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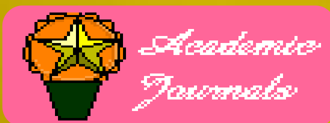

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African Journal of Pharmacy and Pharmacology

Full Length Research Paper

In vivo anti-inflammatory and *in vitro* antioxidant properties of *Hilleria latifolia* and *Laportea ovalifolia*

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Inflammation which is a normal phenomenon involved in healing of injurious tissue can be complicated with reactive oxygen resulting in chronic inflammatory state and subsequent tissue damage. Since there are reports on severe side effects associated with the use of synthetic anti-inflammatory medications, there is the need to search for new medications with milder side effects. The study, therefore, investigated the antioxidant and anti-inflammatory properties of methanol extracts of *Hilleria latifolia* root (HLRE) and *Laportea ovalifolia* leaf (LOLE). The DPPH free radical scavenging, total phenolic content and phosphomolybdenum antioxidant assays were used to assess the *in vitro* antioxidant activity, while the carrageenan-induced foot oedema model in rats was employed to investigate the acute anti-inflammatory activity of the extracts. LOLE and HLRE were able to scavenge 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) free radicals with IC_{50} values of 130.8±0.9 and 233.5±0.5 µg/mL, total phenol content of 56.75±0.3220 and 91.32 ±4.258 mg TAE/g extract whereas total antioxidant capacity of 337.6±6.961 and 408.0±18.70 mg α-TE/g extract, respectively. The extracts at 100 and 300 mg/kg significantly (p<0.001) reduced the induced oedema when administered prophylactically and therapeutically. In conclusion, the methanol extracts of *H. latifolia* and *L. ovalifolia* exhibited *in vitro* antioxidant and *in vivo* anti-inflammatory properties.

Key words: *Hilleria latifolia, Laportea ovalifolia,* inflammation, anti-inflammatory, antioxidant.

INTRODUCTION

Inflammation is a complex natural mechanism used by vascular tissue for regeneration when injured or exposed to dangerous stimuli like pathogens or infections and irritants. It is a defensive action taken by an organism to remove injurious stimuli, initiate healing process and generate new tissues to replace impaired ones (Schmid-Schönbein, 2006; Singh et al., 2008). Typical signs that are cardinal to inflammation are pain, redness, swelling, heat and loss of function (Kumar et al., 2010; Rock and Kono, 2008; Shailasree et al., 2012). An organism's

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ability to mount an inflammatory response is very crucial for its survival when injured or when infected with pathogens and other harmful stimuli.

Though the inflammatory response is a protective mechanism on its own, it may lead to serious tissue destruction resulting in chronic inflammation when implicated with over production of reactive oxygen species (ROS). These ROS are constantly produced in the body of humans through the process of aerobic respiration and from other external sources (Narayanaswamy and Balakrishnan, 2011) and are acted upon and detoxified by endogenous enzyme called antioxidants. In the presence of excess generation of ROS and insufficient antioxidant defense, oxidative stress sets in and may complicate a lot of diseases like diabetes mellitus, cancer, atherosclerosis, hypertension, ocular diseases, haematological diseases, pulmonary diseases, neurological diseases as well as inflammatory diseases (Cui et al., 2004).

However, key orthodox anti-inflammatory medications though effective, have been reported to be associated with severe side effects (Graham, 2006). This has necessitated the search for new and effective antioxidant and anti-inflammatory medications with milder side effects. The search is ongoing and can never be accomplished without looking out for medicinal plants with such properties, since plants have been known to be rich sources of therapeutic agents (Cragg and Newman, 2013). Phenolic compounds and flavonoids widely distributed in plants have been reported to exert numerous biological activities such as anti-inflammatory activity and have also been known as the main antioxidant compounds of fruits and vegetables (Wu and Ng, 2008). *Hilleria latifolia* (Lam.) H. Walt (Phytolaccaceae) is locally known in Ghana as 'Avegboma' and 'Anafranaku' by the Ewes and Asantes, respectively. It is a perennial herb (30 to 120 cm high) with long petiole of 3 to 7 cm high and pink or white flowers. It has ovate-elliptic leaves with numerous short hair-like structures covering the lower surface (Dokosi, 1998; Mshana, 2000). The leaves are used in Ghana for the management of rheumatism, boils and wounds (Agyare et al., 2009). The leaves, added to those of *Piper guineense*, are used to treat general oedema. In Congo, the leaves are employed to treat some skin diseases (Dokosi, 1998; Mshana, 2000). Woode et al. (2011) reported on the presence of phytochemical constituents such as saponins, tannins, glycosides, steroids, terpenoids, flavonoids and alkaloids in the aerial parts of the plant. In another report, phytochemical screening of methanolic fraction of *H. latifolia* revealed the presence of glycosides, coumarins and reducing sugars, as well as small amount of triterpens and sterols. However, saponins, tannins, flavonoids and alkaloids were absent (Assob et al., 2011). The leaf extract of *H. latifolia* has been reported to have anxiolytic and antidepressant-like effects, antimicrobial, antioxidant, anti-nociceptive and some neurobehavioral properties (Abotsi et al., 2012; Assob et al., 2011; Woode et al., 2011; Woode and Abotsi, 2011). The leaf extract has also been found to be able to modify the activity of some selected antibiotics by either enhancing or reducing their activities (Dapaah et al., 2016).

Laportea ovalifolia (Schumach.) Chew (Urticaceae) is known by the Asantes in Ghana as 'akyekyenwonsa', 'abrewa nom taa' or 'Kumasi otuo'. It is a herbaceous weed more often creeping than erect and densely covered with stinging hairs. It has cylindrical stem of greenish or sometimes reddish to brownish in colour (Chew, 1969).There are two varieties of *L. ovalifolia* that is, male and female. These two varieties are related but are different in structure. *L. ovalifolia* (male) has big leaves and (female) possess small leaves (Essiett et al., 2011). The leaves of *L. ovalifolia* are used to heal wounds (Agyare et al., 2009), fruits are used as a poison antidote and the roots boiled in water is taken to prevent excessive menstrual bleeding (Sofowora 1996; Bouch, 2004). Phytochemical screening of the leaf extract of *L. ovalifolia* showed the presence of saponins, tannins, flavonoids, phlobatanins and cardiac glycosides. Anthraquinone was however absent (Essiett et al., 2011). *L. ovalifolia* has been reported to possess antimicrobial, anti-hyperglycemic activity and it is also effective in reducing oxidative stress in diabetes (Iffen and Usoro, 2010; Okwulehie and Akanwa, 2013). It also has anti-diabetic and hypolipidemic effects in alloxan-induced diabetic rats (Momo et al., 2006). Methanol leaf extract of *L. ovalifolia* has been found to exhibit antibiotic resistance modifying activity (Dapaah et al., 2016). The aim of study is to investigate the antioxidant and *in vivo* anti-inflammatory properties of methanol extracts of *H. latifolia* root (HLRE) and *L. ovalifolia* leaf (LOLE).

