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Original Article

POTENTIAL ANTIOXIDANT, ANTI-INFLAMMATORY AND ANTIBACTERIAL EVALUATION OF EXTRACTS OF LEUCAS ASPERA USING *IN VITRO* MODELS

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ABSTRACT

Objective: To evaluate the potential antioxidant, anti-inflammatory and antibacterial activities of aqueous and methanolic extracts of leaves of *Leucas aspera* (Thumbae).

Methods: Phytochemical screening of the leaves of *L. aspera* was followed by analysis of antioxidant activity by means of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity. *In vitro* anti-inflammatory activity was evaluated using lipoxygenase inhibition, albumin denaturation assay, membrane stabilization assay and proteinase inhibitory activity at different concentrations. Aspirin was used as a standard drug for the study of anti-inflammatory activity. Linear regression analysis was used to calculate half maximal inhibitory concentration, IC50 value. The zone of inhibition was performed against common pathogens to determine the antimicrobial activity at different concentrations of plant extracts (60%, 70%, 80%).

Results: The phytochemical analysis revealed the presence of carbohydrates, amino acid, alkaloids, tannins, flavonoids, glycosides, xanthoproteins, and phenols. The total phenolic and flavonoid content was found to be 2.25±0.04 mg GAE/g (gallic acid equivalents) and 1.2±0.05 mg QE/g (Quercetin equivalents) of fresh weight tissue respectively. The IC50 values for hydrogen peroxide scavenging activity were found to be 244.6 µg/ml. The extract inhibited the lipoxygenase enzyme activity with an IC50 value of 356.3 µg/ml. Maximum inhibition of heat-induced protein denaturation of 69% was observed at 400 µg/ml, IC50 249.6 µg/ml. Proteinase activity was also significantly inhibited (IC50 = 421.6 µg/ml). Membrane stabilization assay attributed minor protection by the leaf extract with an IC50 of 206.7. It was observed that *E. coli* were inhibited at all concentrations, followed by *Klebsiella* and *Pseudomonas*.

Conclusion: Results indicate that L. aspera possess anti-inflammatory properties due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins and steroids that serve as free radical inhibitors or scavenger. Compounds of the plant L. aspera may hence be used as lead compounds for designing potent anti-inflammatory drug which can be used for treatment of various diseases.

Keywords: Albumin denaturation, Anti-inflammatory**,** Antimicrobial, Hemolysis, lipooxygenase, Phytochemicals, *Leucas aspera*

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INTRODUCTION

The rich biodiversity of Indian subcontinent contributes to the wealth of medicinal plants, which are very much used in traditional medical treatments [1]. India is one of the 12 mega biodiversity centers with over 18,000 plant species. Over 2,500 species are formally recognized as having true medicinal value. About 7500 plants have been used in local health traditions in rural and tribal villages of India. Out of these, the medicinal efficacy of 4000 plants is either little known or unknown to the mainstream population [1]. About 1500 plants with medicinal uses are mentioned in ancient texts such as Ayurveda, Siddha, Unani and Tibetan medicine. In recent years, the growing demand for herbal products has led to a quantum jump in volume of plant materials traded within and across various countries in the world.

The plants of genus *Leucas* have been used by the tribals in various parts of Asia, Africa, and India. Hot water extract of *L. aspera* is used orally as a stimulant, laxative, and diuretic [2], for the treatment of a headache, asthma, and bronchitis [3]. Hot water extract of the entire plant is also used to treat inflammation, dyspepsia, and jaundice [4]. Entire plant extract is used orally to treat scabies, psoriasis, and snake bite [5]. The plant *L. aspera* is externally used as an insect repellent [6]. *L. aspera* are externally used to fumigate dwellings [5]. A handful of flowers roasted in ghee are given orally (5–10 g once a day) for the treatment of a cough and colds. The flowers are crushed, and aroma is inhaled in the opposite nostril for the relief of a migraine. The juice of leaves is used aurally for ear pain [7] and for pus discharge from ear. The paste of leaves ground with chalk is applied to tooth cavity (periodontal) to prevent decay. The decoction of leaves is used nasally as an antivenin. Infusion of leaves is used externally to treat scabies [8]. Leaf paste mixed with turmeric is used to heal wounds and boils [9]. The decoction of roots, stem, and inflorescence of *L. aspera* are used orally for high fevers [10], for influenza [11] and against the filarial parasite [5].

