use of analgesics thus; it is difficult to state all the situations that require the use of analgesics. Over the years, several medications was used in the treatment of pain (Ahmadiani *et al*., 2018) and herbs are most often used as a result of its' availability, affordability and has less adverse effects; a typical example is *Papaver somniferum;* medicinal plant from which morphine, a prototype of opiate analgesic drug was isolated (Breivik *et al*., 2018).

Pain is an unpleasant sensation which often stands as the only symptom for different stages of ailments (Breivik *et al*., 2018). Conventional analgesics are medications involved in pain treatment starting from mild stage to moderate and severe stage. These medications are saddled with adverse effects such as

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gastrointestinal irritation, tolerance and dependency (Hugo *et al*., 2018). Therefore, there is a need to intensify effort to research with the objective of developing medication with low toxicity profile (Hugo *et al*., 2018).

Fundamental Processes of Pain Mechanisms.

Three main processes that take place in response to unpleasant stimuli can be used to categorize the basic mechanics of pain. According to Mun *et al.* (2018), these are modulation, transmission, and transduction. The nociceptive pathway is involved in the transduction process of pain mechanisms, and it occurs in the following order; stimulus-induced occurrence converts to chemical tissue, chemical and synaptic cleft occurrence converts to electrical one, and synaptic nerve converts electrical occurrence to chemical signals. The next essential step in the pain mechanism after transduction is the transmission mechanism. Neurotransmitters facilitate the transmission of electrical events at the neural circuits by carrying information from the synaptic cleft into the cells of post-synaptic terminal and then to another pre-synaptic cell. Every nociceptive pathway level, such as the primary afferent neuron and brain centers, is subject to up- and down-regulation due to the modulation event (Bei *et al*., 2021). The events began and finished pathway allowed us to experience the negative feeling that the stimulus elicited.

Laportea aestuans (Urticaceae) is commonly known as White Nettle or Tropical Nettle Weed in English. It is herbaceous plant widespread in tropical Africa and the Caribbean nations (Ani *et al*., 2023). The plant is traditionally used to treat various diseases associated with inflammation, pain, fever and infections. Preliminary ethnobotanical surveys and phytochemical analyses suggested the presence of bioactive principles, but the scientific validation of its analgesic potential is limited (Adama, 2021). The study was aimed to evaluate the analgesic property of ethanol leaf extracts of *Laportea aestuans* using standard animal models, identify the active principles and to validate its uses in African ethnomedicine.

MATERIALS AND RESEARCH METHODS

Plant Collection and Authentication

Laportea aestuans leaves were obtained from Abakaliki, Ebonyi State, Nigeria. It was identified by ethno-botanist and authenticated by a taxonomist from the National Institute of Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The research ethics consideration and permission was gotten from Animal Research Ethics Committee of Nnamdi Azikiwe University, Awka, Nigeria, where the research was performed at the Physiology Laboratory of the same institution.

Preparation of Ethanol Leaf Extracts

Laportea aestuans leaves were washed and dried in a room temperature, pulverized and extracted with 90% ethanol using maceration method. The concentration of the filtrate was performed with rotary evaporator and the extracts was stored in refrigerator at 4^{o}C for the experiment.

Trease and Evans (2002) Method of Phytochemical Screening.

The active principle screening test was carried out on lyophilized leaf extracts of *Laportea aestuans* to identify the qualitative presence of saponins, tanins, phenolic compound, alkaloids, flavonoids, steroids and terpenoids according to the standard procedures as described by Kumar, *et al*., (2010).

Different plant-derived bioactive principles have specific functional groups that selectively interact with a particular reagents, to produce visible outcome such as colour changes, precipitate formation, and foam formation which indicate their presence (Akuodor *et al*., 2013). In Trease and Evans phytochemical screening method, ethanol leaf extracts was exposed to specific reagents that interact chemically with targeted classes of phytochemicals.

Experimental Animals

A total of sixty (60) Swiss mice (16-18g) and thirteen (13) Wistar rats weighing 180-200g of both male and female was acquired and kept at Physiology Department Laboratory where the experiment was performed. The animals were kept in good environment throughout the period of the experiment. After seven days of acclimatization to the new surroundings, the animals were fed pelletized animal mash (Premier Feed Mills, Nigeria), given unlimited access to clean water, and fasted the night before the experiment started. The Organization of Economic Cooperation and Development (OECD) (2011) adopted the 425 standards for experimental animals followed by Animal Research Ethics Committee (NAU-AREC) guidelines of Nnamdi Azikiwe University, Awka with reference number NAU/AREC/2024/0117.