MATERIALS AND METHODS

Plant collection

Fresh leaves of *L. ovalifolia* and leaves and roots of *H. latifolia* were collected from Aburi (longitude 0.1729°W and latitude 5.8512°N) in the Eastern region of Ghana in February, 2014. The plants were authenticated by Dr. Alex Asase of the Department of Botany, University of Ghana, and voucher specimen AA 71 and AA 63, respectively deposited in the Ghana Herbarium, Department of Botany, University of Ghana, Legon, Accra, Ghana

Plant extraction

The plant parts collected were washed thoroughly under running tap-water and dried under shade at a temperature of 25 to 28°C for two weeks, after which they were pulverized into coarse powder using the laboratory milling machine (Christy and Norris, Chelmsford, England). Eight hundred grams (800 g) each of the powdered plant materials were soaked in 2.5 L of 70% v/v methanol and extracted with the aid of ultra-turrax (T 25 Janke and Kunkel, Labortenik, Germany) under ice-cooling at a speed of 24000 rpm for 3 to 5 min, and then filtered using a laboratory sieve (Retsch,

Haan, Germany) of mesh number 200 with aperture of 75 μm and Whatmann filter paper Number 1. The filtrates were concentrated with the rotary evaporator (Rotavapor BÜCHI R-200 with heating bath B-490, Büchi, Konstanz, Germany) at 40°C under reduced pressure and allowed to dry in the hot air oven (Gallenkamp, London, UK) at 40°C and then stored in air tight containers at 4 to 8°C in a refrigerator. The yields of the extracts relative to the dry powder used were recorded as 11.29 and 7.50% w/w related to the dried material, respectively.

Determination of antioxidant activity

Three different *in vitro* assays were employed to assess the antioxidant activity of the extracts. These included DPPH free radical scavenging, total phenolic content and phosphomolybdenum antioxidant assays.

DPPH free radical scavenging assay

Different concentrations (15.6 to 1000 μg/mL) of leaf extract of *L. ovalifolia* (LOLE) and root extract of *H. latifolia* (HLRE) and reference antioxidant (α-tocopherol) (Sigma-Aldrich, Taufkirchen, Germany) were prepared in methanol. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) (Sigma-Aldrich, Taufkirchen, Germany) solution (20 μg/mL) was prepared with methanol in the dark. Three milliliters of DPPH solution was added to 1 mL each of the different concentrations of LOLE, HLRE and α-tocopherol, followed by incubation in a dark place for 30 min. Control was prepared by adding 3 mL of DPPH solution to 1 mL methanol and treated in the same way as the test samples. Absorbance of excess DPPH was measured at a wavelength of 517 nm (Braca et al., 2001; Susanti et al., 2007). The experiment was performed in triplicates. The percentage scavenging activity was calculated using the equation below:

% scavenging = [(Absorbance control - Absorbance test)/ Absorbance control] × 100

Total phenolic content

The method described by Škerget et al. (2005) involving the use of Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, Missouri, USA) was employed. LOLE and HLRE (0.5 to 10 mg/mL) and standard drug (tannic acid) (Merck BDH, Poole, UK) with concentration range of 0.0156 to 1 mg/mL, were prepared. A volume of 0.1 mL Folin-Ciocalteu reagent was added to 0.5 mL each of the different concentrations of LOLE, HLRE and tannic acid solution, followed by the addition of 2.5 mL 2% sodium carbonate (Sigma-Aldrich, St. Louis, Missouri, USA). The mixtures were incubated for 20 min at room temperature and absorbance read at 760 nm. The experiment was performed in triplicates (independent). A blank was prepared by adding all the reagents with the exception of extracts/standard drug and treated likewise. The total phenolic content was expressed as milligram tannic acid equivalent (TAE) per gram of extract.

Total antioxidant capacity (Phosphomolybdenum antioxidant assay)

The method described by Prieto et al. (1999) was used to determine the ability of LOLE and HLRE to reduce Mo-VI to Mo-V with subsequent formation of green phosphate-molybdate complex in an acidic pH condition. LOLE, HLRE (0.5 to 10 mg/mL) and reference antioxidant (α-tocopherol) ranging from 0.008 to 0.03 mg/mL were prepared. To 1 mL each of the different concentrations of LOLE, HLRE and α-tocopherol, 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM disodium phosphate and 4 mM ammonium molybdate) (Sigma Aldrich, St. Louis, Missouri, USA) was added and incubated at 95°C for 90 min. A blank (all reagents without extracts/standard drug) was treated in the same manner. The mixture was allowed to cool and absorbance was read at 695 nm.

Determination of anti-inflammatory activity of extracts

The acute inflammation model was employed to examine the anti-inflammatory activity of the extracts. Prophylactic determination (drugs given 1 h before inducing the oedema) was made; therapeutic determination (drugs administered 1 h post oedema induction) was also carried out to evaluate the extent of the extracts' anti-inflammatory effect, since not all drugs or agents that exhibit anti-inflammatory activity when administered prophylactically can do same when administered therapeutically. For instance, as observed with cyclosporine (Kaibara et al., 1983).

Experimental animals

Sprague-Dawley male rats (150 to 200 g) were obtained from the animal house of the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, and kept in stainless steel cages with soft wood shavings as bedding. They were maintained under standard environmental conditions of temperature (30±2°C) and adequate humidity, with a twelve hour cycle of light and darkness. The animals were fed with standard pellet diet and provided with water *ad libitum*.

Ethical approval

The experiments were conducted in accordance with accepted principles for laboratory animal use and care (EU directive of 1986:86/609/EEC) and approval from the Animal Ethical Committee (FPPS-AEC/CA01/13), Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Acute anti-inflammatory activity

The carrageenan induced foot oedema model in rats as described by Winter et al. (1962) was employed to assess the extracts' ability to inhibit/reduce the induced paw swelling. The rats were weighed and assigned randomly into groups consisting of four rats each. The initial paw thickness of the rats were measured using an electronic caliper (Z22855, Milomex Ltd, Bedfordshire, UK) after which reference drug, 100 mg/kg aspirin, (Sigma-Aldrich, St. Louis, Missouri, USA) and extracts (30, 100 and 300 mg/kg), prepared in sterile distilled water, administered orally to the respective groups, with the control group given 0.5 mL sterile distilled water. After an hour of drug administration, oedema was induced by injecting 0.1 ml of 1% w/v carrageenan (Sigma-Aldrich, St. Louis, Missouri, USA) solution (in sterile distilled water) into the sub-plantar tissue of the right foot pads of the rats. paw thickness were again measured at an hourly interval for 6 h. In the therapeutic experimental protocol, eodema was induced and measured 1 h after administering the extracts and reference drug. Inhibition of inflammation was calculated using the relation;

$$\text{\% change in paw thickness} = 100 \text{ x } \left[\frac{(T_f - T_i)}{T_i}\right]$$

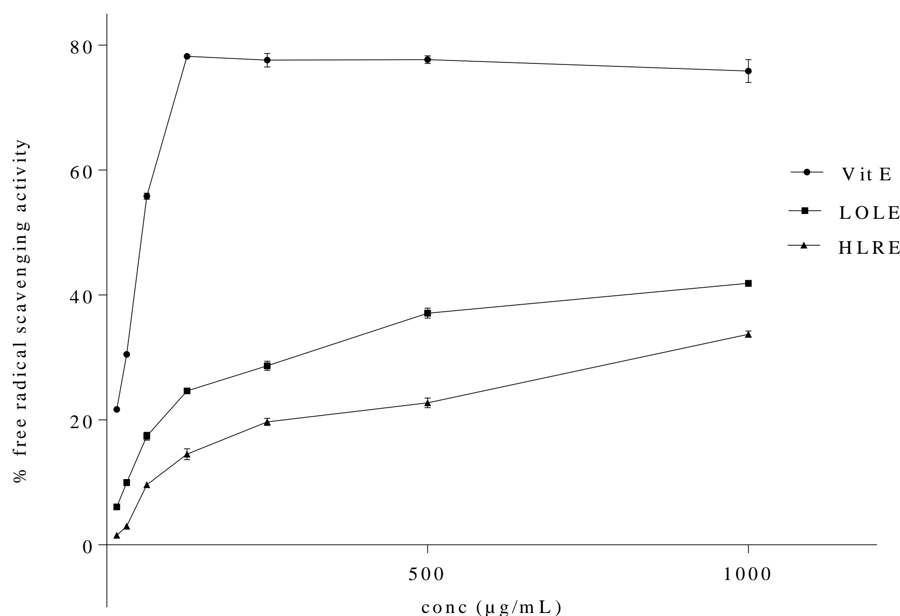

Figure 1. DPPH free radical scavenging activity of LOLE, HLRE and standard drug (Vitamin E). conc: concentration, Vit E: Vitamin E, LOLE: *Laportea ovalifolia* leaf methanol extract, HLRE: *Hilleria latifolia* root methanol extract. Values are mean ± SEM (n=3).