Current research on natural molecules and products primarily focuses on plants since they can be sourced more easily and selected on the basis of their ethnomedicinal use [12]. Primary bioassays are generally designed for rapid screening of large numbers of products or extracts. They are simple, easy to implement and produce results quickly and preferably at low cost. With this under consideration, *L. aspera* was chosen for *in vitro* studies. It is widely used India as foods and also for the nutritional requirement. Mukherjee *et al.*, [13] reported that the ethanol extract of aerial part of *Leucas* significantly reduced the incidence and severity of diarrhoea in the castor oil-induced diarrhoea in rats. Antidiabetic activity has established that the methanol extracts of the whole plant of *Leucas* possess a dose-related strong hypoglycemic activity and have a similar potency to that of glibenclamide at an oral dose of 400 mg/kg [14]. The cold methanolic extract of the whole plant of *Leucas aspera* was found to exhibit significant hepatoprotection in CCl_4 induced liver damage [15]. Various studies using the brine shrimp lethality assay showed that the hydroalcoholic extract of *L. aspera* whole plant exhibited cytotoxicity [16]. *L. aspera* leaf extract exhibited significant larvicidal activity against first, second, third and fourth instar larvae of *Culex quinquefasciatus* [17].

The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents. Multiple drug resistance in human pathogenic micro-organisms has been developed due to indiscriminate use of

commercial antimicrobial drugs commonly used in the treatment of infectious diseases [18]. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of an antibacterial agent, host characteristics and environmental factors. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, but the cost production of synthetic drugs is high and they produce adverse effects compared to plant-derived drugs [19]. The aqueous extract of the leaves of *Leucas* was found to be inactive against fungal strains-*Aspergillus flavus* [20], *Trichophyton rubrum, Aspergillus niger* [21]. Significant antimicrobial activity was also reported for the alkaloidal fraction and the total methanol extract the *Leucas* flowers [15]. Conflicting antimicrobial activities of *Leucas* leaves by a number of researchers may be due to variation in the constituents and/or compositions of the different extracts.

This study aimed to screen the plant for its phytochemical constituents in aqueous and methanolic extracts. It evaluates the antioxidant potential and anti-inflammatory nature of the leaf extract in comparison with the commercial standard antioxidant, ascorbic acid. The study also investigated the antibacterial activity of the extract using the reference antibiotic ciprofloxacin.

MATERIALS AND METHODS

Preparation of plant extract

The plant samples were obtained from University of Agricultural Sciences, GKVK campus, Bengaluru, India. The plant extracts were prepared using aqueous methanol and water. Fresh leaves were washed thoroughly in distilled water 3-4 times and air dried. 500 mg of leaf sample was homogenized in 10 ml of the selected solvent using mortar and pestle. The crude extracts were then centrifuged at 8000 rpm for 10 min; the supernatant was collected and stored in cold condition.

Chemicals, reagents and solvents

All chemicals used were of analytical grade and include: methanol (Sigma-Aldrich, Germany), Quercetin (Sigma-Aldrich, Germany), Bovine serum albumin, distilled water, chloroform, Folin-Ciocalteu reagent, hydrogen peroxide, sodium hydroxide (NaOH), potassium ferricyanide (K3[Fe(CN)6]), trichloroacetic acid (C(Cl3)COOH; TCA), ferric chloride ($FeCl_3$), ascorbic acid, α,α-diphenyl-β-dipicryl-hydrazyl (DPPH) radical, buffer tablets.

Equipment/Instruments

The equipment/instruments used include: electrical weighing balance (Shimadzu, AUW-D Series), analytical weighing balance, digital pH meter, centrifuge tubes, incubator, centrifuge (Remi, India), test tube racks, UV-Visible spectrophotometer (Systronics, μ Controller Based UV-VIS Type: 117), soxhlet apparatus, and rotary evaporator (B. Bran Scientific and Instruments Co., England).