Acute Toxicity Study (LD_{50})

The acute toxicity study was carried out using Lorke's method (1983) (Kuma et al., 2010). The lethality dose study was performed in two phases using 13 Wistar rats of both sexes, weighing 180-200g. The first phase consists of group I to III (n =3). The groups were administered with ethanol leaf extracts, orally at the doses of 10, 100, and 1000mg/kg respectively. The animals were observed for physical changes like signs of toxicity and mortality for 4 hours and then 24 hours. Due to the absence of any observable lethality. The second phase involved 4 groups of one rat in each cage and were intra-gastrically given the ethanol leaf extracts in geometrically increased doses; 1600, 2900, and 5000mg/kg respectively. The rats were caged in the similar conditions and observed for 4, 24 and 72 hour respectively for later toxicity effects. The two phases indicated that ethanol leaf extracts of *Laportea aestuans* were atoxic to the animals.

EVALUATION OF ANALGESIC PROPERTY

Hot Plate-induced Method

The ethanol leaf extracts were evaluated using thermal stimuli models in mice. Thirty (30) Swiss mice were grouped into 5 (n = 6). The mice were then left to fast for the whole

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night. Groups 3, 4, and 5 received the extracts orally at dosages (100, 200 and 400 mg/kg respectively). Group 1 (negative control) received distilled water (10ml/kg) and group 2 (positive control) received aspirin (10mg/kg). After an hour, each mouse from the different groups was carefully put on hot plate set at 55±0.5ºC. The time it took mice to leap off the hot plate or lick their paw was recorded. The response was the amount of time it took the mice to leap off the hot plate or lick their paw. In order to prevent tissue injury, the delay time was set at 15 seconds.

The analgesic effect was calculated thus;

Percentage inhibition = $(V_c - V_t / V_c) \times 100$

Where;

Vc = Mean increase in latency time of the control group and

Vt = Mean increase in latency time of the treated groups.

Evaluation of Analgesic Property using Acetic acid-induced analgesia in Animal Models

The Swiss mice was grouped into five (n = 6). Writhing of the animals was initiated with 0.7% aqueous solution of acetic acid through intra-peritoneal route to create pain sensation (Mamun-Or-Rashid, *et al*., 2017) and the number of writhes in 20 minutes was counted. The animals continued to writhes as long as they feel pain and therefore, each writhing was considered as evidence of pain sensation. Group 3 to 5 received extracts of *Laportea aestuans* orally at 100, 200 and 400mg/kg respectively. Group 1 (negative control) was administered with 10 mL/kg; bwt of distilled water and Group 2 (positive control) was administered with aspirin (standard drug); 10mg/kg; bwt. Evaluation of the analgesic property was counted by the number of writhing at different time interval.

Statistical Analysis

The data was calculated and presented as mean ± SD, after a one way ANOVA. The p-value <0.05 was deemed statistically significant.

RESULT

Phytochemical Screening Result

The result indicated the qualitative presence of flavonoids, phenolic compounds, alkaloids, steroids, tanins, saponins, and terpenoids as shown in Table 1 below.

Table 1: The Bioactive Principles of Ethanol Extracts of *Laportea aestuans*

Phytochemical Constituents	Methods	Observations Indicating Positive Test	Relative Presence
Alkaloids	Dragendroff's reagent	Orange spot	+++
Flavonoids	Shinoda's test 10% FeCl3 Test	A yellow solution turns colourless with Dilute HCl. Pink coloration	++
Phenolic compound	Ferric chloride test Lead acetate test	Deep blue coloration of the spot	+++ +++
Saponins	Frothing test	Presence of froths	+++
Tannins	Braymer's test 10% NaOH test	Greenish grey coloration of the solution	+++
Terpenoids	Salkowski's test	Reddish brown colour of the interface	++
Steroids	Chloroform + Acetic acid + Conc. H_2S0_4	Greenish colour	+

+ = low; ++ = moderate; +++ = large

Phytochemical screening tests on lyophilized ethanol leaf extracts of *Laportea aestuans* indicated the qualitative active principles such as phenolic compounds, alkaloids, steroids, saponins, tannins, flavonoids, and terpenoids. The table 1 above showed the bioactive principles that are present in ethanol leaf extracts of L. *aestuans*.