Where T_i is paw thickness before carrageenan injection and T_f is paw thickness at time T.

Raw scores for right foot thickness were individually normalized as percentage of change from their values at time 0 and then averaged. Total pedal oedema was calculated in arbitrary units as the area under the curve (AUC) and to determine the percentage inhibition of oedema, the following equation was used:

$$\% \text{ inhibition of oedema} = \left[\frac{\text{AUC}_{(\text{control})} - \text{AUC}_{(\text{treatment})}}{\text{AUC}_{(\text{control})}}\right] \text{x } 100$$

Data analysis

Data were presented as mean ± standard error mean (SEM) in the studies. Analysis of results was done using one-way ANOVA followed by the Tukey's *post hoc* test in analyzing the antioxidant activity and Dunnett's *post hoc* test in the anti-inflammation analysis. Graphs were plotted with Graph Pad Prism for windows version 6 (Graph Pad, San Diego, CA, USA).

RESULTS

Antioxidant activity of extracts

DPPH free radical scavenging activity of LOLE and HLRE

LOLE, HLRE and vitamin E (α-tocopherol) showed antioxidant activity at the test concentrations (0.0156 to 1 mg/mL) (Figure 1). The IC_{50} values obtained for vitamin E, LOLE and HLRE were 18.9 ± 1.3, 130.8 ± 0.9 and 233.5 ± 0.5 µg/mL, respectively (Table 1).

Total phenol content of LOLE and HLRE

LOLE and HLRE at the test concentrations (0.5 to 10 mg/mL) showed an increased phenol content with increasing concentration. LOLE ranged from 7 to 122 mg TAE/g of the extract as the concentration increased and HLRE was from 27 to 175 mg TAE/g of the extract with increasing concentration (Figure 2). The total phenol content in each of the extracts (HLRE and LOLE) was calculated as mean ± SEM as 56.75 ± 0.3220 and 91.32 ± 4.258, respectively.

Total antioxidant capacity of LOLE and HLRE

Total antioxidant capacity of LOLE and HLRE was expressed as α-tocopherol equivalence (α-TE) from the α-tocopherol calibration curve. The total antioxidant capacity of LOLE was 337.6 ± 6.961 mg α-TE/g extract and that of HLMR was 408.0 ± 18.70 mg α-TE/g. At the test concentrations (0.5 to 10 mg/mL) of LOLE and HLRE, it was observed that, antioxidant capacity decreased as the concentrations reduced.

Acute anti-inflammatory activity of LOLE and HLRE

LOLE (30, 100 and 300 mg/kg), when administered

Table 1. IC_{50} of extracts and vitamin E (α-tocopherol).

Extract/reference compound	LOLE	HLRE	vitamin E
IC_{50} (µg/mL)	130.8 ± 0.8511	233.5 ± 0.4933	18.90 ± 1.312

LOLE: *Laportea ovalifolia* leaf methanol extract, HLRE: *Hilleria latifolia* root methanol extract. Values are mean ± SEM.

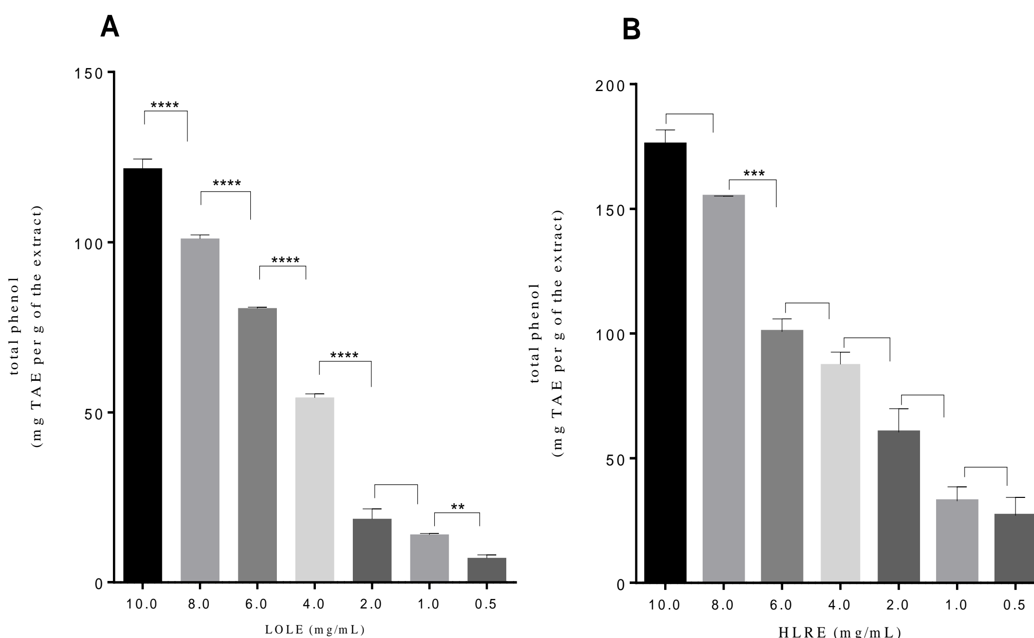

Figure 2. Total phenol content in different concentrations of LOLE (**A**) and HLRE (**B**) expressed as tannic acid equivalent. LOLE: *Laportea ovalifolia* leaf methanol extract HLRE: *Hilleria latifolia* root methanol extract. Values are mean ± SEM. ****p<0.0001; **p< 0.01 (Tukey's *post hoc* test).

before the induction of the carrageenan paw oedema (prophylaxis), showed a significant (p<0.001) reduction of the oedema with the mean maximal swelling attained at 2 h reduced respectively to 55.38 ± 0.57%, 51.15 ± 2.30% and 45.05 ± 2.81% from the inflamed control response of 61.50 ± 1.44% (Figure 4A). The total paw swellings induced over the 6 h were also significantly (p<0.001) suppressed to 86.96 ± 2.14%, 76.37 ± 3.70% and 67.92 ± 3.77% respectively of the inflamed control response (Figure 4B). Also, HLRE (30, 100 and 300 mg/kg) showed a significant (p<0.001) reduction of the oedema with the mean maximal swelling attained at 2 h reduced to 54.04 ± 1.40%, 48.33 ± 0.54% and 41.30 ± 2.36% respectively, from the inflamed control response of 61.50 ± 1.44% (Figure 4C). The total paw swellings induced over the 6 h were also significantly (p<0.001) suppressed to 88.51 ± 4.30%, 73.38 ± 2.99% and 61.78 ± 3.64% respectively (Figure 4D).

