The second geoelectric layer constitutes sandstone having resistivity value that ranges from 2044 – 3970Ωm with thickness values that range from 1.3–5.8 m within the depth range of 2.2 to 7.0 m. The third geoelectric layer has resistivity values that range from 758 – 6077Ωm with thickness value that ranges from 3.6 to 31.9m. This layer is composed of fine-grained sand/ Tar sand. The fourth geo-electric layer was found in VES 2, VES 3 and VES 4 which constitutes sandwith resistivity values that ranges from 994 - 1479 Ωm. Only VES 1 and VES 5 has the fifth geoelectric layer constitutes sand with resistivity values that range from 801 - 1387 Ωm.According to Odunaike *et al.* (2010) and Akinmosin *et al.* (2011) in search for tar sands in Southwestern region, the results obtained in the study area fall within the range and thereby establishing the presence and also in abundance at an average depth of 35.5m.

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ANALGESIC AND ANTI-INFLAMMATORY POTENTIAL OF THE ETHANOL EXTRACT OF LEAVES OF *Laportea aestuans* (L.) CHEW IN ALBINO WISTAR RATS

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ABSTRACT

In traditional medicine, *Laportea aestuans* (*LA*)is used in treatment of several ailments including pain and inflammatory conditions. This study evaluates the analgesic and anti-inflammatory potential of the ethanol extract of the leaves of *LA* using hot–plate induced analgesia and carrageenan–induced acute inflammatory model in Albino Wistar rats (120g-160g). The analgesic activity was assessed by oral administration of the extract doses; 50, 100 and 150 mg/kg bwt to separate groups of rats, 100 mg/kg bwt of ibuprofen (positive control) and 10 mL/kg bwt of distilled water (negative control).0.1mL of 1% carrageenan suspended in distilled water was injected intra-peritonially into the sub-plantar region of the right-hind paw of the rats to induce inflammation. Similar doses were given to test its anti-inflammatory activity but 10 mg/kg of diclofenac sodium was given as the positive control. The results obtained showed significant ($p<0.05$) dose -dependent difference among the groups. 150 mg/kg of the extract gave promising analgesic and anti-inflammatory effect which could be relatively compared with the control. LA reduced the activity of the enzymes supporting inflammation; a stable oedema formation was experienced at the second hour which could signify maximum level of carrageenan activity while a decline in oedema formation set in at the third hour up to the twenty-fourth hour. Phytochemical screening showed diverse secondary metabolites which could account for its wide therapeutic spectrum.
These results justify the rational use of the plant in local management of pain and inflammation.

Keywords: *Laportea aestuans (LA),* Analgesic, anti-inflammatory activities, Carrageenan, Ibuprofen,Diclofenacsodium

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INTRODUCTION

Inflammation is a protective response of mammalian tissues against diverse stimuli, giving rise to a series of complex events which are facilitated by a number of inflammatory mediators like the prostaglandins, prostacyclin and leukoterines which persuade, uphold and exaggerate many associated disorders (Ricciotti and Fitzgerald, 2011). It is key to pathophysiological processes of cancer, stroke, arthrithis, neurodegenerative and cardiovascular disease (Coussens and Werb,2002; Gil, 2002). It is characterised by pain, swelling, redness oedema and heat (Mantovani. 2010). Pain affects the proper functioning of the body such that when a part of the body is injured, all other parts are affected (Breivik et al., 2008). It is a complex biological and localized response of the vascular tissues to unwanted agents or toxins released in the body (Laupattarakasem et al., 2003; Schmid-Schönbein, 2006; Ferrero et al., 2007) giving a feverish condition as one of its symptoms (Tomlinson et al., 1994).
Uncontrolled inflammation is very detrimental to tissues, as such, there are increasing drive to explore natural agents even from marine environment to search for novel anti-inflammatory agents. One such is the recent identification of nitrosporeusine A and B, which are marine natural products that

reduced levels of nitric oxide (NO), reactive oxygen species (ROS) and pro-inflammatory cytokines (Philkhana et al., 2017).