Acute Toxicity

No mortality or signs of toxicity were observed at both low (10mg/kg) and high dose (5000mg/kg) of the ethanol extracts of *Laportua aestuans*, indicating that the extract is relatively safe.

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Analgesic Activity
Hot Plate-induced Test

Table 2: The Effects of Ethanol Leaf Extracts on Hot Plate-induced Analgesia in Mice

n = 6

Group (s)	Dose (mg/kg)	Before (s) Mean ± SD	After (s) Mean ± SD	% Inhibition	P-value
Neg. Ctrl (distilled water)	10	1.35±0.21	1.38±0.25	8.99	-
Positive Ctrl (Aspirin)	10	1.37±0.12	4.15±0.37*	27.04	< 0.0001
EtOH in low Dose	100	1.38±0.21	2.12±0.25	13.81	˜ 0.01
EtOH in Medium Dose	200	1.66±0.23	3.57±0.32*	23.26	< 0.001
EtOH in High Dose	400	1.80±0.30	4.13±0.35*	26.91	< 0.0001

The treated groups indicated a percentage inhibition that can be compared to the standard drug; aspirin and negative control at a dose-dependent manner. The high dose (400mg/kg) of the extracts with a percentage inhibition of 26.91 indicated an increased latency time of 4.13 seconds which is compared with aspirin with percentage inhibition of 27.04 with an increased latency time of 4.15 seconds as shown in Table 2 above. The thermal responses of the animals on administration of the ethanol leaf extracts was dose dependent and inhibited the effects of analgesia.

Acetic Acid-induced Test

Table 3: The Percentage Inhibition of Ethanol Leaf Extracts of *Laportea aestuans* on Induced Analgesia.

n = 6

Test Group(s)	Dose (mg/kg)	Mean ± SD	% Inhibition	P - value
Distilled water	10	-	-	-
Positive Control (Aspirin)	10	17.00±3.83	15.67	< 0.0001
EtOH in High Dose	400	13.00±3.37	11.98	< 0.0001
EtOH in Medium Dose	200	21.00±2.95	19.35	< 0.001
EtOH in Low Dose	100	22.00±4.97	20.28	< 0.0001

The percentage inhibition of ethanol leaf extracts of *Laportea aestuans* on induced analgesia in mice were shown in Table 3 above. The writhing in the treated groups were significantly (P< 0.0001) reduced that can be compared to the standard drug; aspirin. The inhibition rate in the treated groups reached 11.98%, 19.35%, and 20.28%, respectively. Aspirin treatment (10mg/kg) also inhibited the writing at 15.67%. It can be deduced that the ethanol leaf extracts reduced writhing responses significantly.

DISCUSSION

Ethanol leaf extracts of *Laportea aestuans* exhibited potent analgesic property in both peripheral and central pain models (Marrelli, 2021). Acetic acid-induced test indicated of peripheral analgesic effects, typically mediated through inhibition of prostaglandin synthesis (Dahiru *et al*., 2016). The ethanol leaf extracts significantly reduced writhing, suggesting analgesic property.

The hot plate test evaluated central nociceptive pain, where increased latency indicated involvement of opioid-like mechanisms (Cohall and Carrington, 2012). The ethanol leaf extract's dose-dependent activity implies central action, possibly through interaction with pain-modulating neurotransmitter pathways.

The presence of bioactive principles supported this observations. Flavonoids and alkaloids are known to contribute to analgesic effects via antioxidant and receptor-mediated pathways (Edeoga *et al*., 2015). These results align with previous studies on other members of Urticaceae family that indicated analgesic property.

The ethanol leaf extracts of L. *aestuans* have shown effectiveness in the reduction of visceral pain induced by hot plate, and nociceptive pain induced by acetic acid at both peripheral and central nervous system.

CONCLUSION

The research study demonstrated that leaf extracts of *Laportea aestuans* possesses significant analgesic property in animal models. The effects observed are related to qualitative presence of flavonoid and alkaloid content. These findings scientifically validated the traditional use of *Laportea aestuans* for pain relief and supported further studies on its bioactive constituents for potential drug discovery and development.

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