When administered after the induction of the carrageenan paw oedema (therapeutic), LOLE (30, 100, 300 mg/kg) showed a significant (p<0.001) inhibition of the oedema with the mean maximal swelling at 4 h reduced respectively to 45.51 ± 0.6827%, 43.91 ± 1.431% and 38.19 ± 0.9960% of the inflamed control response of 105.2 ± 4.553% (Figure 5A). The total paw swellings induced over the 6 h were also significantly (p<0.001) suppressed to 48.04 ± 1.37%, 45.38 ± 1.57% and 40.79 ± 2.40% respectively of the inflamed control response (Figure 5B). Similarly, HLRE (30, 100, 300 mg/kg) showed a significant (p<0.001) inhibition of the oedema with the mean maximal swelling at 4 h reduced respectively to 63.70 ± 5.490%, 75.22 ± 7.452% and 65.70 ± 4.943% of the inflamed control response of 105.2 ± 4.553% (Figure 5C). The total paw swellings induced over the 6 h were also significantly (p<0.001) suppressed to 64.30 ± 4.66%, 75.05 ± 9.59% and 62.00 ± 4.90% respectively of the inflamed control response (Figure 5D).

DISCUSSION

Antioxidant activity of the extracts was determined by assessing their DPPH free radical scavenging properties, total phenol content and total antioxidant capacity. LOLE

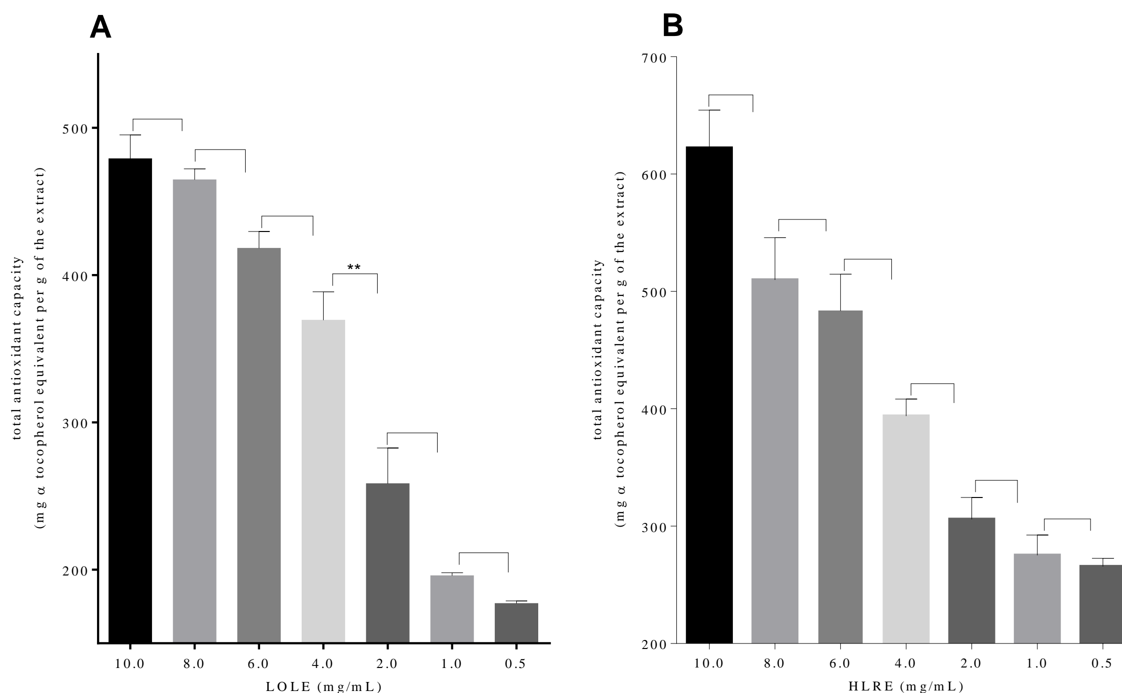

Figure 3. Total antioxidant capacity in different concentrations of LOLE (A) and HLRE (B) expressed as α-tocopherol equivalent. LOLE: *Laportea ovalifolia* leaf methanol extract, HLRE: *Hilleria latifolia* root methanol extract. Values are mean ± SEM. **p< 0.01 (Tukey's post hoc test).

and HLRE were able to scavenge free radicals of DPPH at the various test concentrations (Figure 3), with IC_{50} values of 130.8 ± 0.9 and 233.5 ± 0.5 µg/mL, respectively. The IC_{50} gives a notion on the ability of an agent to mop up free radicals indicating its potency as an antioxidant. Lower IC_{50} values indicate potent antioxidant activity (Apenteng et al., 2014). This implies that, LOLE exhibited a higher antioxidant activity as compared to HLRE. Wu and Ng (2008) report that antioxidant activity exhibited by plant extracts may be due to their phenolic and non-phenolic contents.

For this reason, the total phenol content of LOLE and HLRE was determined. HLRE had higher total phenol content (91.32 ± 4.258 mg/g) as compared to LOLE of (56.75 ± 0.3220 mg/g). This indicates that the higher free radical scavenging activity of LOLE as compared to HLRE may be due to other non-phenolic compounds present (Conforti et al., 2008).

The total antioxidant capacity of LOLE and HLRE (337.6 ± 6.961 and 408.0 ± 18.70 mg/g, respectively) correlated with their respective total phenol content. This implies that the higher the total phenol content the greater the total antioxidant capacity of the extracts. This confirms the report that phenolic compounds significantly cause the reduction of Mo+6 to Mo+5 (Khan et al., 2012). The acute anti-inflammatory activity of LOLE and HLRE was determined using the carrageenan-induced rat foot oedema model, since the events involved in the vascular response to carrageenan-induced oedema are similar to the early exudative stage of inflammation (Winter et al., 1962; Ozaki, 1990) and hence the use of anti-inflammatory agent to inhibit this acute phase of inflammation. The molecular response to carrageenan-induced oedema is bi-phasic involving the release of diverse inflammatory mediators, characterised by marked oedema formation. Inflammatory mediators such as histamine, serotonin and bradykinin are released during the first phase (1 to 2 h), and sustained by the release of prostaglandins and nitric oxide in the second phase (Thomazzi et al., 2010; Abotsi et al., 2012).