Non-steroidal anti-inflammatory drugs (NSAIDs) are the frontline drugs used to reduce the noxious events associated with inflammation. However, these drugs exhibit devastating adverse events ranging from gastric irritation and ulcers to hepatotoxicity and renal failure on chronic administration (Kunanusorn et al., 2009). These negatives of the NSAIDs can be by-passed or lessened by substituting them with relatively safer and efficient plant drugs (Khan et al., 2011). Moreover, the limitations of the drugs available for treatment of chronic inflammatory conditions like arthritis, has driven the search for new natural products from plant sources (Khan et al., 2013). In search for newer plant medicines with minimal or no side effects. An annual herbaceous plant, Laportea aestuans (synonyms Urtica aestuans, Fleurya aestuans, English names; stinging nettle, West Indian wood nettle, Yoruba name; 'Fuafua', family; Urticaceae)which has stems that are one and a half long, slightly woody at base, densely covered with stinging hairs up to 1mm long and soft glandular hairs is considered in this study.

Fig 1: Laportea aestuans(L) Chew

MATERIALS AND METHODS

Plant collection and identification

The fresh leaves of *L. aestuans* were collected at the mini-campus of Olabisi Onabanjo University, Ago-Iwoye, Ogun State, identified and authenticated at the taxonomy section of the Forestry Research Institute of Nigeria (FRIN), Oyo State and ELIKAF herbarium of the Department of Plant Science, Olabisi Onabanjo University, Ago-Iwoye with Voucher number: ELK234012.

Preparation of extract

The dried plant of *L. aestuans* was ground with an electric blender. The ground plant, 0.31 kg was macerated in 5 L ethanol for a period of one week. The resulting extract was filtered and the filtrate was concentrated to dryness using a water bath at 50°C. After determination of percentage yield, the extract was stored in the refrigerator (4°C) until needed for analysis.

Experimental animals

A total of sixty (60) adult Wistar rats of both sexes were purchased at the University of Ibadan, Oyo State. The weight of the rats ranges from 120-160 g. The animals were housed in clean cages of three animals in a well ventilated room with suitable temperature and relative humidity. They were allowed to acclimatize with the new environment for 2 weeks, fed with pelletized feed, allowed clean water *ad libitum* and fasted overnight before the experiment commenced.

Chemical reagents and reference drugs

Absolute Ethanol, Carrageenan, Diclofenac sodium (SWIPHA, London), Ibuprofen (reference drug), Hydrochloric acid (HCl), Distilled water, Ammonia solution (BDH, Frankfurt), Dragendorff reagents, Chloroform, Lead acetate solution, concentrated Sulphuric acid (H_2SO_4), Glacial acetic acid, and Ferric chloride solution ($FeCl_3$). Absolute ethanol (BDH, Frankfurt).

Phytochemical screening of the plant

Phytochemical screening was carried out on the powdered sample of the leaves of the plant to test for alkaloids, anthraquinone glycosides, cardiac glycosides, tannins, saponins, flavonoids, phenols and cardenolides according to standard procedures (Sofowora, 1993; Harborne, 1973; Trease and Evans, 2002).

Test for alkaloids

One gramme (1.00 g) of the powdered sample was extracted with 10 mL of 10% HCl on a water bath for five minutes. The extract was filtered and allowed to cool adjusting the pH to about 6-7 by adding 10% ammonia and using litmus paper. 5 mL of the filtrate was taken into separate test tubes and small quantity each of Wagner's, Mayer's and Dragendorff reagents were added and observed. The presence of turbidity or precipitation indicates presence of alkaloids (Sofowora, 1993).

Test for anthraquinone glycosides

One gramme (1.00 g) of powdered sample was extracted with 2 mL of 10% HCl by boiling for five minutes and filtered while still hot, then allowed to cool. The filtrate was partitioned with equal volume (aliquot) of chloroform and shaken gently. The chloroform layer (lower layer) was transferred to a clean test tube and aliquot 10% ammonia solution was added and shaken gently. The presence of delicate rose-pink layer on the test solution indicates the presence of anthraquinones glycosides (Sofowora, 1993).

Tests for cardiac glycosides

One gramme (1.00 g) of powdered sample was extracted with 10 mL of ethanol for five minutes on a steam bath and filtered. 2-3 drops of lead acetate was added to the filtrate solution, shaken gently and filtered. To the filtrate, 2 mL of chloroform was added and 1mL concentrated H_2S0_4 was carefully added to form a lower layer. A reddish-brown colour at interface was observed for cardiac glycosides (Sofowora, 1993).

One gramme (1.00 g) of powdered sample was extracted with water and added with 2ml of glacial acetic acid containing a drop of Ferric chloride solution. 1mL of concentrated H_2SO_4 was gently added to form an under layer. A brown or purple or reddish-brown ring formed at the interface and green colour in the acetic acid layer indicates cardiac glycosides and cardenolides respectively present (Ajaiyeoba, 2002).