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods [22-24].

Total phenolic content

Total phenolic contents were estimated according to the spectrophotometric method [25]. A methanolic solution of the extract in the concentration of 1 mg/ml was taken for estimation. The reaction mixture was prepared by mixing 0.5 ml of a methanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent and 2.5 ml 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45 °C for 45 min. The absorbance was determined using spectrophotometer at λ_{max} = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The above procedure was repeated for the standard solution of gallic acid, and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GAE/g of tissue).

Total flavonoid content

The aluminum chloride colorimetric method was used for determination of total flavonoids [26]. The plant extract in methanol was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It was incubated at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm in triplicate. Sample blank was prepared in a similar way by replacing aluminum chloride with distilled water. The calibration curve was prepared by preparing quercetin solutions at concentrations 6.25, 12.5, 25, 50, 80 and 100 μg/ml. 10 to 100 μg/ml in methanol. The flavonoid content in extracts was expressed in terms of quercetin equivalent (mg of QE/g of tissue).

Evaluation of antioxidant activity

DPPH radical scavenging activity

The procedure of Braca [27] was used for determination of DPPH scavenging capacity of various fractions. A stock solution of ascorbic acid (1000 μg/ml) was diluted ranging from 10-100 μg/ml. 0.1 ml solution from different dilutions was pipetted out in respective tubes. The volume in each tube was made up to 3 ml with DPPH (20 μg/ml). The test tubes were incubated for 10 min at room temperature. The contents of each tube were mixed well and the absorbance was measured at 517 nm against a blank. A control (3.1 ml DPPH) was prepared. The percentage inhibition of DPPH by the samples was calculated as follows:

$$\text{Scavenging effect (\%)} = \frac{(\text{OD of control} - \text{OD of sample})}{\text{OD of control}} \times 100$$

Hydrogen peroxide scavenging activity

Scavenging of hydrogen peroxide was assayed according to the method of Ruch [28]. Plant extract at 30 μg/ml concentration in 3.4 ml phosphate buffer was added to 0.6 ml of 45 mmol H_2O_2 solution absorbance of the reaction mixture was recorded at 230 nm. A blank solution contained the sodium phosphate buffer without H_2O_2. Ascorbic acid was used as a standard. The concentration of H_2O_2 (mM) in the assay medium was determined using a standard curve ($y = 0.123x+2.2$; $R^2 = 0.9583$). H_2O_2 scavenging ability was calculated as IC50. The scavenging effect was calculated as follows:

$$\text{Scavenging effect (\%)} = \frac{(\text{OD of control} - \text{OD of sample})}{\text{OD of control}} \times 100$$

Evaluation of *in vitro* anti–inflammatory activity

Lipoxygenase inhibition

Soybean lipoxygenase activity was assayed by the procedure of Axelrod [29]. Briefly, the reaction was carried out in a final volume of 3 ml containing 2.9 ml of 0.1M borate buffer pH 9.0 and 50 μl of 10 mmol Linoleic acid. The reaction was started by the addition of 50 μl of the soybean enzyme extract. The enzyme activity was measured by following the formation of the product, 12-HETE at 234 nm for up to 1 min. The enzyme inhibition was determined by preincubating the enzyme with the plant extract or standard phytochemicals prior to determining its 12-LOX activity. The results are expressed as percent inhibition of the 12-LOX activity.

Inhibition of heat-induced protein denaturation

The anti-inflammatory activity was studied by using inhibition of albumin denaturation technique [30, 31] followed with minor modifications. The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using a small amount of 1N HCl. The sample extracts were incubated at 37 °C for 20 min and then heated to 51 ° C for 20 min, after cooling the samples the turbidity was measured at 660 nm. The experiment was performed in triplicate. The Percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inibition (\%)} = \frac{(\text{OD of control} - \text{OD of sample})}{\text{OD of control}} \times 100$$