LOLE and HLRE at 300 and 100 mg/kg, significantly ($p<0.001$) reduced the induced oedema during both prophylactic and therapeutic treatments. This indicates the presence of compounds that can reduce the inflammatory responses. Even though the mechanism(s) of action of LOLE and HLRE are not known, they could be acting by inhibition and/or interference with the role of inflammatory mediators (such as histamine, serotonin, bradykinin, prostaglandins and other cyclooxygenase products) involved in the carrageenan induced oedema (Abotsi et al., 2012; Obiri et al., 2013). Also, the antioxidant potential of LOLE and HLRE may be a contributing factor to the extracts' anti-inflammatory activity. ROS are released from activated neutrophils and macrophages during inflammatory injury and their overproduction leads to tissue injury or damage. ROS

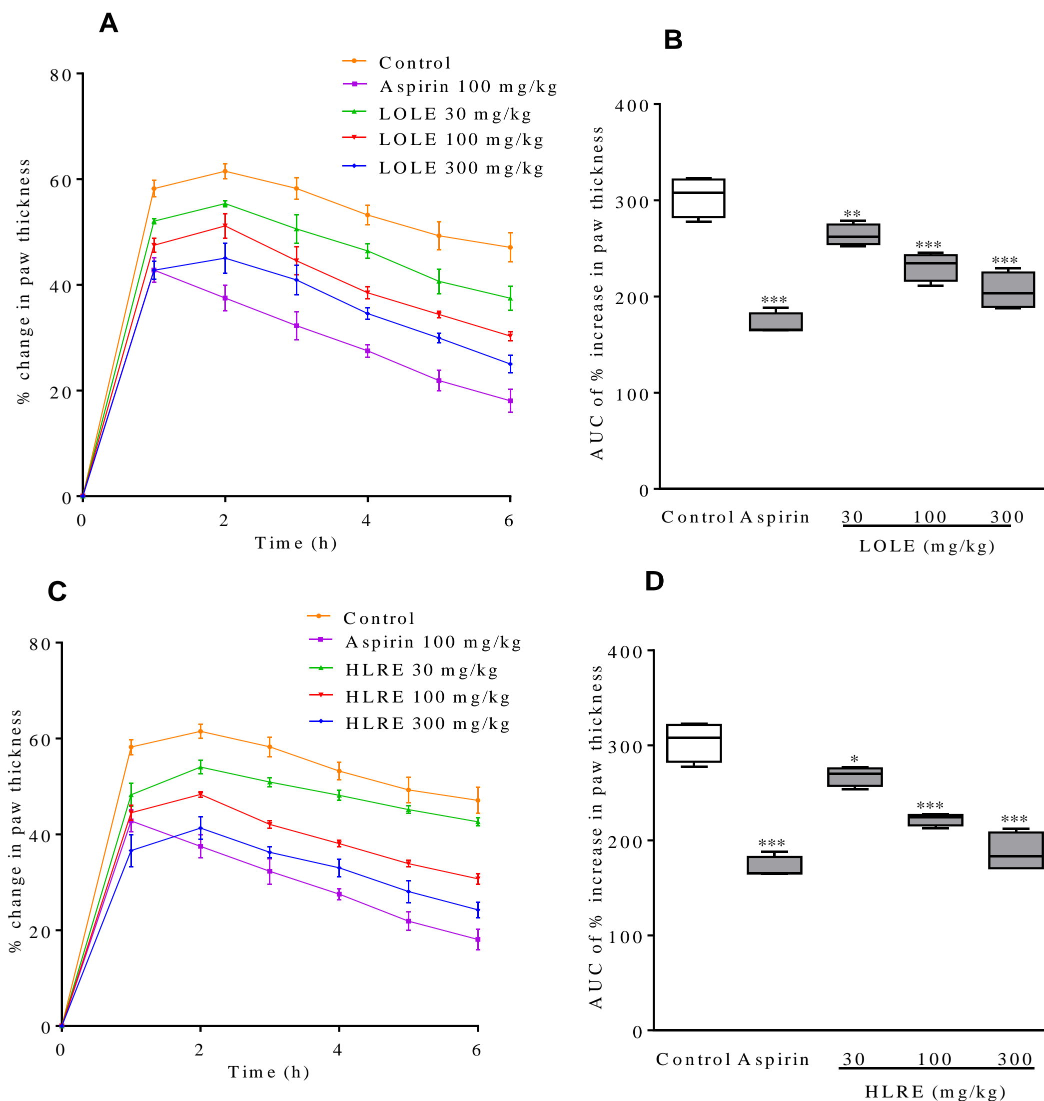

Figure 4. Prophylaxis anti-inflammatory activity of LOLE and HLRE on carrageenan-induced oedema in rats. **A**: Time-course curve of LOLE, **B**: AUC of carrageenan-induced oedema in LOLE treated group, **C**: Time-course curve of HLRE, **D**: AUC of carrageenan-induced oedema in HLRE treated group, LOLE: *Laportea ovalifolia* leaf methanol extract, HLRE: *Hilleria latifolia* root methanol extract. Values are means ± SEM (n=4); ****p*<0.001; ***p*<0.01; **p*<0.05 compared to control group (Dunnett's *post hoc* test).

can also cause the release of pro-inflammatory cytokines such as IL-1β and TNF-α which directly enhance inflammatory response (Dinarello, 2000; Conforti et al., 2008). Hence, the extracts ability to counteract the effects of ROS and free radicals can contribute to their anti-inflammatory activities.

Abotsi et al. (2012) used different *in vitro* antioxidant models to assess the antioxidant activity of the aerial parts of *H. latifolia* as well as its anti-inflammatory activity, using the carrageenan induced oedema in 7-day old chick model. The study reported the antioxidant activity and significant reduction of induced oedema (anti-inflammatory activity) by the aerial parts of *H. latifolia* comparable to what was observed in this study conducted on the root extract. There is need to isolate and characterize the bioactive agent(s) or compound(s) from these two plants responsible for the above pharmacological or biological activities.

Conclusion

The leaf extract of *L. ovalifolia* and root extract of *H. latifolia* exhibited antioxidant and acute anti-inflammatory activity at the test concentrations, when administered in both preventive and curative protocols of carrageen-