Tests for flavonoids

One gramme (1.00 g) of the powdered sample was added to 10 mL of ethanol and 3 drops of $FeCl_3$ solution was added. A dark green colour indicates the presence of flavonoids.

One gramme (1.00 g) of powdered of sample was extracted with ethyl acetate and heated for 3 minutes. The residue was treated 10% NH_3. A yellow coloration indicates the presence of flavonoids.

Test for Tannins

One gramme (1.00 g) of the powdered sample was decocted with 10 mL of distilled water by boiling for 10 minutes, filtered while hot and allowed to cool. 0.1% Ferric chloride reagent was added to the filtrate. A blue-black, green or blue green precipitate indicates the presence of tannins (Sofowora, 1993).

Tests for saponins

Frothing test

One gramme (1.00 g) of powdered sample was transferred into a test tube containing 10 mL of distilled water, boiled for five minutes and then filtered. The filtrate was shaken vigorously and observed. The presence of froths indicates the presence of saponins (Sofowora, 1993).

Emulsion test

Three drops of olive oil was added to the frothing and shaken vigorously, the formation of emulsions indicates the presence of saponins (Sofowora, 1993).

Test for phenols

One gramme (1.00 g) of powdered of sample was added to 10 mL of ethanol and 3 drops of phenol solution was added. A dark green colour observed indicates the presence of phenol (Sofowora, 1993).

Administration of extract

Evaluation of analgesic activity

Hot plate-induced analgesia was used (Ilodigwe, & Akah, 2009). The total number of 30 rats were fasted overnight and divided into five groups of 6 rats per groups. The extract (50, 100 and 150 mg/kg bwt) was administered orally into group 1-3 respectively. Group 4 received oral administration of distilled water (10 ml/kg bwt) as negative control while group 5 received oral administration of ibuprofen (100 mg/kg bwt) as positive control. After about one hour, each rat was gently placed on a hot plate maintained at 55±0.5°C and the time required by the rat to lick the paw or jump was taken as the response. The cut off time or latency response was 15 seconds to avoid tissue damage.

The percentage inhibition was calculated using the formula:

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

V_c and V_t represents mean increase in latency time of the control and treated groups.

Evaluation of anti-inflammatory activity

The carageenan-induced acute inflammation model was employed (Winter *et al*., 1962). Total number of thirty (30) rats were fasted overnight and divided into 5groups of 6 rats each. The extract (50, 100 and 150 mg/kg bwt) was administered orally to groups 1-3 respectively. Group 4 received oral administration of distilled water (10 ml/kg) as negative control while group 5 received oral administration of diclofenac sodium (10 mg/kg) as positive control. One hour later, 0.1mL of freshly prepared 1% carageenan suspended in distilled water was injected into the sub-plantar region of the right hind paw of the rats. Paw measurements were taken before (initial paw volume) and after the injection of carageenan at 30mins, 1, 2,3,4,5 and 24 hours using a vernier calliper. The results were expressed as percentage inhibition in relation to the control group.

Percentage inhibition = $(1-V_t / V_c) \times 100$

Where V_t and V_c represent the mean change in paw size of the treated rats and control groups respectively.

Statistical analysis

The values gotten from the experiment were expressed as mean ± standard error mean (SEM). Numerical data were analysed for homogeneity of variance using Bartlett's test. The data were then analysed using one-way ANOVA to determine whether results in a particular group is significantly different from those in the corresponding control groups. The analysis of variance was followed by Tukey post hoc for intergroup comparisons. $P<0.05$ were considered as significant.

RESULTS

The preliminary phytochemical screening of the ethanol extract of *L. aestuans* (Table 1) showed the plant is rich in saponin. Flavonoid, phenols and tannins are equally present, with alkaloids in trace amount. Other constituents like cardenolides, cardiac glycoside and anthraquinones were absent.

Effect on hot plate analgesia

A significant ($p<0.05$) and dose-dependent elevation of the treatment reaction time to thermal pain was evident in the extract-treated animals. The effect of the extract (150mg/kg) was comparable to that produced by 100 mg/kg of ibuprofen (standard drug) as displayed in Table 2. Highest percentage of the inhibitory effect of the extract(33.3%) was observed at a dose of 150 mg/kg. The Ibuprofen was more active with percentage inhibition of 53.47%. at a dose of 100 mg/kg (67.15%).