***Proteinase Inhibitory Action*:** The test was performed according to the modified method [32]. The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml 20 mmol Tris-HCl buffer (pH 7.4) and 1 ml test sample of different concentrations (100-500 µg/ml). The mixture was incubated at 37 °C for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged, and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated.

$$\text{Percentage inibition (\%)} = \frac{(\text{OD of control} - \text{OD of sample})}{\text{OD of control}} \times 100$$

***hRBC (human red blood cell) membrane stabilization act*ivity**

The test was performed according to the method of Azeem [33]. Different concentration of extract (100-500 µg/ml), reference sample, and control were separately mixed with 1 ml of phosphate buffer, 2 ml of hyposaline and 0.5 ml of hRBC suspension. Diclofenac sodium (100 µg/ml) was used as a standard drug. All the assay mixtures were incubated at 37 °C for 30 min and centrifuged at 3000 rpm. The supernatant liquid was decanted, and the hemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in control as 100%.

$$\text{Percentage inibition (\%)} = \frac{(\text{OD of control} - \text{OD of sample})}{\text{OD of control}} \times 100$$

Screening of antimicrobial activity

The antibacterial activity was carried out by employing 48 h cultures of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The activity of aqueous and methanolic extracts of different concentrations 60, 70, 80 % of *L. aspera* was tested separately using Agar well diffusion method. The medium was sterilized by autoclaving at 120 °C (15 lb/in^2). About 30 ml of the Agar medium was poured in respective sterile Petri plates and were left at room temperature for solidification. 1 ml of the respective test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. A well of 6 mm diameter was made using a sterile cork borer. The standard drug and extracts were placed in the well. Antibacterial assay plates were for overnight incubation. Ciprofloxacin (50 µg/disc) was used as a positive control for antibacterial activity. After 24 h of incubation at 37 °C, Zone of Inhibition (ZOI) was observed and diameter measured.

Statistical analysis

Data of *in vitro* assays recorded were analyzed using Microsoft Excel to determine IC50. One-way analysis of variance (ANOVA) was conducted and *P* value less than 0.05 was considered as significantly different.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Secondary metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, anticholinergic, antileprosy activities, etc. [34]. The qualitative analysis of extracts of *L. aspera* was carried out and extracts showed the presence of various chemical constituents such as alkaloids, carbohydrates, saponins, glycosides, steroids, flavonoids, phenolics and tannins (table 1).

Our results indicate the presence of phytochemicals in the order of methanolic>aqueous extract (table 1). This shows a high level of its possible medicinal value. Our results are similar to those reported in *Tinospora cordifolia* [35]. The presence of tannins in *L. aspera* has been attributed to the plant's ability to hasten the healing of wounds and inflamed mucous membranes. Flavonoids are free radical scavengers that prevent oxidative cell damage, possess strong anticancer activity and diabetes-induced oxidative stress [36]. Saponins, seen only in the methanolic extract possess the unique property of precipitating and coagulating red blood cells [37]. Steroids are known to regulate the immune response as well as reduce cholesterol [38]. The relatively higher amounts of alkaloids seen in methanolic extract have been implicated for its diuretic role and in the reduction of appetite [39]. Thus preliminary phytochemical screening has shown the presence of antioxidant components and other bioactive compounds which might be responsible for the use of the plant in ameliorating inflammatory ailments [40].

Table 1: Phytochemical investigation of *L. aspera* leaves (n=3)

S. No.	Phytochemical test	Reagents used (test performed)	Results	
			Aqueous extract	Methanolic extract
1	Alkaloid test	Mayer's test	+	++
		Wagner's test	+	++
		Dragendoroff's test	+	++
2	Carbohydrate test	Molish's test	+	+
		Benedict's test	+	+
		Fehling's test	+	+
3	Saponin test	Foam test	-	+
4	Glycosides test	Borntrager's test	+	++
5	Steroid test	Salkowski test	+	+
6	Flavonoid test	Lead acetate test	+	++
7	Proteins and amino acids	Xanthoproteic test	+	+
8	Tannins test	Ferric chloride test	+	++
9	Terpenoids test	Salkowski test	-	-

-indicates absence,+denotes average,++means abundance of phytochemicals

Total phenolic, flavonoid contents, and DPPH scavenging potential

The role of phenolics in biological activity has been reported by several researchers [41]. They are a good electron and hydrogen atom donors having the ability to terminate radical chain reaction involved in lipid peroxidation by converting free radicals and reactive oxygen species to more stable products. The content of phenolic compounds in the methanolic extract was calculated using the standard curve of Gallic acid ($y = 1.03x-0.0067$; $R^2 = 0.999$) and is expressed as Gallic acid equivalent (GAE) per gram of plant extract (fig. 1A).