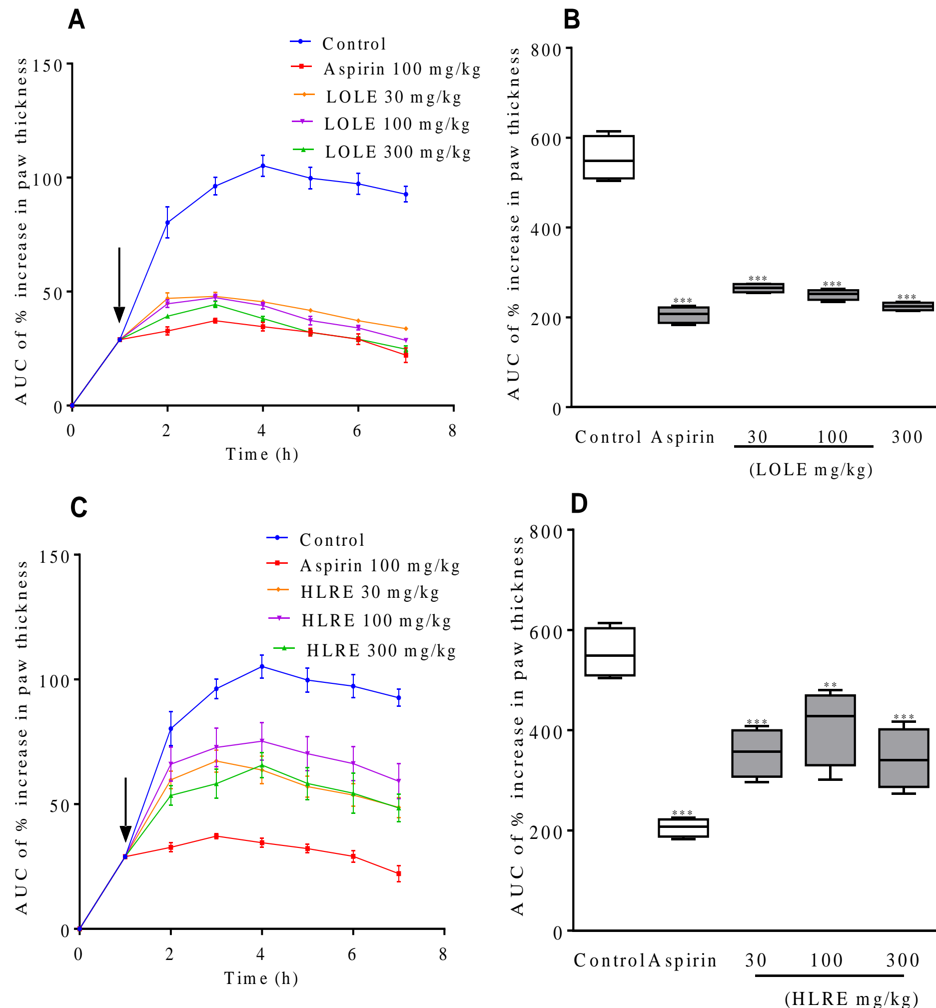

Figure 5. Therapeutic anti-inflammatory activity of LOLE and HLRE on carrageenan-induced oedema in rats. **A:** Time-course curve of LOLE, **B:** AUC of carrageenan-induced oedema in LOLE treated group, **C**: Time-course curve of HLRE, **D**: AUC of carrageenan-induced oedema in HLRE treated group, LOLE: *Laportea ovalifolia* leaf methanol extract, HLRE: *Hilleria latifolia* root methanol extract. Values are means ± SEM (n=4) ****p*<0.001; ***p*<0.01 compared to control group (Dunnett's *post hoc* test). Arrow indicates point of Aspirin or LOLE administration.

induced oedema.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Studies on gastrointestinal properties of ethanolic leaf extract of *Salacia lehmbachii* in Wistar rats

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Salacia lehmbachii **Loes, is used traditionally in Nigeria for the treatment of gastrointestinal disorders. The aim of this study was to investigate the anti-ulcer and anti-diarrhoeal activities of the ethanolic leaf extract of *S. lehmbachii*. The ethanolic leaf extract was evaluated for castor oil-induced diarrhoeal, intestinal transit as well as intestinal fluid accumulation in rats, while indomethacin, water immersion stress-induced and histamine were used for anti-ulcer tests. The extract at the doses used significantly (*P*<0.05) decreased castor oil-induced diarrhoea in rats as judged by a decrease in the number of wet faeces in the extract treated rats. Moreso, the leaf extract inhibited the propulsive movement of intestinal contents. *S. lehmbachii* also showed a dose-related inhibitory activity on castor oil-induced intestinal fluid accumulation in rats. The leaf extract of *S. lehmbachii* significantly (*P*<0.05) reduced the ulcer index in all assays used. The results of the current study support the folkloric usage of *S. lehmbachii* leaf extract in the management of gastrointestinal disorders in Nigerian herbal traditional medicine.**

Key words: *Salacia lehmbachii*, leaves, herbal medicine, antidiarrhoea, antiulcer, rats.

INTRODUCTION

Medicinal plants are known to possess a variety of substances and are used in the treatment of many kinds of ailments in traditional medicine. The beneficial effects of medicinal plant materials result from the combinations of secondary products present in the plant. These constituents are capable of producing definite physiological action on the human body (Erdogrul, 2002). Natural products from plant materials are being continuously used to treat different diseases. Investigation of natural and synthetic compounds has been the source of many therapeutic agents (Mahesh and Satish, 2008). Plant products play essential role in

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drug development programs of the pharmaceutical industry (Parke and Sapota, 1996). In traditional medicine, several plants and herbs have been used to treat gastrointestinal disorders. These agents have fewer side effects, easily accessesible and also affordable (Edeoga et al., 2005).

Salacia lehmbachii, which belongs to the family Celestraceae is commonly found in the tropical rain forest region of West, Central and East Africa. The decoction of the leaves and roots are used traditionally in the folk medicine in Nigeria for the treatment of a number of diseases including renal dysfunction, pain, fever, and gastrointestinal disorders (Essien et al., 2015a, b; Lapah et al., 2014). In present study, the ethanolic leaf extract of *S. lehmbachii* was investigated for its antidiarrhoeal and antiulcer activities to ascertain the folkloric claim made by the indigenes.

MATERIALS AND METHODS

Plant collection

The leaves of *S. lehmbachii* were collected from Akwa Ibom State, Nigeria. The plant was identified and authenticated by a taxonomist in the Department of Botany, University of Calabar, Nigeria, where a voucher specimen (No.688) was deposited at the herbarium for reference.

Extraction

The leaves were cleaned and taken to the laboratory, where they were cut into pieces and air-dried at room temperature for 7 days and ground to powder using mortar and pestle. 500 g of the ground leaf powder was then macerated in 1.5 L of ethanol for 24 h and was filtered. The filtrate was dried on a water bath at reduced temperature to recover the extract and the yield was calculated to be 12.5% w/w. The leaf extract was subsequently reconstituted in normal saline at appropriate concentration for the experiment.

Phytochemical screening

Phytochemical analysis of the ethanolic leaf extract was carried out employing standard procedures to determine the following compounds: flavonoids, tannins, saponins, terpenoids, alkaloids, cardiac glycosides, steroids, resins, anthraquinones and phlobatanins (Mukherjee, 2006; Sumitra et al., 2006).

Animals

Adult Wistar rats (180 to 220 g) of both sexes obtained from Animal House, Department of Pharmacology, College of Medical Sciences, University of Calabar, Nigeria, were used for the study. The animals were housed in cages at room temperature and moisture under naturally illuminated environment of 12:12 h dark/light cycle. The animals were fed on standard pellets and had free access to water. NIH guide for the care and use of Laboratory Animal was employed in this study (NIH, 1985).

Acute toxicity study of the extract

The LD_{50} of the leaf extract was tested to determine the safety of the agent according to the guidelines set by OECD (2010). These studies were done in two phases. Nine rats, randomized and divided into three were used in the first phase. The rats were orally administered with 100, 600 and 1000 mg/kg of the leaf extract, respectively. The animals were observed for the first 4 and 24 h for signs of toxicity and mortality. This was followed by the second phase in which 2000, 3000 and 5000 mg/kg of the extract was administered to the next three groups of three rats per cage. The signs of toxicity and mortality were observed for 24, 48 and 72 h, respectively.

Induction of diarrhoea with castor oil

Anti-diarrhoea activity of the extract was evaluated using the castor oil-induced diarrhoea model in rats (Akuodor et al., 2011; Capasso et al., 2008). Thirty rats fasted for 24 h were randomly grouped into five with six rats in each. Normal saline (20 ml/kg) was given to rats in group 1. The extract (100, 200, and 400 mg/kg) was given rats in groups 2, 3, and 4, while group 5 received 4 mg/kg loperamide. All were administered orally via cannula. One hour after the treatment, rats in all the groups were orally challenged with 1 ml castor oil. The rats in each group were then placed singly in cages with adsorbent paper on their floors. The diarrhoea episodes were observed for 4 h and the cumulative frequency of wet and formed stools was noted. Percentage protection was calculated using the mean stool frequency and anti-diarrhoea activity.

Intestinal transit test

The effect of the extract on gastrointestinal motility was determined as previously described (Akuodor et al., 2012). Thirty rats were randomly divided into five groups of six rats each and they fasted for 24 h prior to the test and had water *ad libitum*. Group 1 (control) was treated with 10 ml/kg normal saline. Group 2, 3 and 4 received 100, 200 and 400 mg/kg of ethanol leaf extract of *S. lehmbachii* orally, respectively. Group 5 was treated with 5 mg/kg Atropine (standard drug) orally. Thirty minutes after, 1 ml of charcoal (5% deactivated charcoal suspension in 10% tragacanth) was orally given to all animals and thirty minutes later, all were sacrificed. The distance travelled by the marker (charcoal) was then measured and expressed as a percentage of the total length of the small intestine (pylorus to caecum).