Carrageeenan-induced oedema

The effect of *L. aestuans* ethanol extract on carrageenan-induced oedema is shown in Table 3. The extract produced a dose-dependent inhibition of carrageenan-induced oedema. The percentage inhibition due to the extract at 150 mg/kg after induction of inflammation is comparable with that of diclofenac sodium 10 mg/kg after 4h of treatment (50.40-52.03% inhibition). No noticeable reduction in inflammation was recorded for the extract until 1hr after treatment. Meanwhile, the extract at 150 mg/kg and the diclofenac sodium already showed mild inhibition after 30 minutes of treatment 1.29 and 3.98% respectively.

Table 1: Phytochemical screening of the leaves of *L. aestuans*

Secondary metabolites	Inference
Alkaloids	+
Cardenolides	–
Cardiac glycosides	-
Anthraquinone glycosides	-
Flavonoids	++
Phenols	++
Tannins	++
Saponins	+++

Note: Trace = +, present = ++, Abundantly present = +++, absent = -

Table 2: Analgesic activity of *L. aestuans* on thermal pain using hot–plate method

Treatment dose (mg/kg bwt)	Mean latency (time spent on the hot plate in seconds±standard error mean (SEM)		
	Pre-treatment (sec)	Post- treatment (sec)	% Pain inhibition
Distilled water (10 ml)	0.48±0.16	0.96±0.33	-
Ibuprofen (100 mg/kg)	0.60±0.03	1.29±0.15	53.47
ELA 50	0.60±0.02	0.81±0.06	26.17
ELA 100	0.86±0.18	1.19±0.18	27.73
ELA 150	1.02±0.20	1.53±0.29	33.33

Note: ELA = extract of *L. aestuans*. Percentage inhibition produced by the extract at 150mg/kg was 33.33% and the standard drug (ibuprofen); 53.47%. These percentages revealed the potent efficacy of the extract and the standard drug in inhibiting pain. All values are expressed as mean latency or cut-off times in second ± standard error mean (SEM). This result shows that the extract is dose dependent in pain reduction

Table 3: Anti-inflammatory activity of L.aestuans using carragenan induced oedema methodz

T.D (mg/kg bwt)	Mean paw size (cm) at time interval (hour) ± standard error mean							
	I.M	0.5hr	1hr	2hrs	3hrs	4hrs	5hrs	24hrs
Distilled water (10 mL)	0.59±0.02	0.77±0.02	0.81±0.04	0.82±0.06	0.84 ±0.07	0.96 ±0.02	0.96±0.02	1.23±0.09
Diclofenac solution (10mg/kg)	0.58±0.01	0.74±0.01 (3.89)	0.71±0.01 (12.34)	0.67±0.03 (17.48)	0.63±0.02 (25.00)	0.60±0.02 (37.50)	0.59±0.01 (38.54)	0.59±0.01 (52.03)
ELA 50	0.59±0.01	0.77±0.01 (0.00)	0.78±0.01 (3.70)	0.76±0.01 (7.31)	0.75±0.02 (10.71)	0.75±0.01 (21.87)	0.72±0.01 (25.00)	0.68±0.01 (44.71)
ELA 100	0.60±0.01	0.77±0.01 (0.00)	0.77±0.02 (4.93)	0.76±0.01 (7.31)	0.75±0.02 (10.71)	0.71±0.01 (26.04)	0.68±0.03 (29.16)	0.65±0.01 (47.15)
ELA 150	0.61±0.01	0.76±0.02 (1.29)	0.75±0.02 (7.40)	0.75±0.01 (7.40)	0.74±0.01 (11.90)	0.71±0.01 (26.04)	0.65±0.01 (32.29)	0.61±0.01 (50.40)

Note: T.D = treatment dose, I.M= initial paw measurement and ELA = extract of *L. aestuans.* All values are expressed as mean paw size in centimetres (cm)±standard error mean (SEM) values in the parenthesis are percentage inhibition of oedema calculated relative to control. The extract produces a dose dependent inhibition of oedema induced by carrageenan.

DISCUSSION

In spite of the extensive research to ensure that synthetic analgesic and anti-inflammatory drugs exhibit no side effects, they are still detected to have one or more unfavourable effects. A strategic approach is therefore needed to screen as many plants as possible for their analgesic and anti-inflammatory activity since they exhibit less side effects compared to synthetic drugs.