The total phenolic contents of *L. aspera* extract was found to be 2.25±0.04 mg GAE/g of fresh weight tissue (table 2). Consumption of leaves of *L. aspera* rich in these polyphenolic constituents may have the property to counteract oxidative stress related disorders.

Total flavonoid content was calculated using the standard curve of quercetin (y = 0.0035x-0.0086; R^2 = 0.988) (fig. 1B) and found to be 1.2±0.05 mg QE/g of fresh weight tissue (table 2).

In the quantitative phytochemical analysis, phenolic content was much more than the flavonoid content (table 2). Flavonoids have been implicated for its role in preventing lipid peroxidation, in iron-chelation and free radical scavenging [42-44].

Total antioxidant capacity of the extracts was calculated using % inhibition against concentration of ascorbic acid (y = 0.8561x; R^2 = 0.826) (fig. 1C). At 100 µg/ml concentration, *L. aspera* (40 %) exhibited maximum DPPH radical scavenging activity that was found to be 80.69±3.68 (table 2). The presence of secondary metabolites in the extract resulted in a significant decrease in *in vitro* DPPH/ concentration by reducing the stable DPPH radical to a yellowish colored diphenyl picrylhydrazine derivative [45].

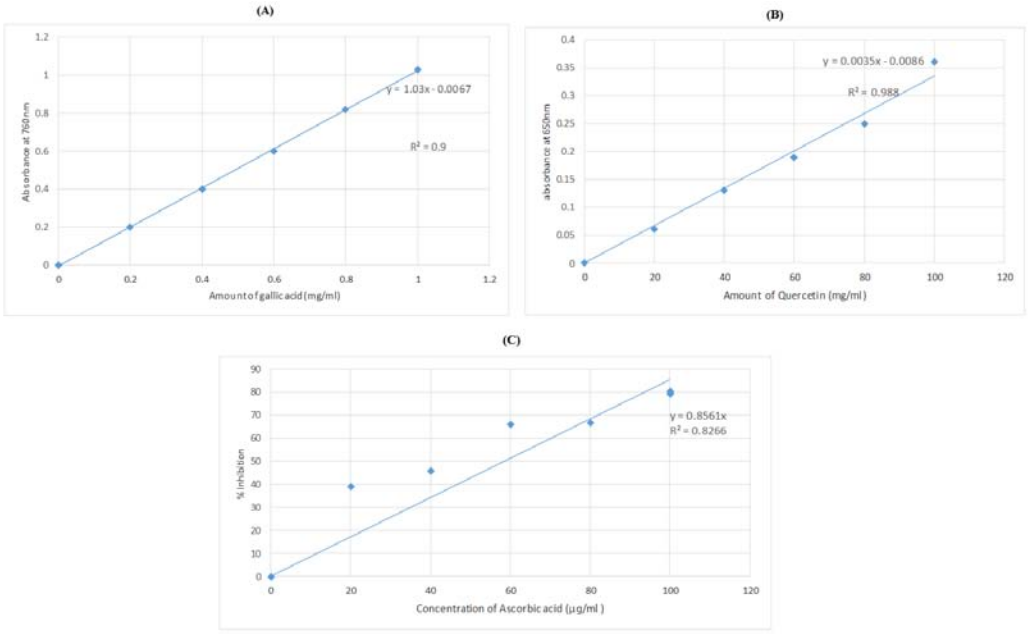

Fig. 1: **Calibration curve for gallic acid (A), quercetin (B) and DPPH radical scavenging activity (C)**