Intestinal fluid accumulation test

Intestinal fluid accumulation was determined by the method as described by Robert et al. (1976) with slight modification. Thirty rats of both sexes were divided into five groups of six each. Normal saline (20 ml/kg) was administered to group 1, while the extract group (2, 3 and 4) was treated with 100, 200 and 400 mg/kg, respectively. Group 5 was given 4 mg/kg loperamide. All administered orally. One hour later, 1 ml castor oil was orally given to all the test animals and 1 h after, they were sacrificed and their small intestines were removed after ligating the ends. Intestinal contents were collected by milking into a graduated tube and the measured volumes were recorded. Percentage inhibition was then determined by calculating the mean volume of intestinal contents and comparing it with values obtained from control group.

Indomethacin-induced gastric ulcer in rats

The method as described by Anosike and Ofoegbu (2013) was adopted with slight modification. Rats used for this experiment were fasted for 48 h having access to water *ad libitum*. They were

Table 1. Effect of the ethanolic leaf extract of *S. lehmbachii* on castor oil-induced diarrhoea in rats.

Treatment	Dose (mg/kg)	Frequency of diarrhoea in 4 h	% Inhibition
Normal saline	20 ml/kg	13.5±0.81	-
S. lehmbachii	100	5.5±0.67*	59
	200	3.67±0.21*	72
	400	2.5±0.67*	81
Loperamide	4	1.17±0.48*	91

Data are mean ± SEM (n=6). *P < 0.05 compared to control group (ANOVA, Tukey's test).

grouped into five of six rats per cage. Group 1 was treated with 20 ml/kg normal saline. Groups 2, 3 and 4 were treated with 100, 200 and 400 mg/kg of the ethanolic leaf extract, while the standard drug ranitidine (20 mg/kg) was administered to group 5, respectively. All drugs were given orally. In this model, gastric ulcers were induced by indomethacin after 1 h of drug treatment. The animals were sacrificed by ether anaesthesia after 5 h for the determination of ulcerative index.

Water immersion stress-induced ulceration in rats

The experiment was performed according to the method of Akuodor et al. (2013). In this model, animals were fasted for 48 h prior to the study. The animals under study were grouped into 5 with 6 rats in each and treated with normal saline (20 ml/kg) in group 1. Those in groups 2, 3 and 4 were treated with the leaf extract (100, 200 and 400 mg/kg), respectively. Standard drug ranitidine (20 mg/kg) was administered to group 5. All administered by oral route. One hour after drug administration, rats were made to swim in a cylinder containing water to the height of 35 cm and maintained at 30±1°C for 1 h. Thereafter, animals were removed, dried and injected intravenously via the tail vein with 30 mg of Evans blue. Ten minutes later, they were all sacrificed under ether anaesthesia and their stomachs removed. Formolsaline (2% v/v) was then injected into the ligated stomachs for storage overnight. The next day, each stomach was opened, washed in warm water and examined under a dissection microscope.

Histamine-induced ulceration in rats

The effect of *S. lehmbachii* leaf extract on histamine-induced ulcers was carried out following the method of Bodhankar et al. (2006) with slight modification. Animals were fasted for 48 h prior to the experiment. After the fasting period, they were divided into five groups of six rats per cage. The control (group1) was treated with 20 ml/kg of normal saline. The ethanolic leaf extract of *S. lehmbachii* (100, 200 and 400 mg/kg) was administered to groups 2, 3 and 4. Group 5 was treated with standard drug, ranitidine (20 mg/kg). The drugs were all given by oral route. Gastric ulcers were induced after 1 h by subcutaneous administration of 100 mg/kg of histamine. All the animals were sacrificed by ether anaesthesia 4 h later for the determination of ulcerative lesion index.

Measurement of ulcer index

Ulcer index of each rat was calculated following the methods as described by Malairajan et al. (2007). Ulcer index of the experimental rats were calculated by adding the values and their mean values were determined as follows: (i) 0 = no ulcer, (ii) 1 = hemorrhagic and slightly dispersed ulcers less than 2 mm length, (iii) 2 = 1 ulcer, hemorrhagic and up to 5 mm length, (iv) 3 = more than 1 ulcer, each up to 5 mm length, (v) 4 = 1 ulcer above 5 mm in length, (vi) 5 = more than 1 ulcer above 5 mm in length. Percentage of ulcer protection index was calculated by adopting the following formula: % Protection = (Uc - Ut/Uc) × 100, where Uc is the ulcer index in control group, and Ut is the ulcer index in treated groups.

Statistical analysis

The data are expressed as the mean ± standard error of mean (SEM) for each group. The results were statistically analyzed using one-way analysis of variance (ANOVA), followed by Tukey's test. Differences were considered significant at $P<0.05$.

RESULTS

Phytochemical screening

Phytochemical screening of the leaf extract revealed the presence of tannins, saponins, flavonoids, steroids, terpenoids, alkaloids, resins and cardiac glycosides, while phlobatannins and anthraquinones were absent.

Acute toxicity test

The acute oral toxicity test showed normal behaviour of the treated rats. There were no lethality or toxic reactions observed. However, the experimental doses used (100, 200 and 400 mg/kg) were orally within safe margin.

Effect of *S. lehmbachii* on castor oil-induced diarrhoea

The ethanolic leaf extract of *S. lehmbachii* exhibited dose-dependent anti-diarrhoea activity in the study. The extract significantly ($P<0.05$) decreased both the frequency of defaecation as well as the wetness of faecal dropping in rats. However, the effect of the leaf extract was less potent in comparison to the standard drug, loperamide (Table 1).

Table 2. Effect of the ethanolic leaf extract of *S. lehmbachii* on intestinal motility in rats.

Treatment	Dose (mg/kg)	Mean intestinal length (cm)	Mean distance travelled by marker(cm)	% Inhibition
Normal saline	20 ml/kg	88.17±0.79	85.17±1.19	-
	100	87.5±1.3.45	45.83±2.71*	46
S. lehmbachii	200	80.17±2.36	35.17± 1.70*	59
	400	82.0±1.48	31.33±2.27*	63
Atropine	5	88.67±1.26	29.17±1.47*	66

Data are mean ± SEM (n=6). *P < 0.05 compared to control group (ANOVA, Tukey's test).

Table 3. Effect of the ethanolic leaf extract of *S. lehmbachii* on castor oil-induced enteropooling in rats.

Treatment	Dose (mg/kg)	Volume of intestinal content (ml)	% Inhibition
Normal saline	20 ml/kg	4.33±0.13	-
	100	1.58±0.14*	41
S.lehmbachii	200	1.42±0.09*	47
	400	1.18±0.09*	56
Loperamide	4	0.47±0.05*	83

Data are mean ± SEM (n=6). *P < 0.05 compared to control group (ANOVA, Tukey's test).

Effect of *S. lehmbachii* on intestinal transit

S. lehmbachii leaf extract significantly (*P*<0.05) reduced distance travelled in the intestine by the marker in a dose-dependent manner. The standard drug, atropine at 5 mg/kg compared favourably with the extract at 400 mg/kg (Table 2).