The phytochemical screening of the crude plant extract of *L.aeustuans* showed the presence of alkaloids, flavonoids, phenols, tannins and saponins which might be responsible for the observed analgesic and anti-inflammatory activity of the plant. It has been reported that this class of metabolites (catechin, catechin derivatives, proanthocyanidin) have potential anti-nociceptive and anti-inflammatory effects (Wagner and Lacaille-Dubois, 1995; Calixto *et al*., 2000; Dongmo *et al*., 2001).

Analgesic and anti-inflammatory effects have been reported with flavonoids and tannins (Ahmadiani *et al*., 1998; Ahmadiani *et al*., 2000), alkaloids (Reanmongkol *et al*., 2005), saponins (Hikino *et al*., 1984; Choi *et al*., 2005). Some flavonoids have strong inhibitory activity against several enzymes including those that contribute to pain and inflammation (Middleton, 1998; Manthey *et al*., 2001). Therefore, the significant analgesic and anti-inflammatory activity of the extract could be due to the presence of these phytochemical constituents.

Hot-plate method is the means by which potent and centrally analgesics are determined (Parkhouse and Pleuvry, 1979; Prado *et al*., 1990). The control negative rats that only received distilled water could not bear the pain induced by the hot–plate, the mean time taken to lick the paw or jump off the plate was 0.96 second. Considering the results of the extract doses given to the test rats; 50 mg/kg gave a low percentage inhibition value of 26.17% while 150 mg/kg of the extract produced significant increase in the ability of the rats to withstand pain by giving a percentage inhibition of 33.33%. This value (33.33%) may not be relatively compared with the standard drug (ibuprofen) which gave 53.47%. However, considering the fact that the ethanol extract is a mixture of various metabolites, the observed 33.33% inhibition will suggest the suitability of the plant for further purification with a view to isolating single chemical entities which could be responsible for the analgesic potential.

Although, it is dose-dependent. These results suggest that the ethanol extract of *L. aestuans* possess moderate inhibitory effects on the release of histamine, serotonin, bradykinin, prostaglandin and leukotriene which are the main mediators of inflammation, pain and fever (Vergne *et al*., 2000). Again, it confirms the ethnomedicinal report on the plant as (Boye and Ampofo, 1990; Nadine, 2004).

Carrageenan induced paw oedema is suitable for evaluating anti-inflammatory drugs and it has been used to check anti-oedematous effects of natural products (Panthong *et al*., 2003). The mean paw volumes of each rat were taken at 30 minutes and every hour up to 24 hours. Since carrageenan induced paw oedema is a two-phase mechanism (Vinegar, 1969; Patra *et al*., 2009); histamine, serotonin and kinins were the inflammatory mediators secreted at the first one hour of carrageenan injection while prostaglandins and lysosome enzymes were secreted at the second hour. A stable oedema formation was experienced at the second hour which could signify the maximum level of carrageenan activity while a decline in oedema formation began to set in at the third hour up to the twenty-fourth hour showing the activity of the ethanol extract of *L. aestuans* in inhibiting oedema.

The action of the ethanol extract of *L. aestuans* in inhibiting oedemais dose-dependent. At 50 mg/kg, the activity of the extract was not really pronounced, although, the swollen paw reduced with time while at 150 mg/kg, the rate of reduction was so visible from the 3rd hour (which gave a mean paw value of 0.74cm) to 24 hours (which gave a low mean paw size of 0.61cm). The dose of 50 mg/kg was not enough to block the release of inflammatory components but to a larger extent 150 mg/kg was able to do so. This pattern is compares with the effect of ethanol extract of *Elytraria marginata* (Ashidi *et al*., 2015).

Considering the inhibitory percentages, the dose of the extract at 150 mg/kg gave the highest value of 50.40% at 24hrs compared to ibruprofen which gave 52.03% at 24hrs. Comparing the difference between these inhibitory values shows that the ethanol extract of *L. aestuans* is effective.

This study provides some justification for the traditional use of *L. aestuans* in treating ailments present in pain and inflammatory conditions. Further studies that involve the purification of the chemical constituents of this plant and the determination of their mechanism of action should be carried out in order to develop a strong analgesic and anti-inflammatory agent with little or no side effects. This study provides some justification for its use as suggested in folklore medicines.

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Conflicts of interest

The authors have none to declare.

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