Effect of *S. lehmbachii* on castor oil-induce enteropooling

The ethanolic leaf extract of *S. lehmbachhii* was found to possess anti-enteropooling activity. The extract significantly (*P*<0.05) decreased intestinal fluid volume in rats. However, this effect was less potent in comparison with the standard drug, loperamide (Table 3).

Effect of *S. lehmbachii* on indomethacin-induced ulcers

Oral administration of ethanol leaf extract of *S. lehmbachii* at a dose of 100, 200 and 400 mg/kg exhibited dose dependent ulcer protection of 57, 72 and 82%, whereas the standard drug, ranitidine (20 mg/kg) had 89% inhibition (Table 4).

Effect of *S. lehmbachii* on water immersion stress-induced ulcers

Pre-treatment of ethanolic leaf extract of *S. lehmbachii* 1 h before water immersion stress-induced ulcers showed dose dependent protection of 59, 74 and 84% at doses of 100, 200 and 400 mg/kg, respectively. Ranitidine (20 mg/kg) showed an inhibition of 88% (Table 5).

Effect of *S. lehmbachii* on histamine-induced ulcers

The ethanolic leaf extract of *S. lehmbachii* significantly protected gastric mucosal against damage induced by histamine. The extract (100, 200 and 400 mg/kg) was found to possess remarkable ulcer protective properties of 52, 73 and 77%, respectively, whereas ranitidine (20 mg/kg) exhibited 88% protection (Table 6).

DISCUSSION

The phytochemical evaluation of the ethanolic leaf extract of *S. lehmbachii* revealed the presence of alkaloids, tannins, saponins, terpenoides, flavonoids, steroids and resins which showed that the plant is of high pharmacological importance. These classes of

Table 4. Effect of ethanolic leaf extract of *S. lehmbachii* on indomethacin-induced ulcers in rats

Treatment	Dose	Ulcer index (UI)	% protection of ulceration
Normal saline	20 ml/kg	4.25±0.36	-
	100 mg/kg	1.83±0.32*	57
S. lehmbachii	200 mg/kg	1.18±0.24*	72
	400 mg/kg	0.78±0.35*	82
Ranitidine	20 mg/kg	0.43±0.28*	89

Data are mean ± SEM (n=6). *P < 0.05 compared to control group (ANOVA, Tukey's test).

Table 5. Effect of ethanolic leaf extract of *S. lehmbachii* in water immersion stress- induced ulcers in rats

Treatment	Dose	Ulcer index (UI)	% protection of ulceration
Normal saline	20 ml/kg	4.38±0.31	-
	100 mg/kg	1. 80±0.27*	59
S. lehmbachii	200 mg/kg	1.15±0.24*	74
	400 mg/kg	0.72±0.32*	84
Ranitidine	20 mg/kg	0.53±0.34*	88

Data are mean ± SEM (n=6). *P < 0.05 compared to control group (ANOVA, Tukey's test).

Table 6. Effect of ethanolic leaf extract of *S. lehmbachii* on histamine-induced ulcers in rats.

Treatment	Dose	Ulcer index (UI)	% protection of ulceration
Normal saline	20 ml/kg	4.28±0.29	-
	100 mg/kg	2.07±0.39*	52
S. lehmbachii	200 mg/kg	1.17±0.24*	73
	400 mg/kg	0.97± 0.31*	77
Ranitidine	20 mg/kg	0.52±0.23*	88

Data are mean ± SEM (n=6). *P < 0.05 compared to control group (ANOVA, Tukey's test).

compounds have been reported to show important biological effects (Longanga-Otshudi et al., 2000; Ghoghari and Rajan, 2006; Panda and Kar, 2007) and the presence of these constituents may be responsible for the anti-diarrhoeal properties seen in the extract. Earlier studies have shown that antidiarhoeal potential of medicinal plants were due to its secondary metabolites (Kouitcheu et al., 2006). The anti-diarrhoeal effect of the plant extract may also be due the precipitation of proteins in enterocyte and production of protein tannates which result in decreased secretion and peristaltic movement (Salawu et al., 2007). The remarkable dose-dependent reduction in castor oil-induced diarrhoea in rats is a demonstration of the effect of *S. lehmbachii* leaf extract as anti-diarrhoeal agent. The absence of death at oral treatment of over 5000 mg/kg suggests that ethanol leaf extract of *S. lehmbachii* is non-toxic acutely. It is therefore safe for oral use in the therapeutic treatment of diarrhoea.

The high safety profile observed may be responsible for its wide spread use in gastrointestinal disorders. The leaf extract has shown significant activity in reducing the frequency of castor oil-induced diarrhoea, a feat comparable to loperamide. Loperamide is an anti-diarrhoeal agent whose action is by increasing colonic phasic segmenting effects which inhibits presynaptic

cholinergic nerves in submucosal and myetheric plexuses. These activities lead to increased colonic transit time and faecal water absorption, hence reducing the frequency of defaecation (Yu et al., 2000; Cammilleri, 2004). Atropine and various doses of the leaf extract of *S. lehmbachii* reduced the propulsive movement in the charcoal meal study, atropine being more potent than the leaf extract at the doses used. The observed effect of castor oil-induced enteropooling suggests that the ethanolic leaf extract of *S. lehmbachii* exhibited significant antidiarrhoea by spasmolytic activity and also anti-enteropooling effects. However, *S. lehmbachii* may have supported Geiger's criteria for the classification of a drug as an antidiarrhoeal (Aniagu et al., 2005).

Ulcer formation induced by indomethacin is known to be related with inhibition of cyclooxygenase which prevent prostaglandin biosynthesis and this in turn inhibits the release of mucus, which is a defensive factor against gastrointestinal damage (Dengiz and Gursan, 2005). However, it is believed that *S. lehmbachii* exert its antiulcer activity by increasing the formation of endogenous prostaglandin synthesis, which in turn promote mucus secretion and enhance the mucosal barrier against the actions of various damaging agents (Malairanjan et al., 2007).

Stress induced ulcers are due to autodigestion of gastric mucosal barrier, presence of HCL and production of free radicals (Olaleye and Farombi, 2006). The ulcers are generated due to the release of histamine which results in increase in acid secretion and reduction in mucus production (Dengiz and Gursan, 2005). Stress may also cause an increase in gastrointestinal motility resulting in stomach folds which are more susceptible to damage when they come in contact with acid (Demirbilet et al., 2004). The ethanolic leaf extract of *S. lehmbachii* showed a dose dependent activity in stress induced ulcers. The reduction in ulcer index of the leaf extract was comparable to the standard drug ranitidine, which suggest that the ethanolic leaf extract may follow ranitidine inhibitory mechanism.

Histamine-induced gastric ulcers have long been recognized and mediated through stimulation of H_2 receptors and may result in enhanced gastric acid secretion and vasodilatation (Adinortey et al., 2013). Histamine does not only increase gastric acid secretion, but also causes disturbances of the gastric mucosa, abnormal motility and reduction in mucus production (Ghodekar et al., 2010). The leaf extract of *S. lehmbachii* significantly reduced histamine induced ulcers by probably blocking H_2 receptors, thus inhibiting gastric acid secretion.

In conclusion, the present study has shown that the ethanolic leaf extract of *S. lehmbachii* possesses significant gastrointestinal activities, thus justifying the wide spread use of this plant in traditional medicine for the treatment of gastrointestinal disorders. The bioassay-guided fractionation, identification and characterization of the active principle(s) responsible for the gastrointestinal potential of the plant are in progress in our laboratory.

Conflict of interest

The authors have not declared any conflict of interest.

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