Table 2: Quantitative analysis of total phenols, total flavonoids, and antioxidant potential of methanolic extract of *L. aspera* leaves

Sample	Total phenols (mg GAE/g Fwt)	Total flavonoids (mg QE/g Fwt)	DPPH (% Inhibition)
L. aspera	2.25±0.04	1.2±0.05	80.69±3.68

Results are mean±SEM obtained from three replicates (n=3); GAE–Gallic acid equivalents; QE–Quercetin equivalents

H_2O_2 scavenging activity

Hydrogen peroxide is an important reactive oxygen species because of its ability to penetrate biological membranes. The toxicity of the hydroxyl radicle is reduced by scavenging of H_2O_2 by phenolics present in the plant extracts. The extract was capable of scavenging hydrogen peroxide in a concentration-dependent manner. The percentage inhibition (% inhibition) at various concentrations of the *L. aspera* extracts as well as standard Ascorbic acid (100-400 µg/ml) was calculated. The IC50 values were found to be 224.6 µg/ml.

Lipoxygenase (LOX) inhibiting activity

The *in vitro* inhibition of lipoxygenase an equivalent to the 'arachidonic acid cascades' in animals serves as a good model for screening the anti-inflammatory potential of plants [46, 47]. LOX inhibits lipid hydroperoxide formation due to scavenging of lipidoxy or lipid peroxy radical formed in the course of enzyme peroxidation. Methanolic extracts of *L. aspera* leaves were studied at 100-400 µg/ml, the strongest inhibition being recorded at 400 µg/ml (table 3). The IC50 values were found to be 328.2 µg/ml (Ascorbic acid) and 389.4 µg/ml. The results obtained from studies on the leaf samples have shown a potential anti-inflammatory activity and may be used in various related physiological studies, aging and diseases such as cancer, neurological disorder, etc.

Inhibition of heat induced albumin denaturation

Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammatory activity, the ability of the extract to inhibit protein denaturation was studied. *L. aspera* was found to be effective in inhibiting heat induced albumin denaturation with a maximum inhibition of 69 % observed at 400 µg/ml against that of 67.3 % for aspirin (table 3). The IC50 values were found to be 255.5 µg/ml (Ascorbic acid) and 249.62 µg/ml (*L. aspera*).

Proteinase inhibitory action

Proteinases have been implicated in arthritic reactions while leukocytes proteinase plays an important role in the development of tissue damage during inflammatory reactions. Proteinase inhibitors were found to provide protection against such inflammation-induced damage [48]. *L. aspera* leaf extracts exhibited significant antiproteinase activity at different concentrations as shown in table 3. Maximum inhibition of 50.4% at 400 µg/ml for *L. aspera* against that of 77.6% for Aspirin. The IC50 values were found to be 216.0 µg/ml (Ascorbic acid) and 421.6 µg/ml (*L. aspera*).

hRBC membrane stabilization activity

Stabilization of RBCs membrane was studied to further establish the mechanism of anti-inflammatory action of the extract of *L. aspera* as the RBC membrane is analogous to the lysosomal membrane [49]. Acute or chronic inflammation occurs when the lysosomal enzymes enter the extracellular matrix. Membrane stabilization thus prevents further tissue inflammation. Inhibition of 64.1% for *L. aspera* against that of 71.3% for Aspirin was obtained. The IC50 values were found to be 206.2 µg/ml (Ascorbic acid) and 206.7 µg/ml (*L. aspera*). Research on the bioactivity of tannins, flavonoids and saponins have demonstrated their ability to bind to cations resulting in the stabilization of *in vivo* and *in vitro* lysosomal and RBC membranes as well as other biological molecules [50, 51].

Antioxidant potentials of aqueous extract obtained from leaves of *L. aspera* were evaluated. The extracts were found to possess anti-inflammatory and antioxidant effects, as determined by protein denaturation, membrane stabilization, proteinase inhibition assay, scavenging effect on the DPPH and LOX inhibition. In general, results indicated that the extract possess potent bioactivities due to the presence of substantial amount of phenolics, flavonoids, saponins and tannins.

Table 3: Evaluation of *In vitro* anti-inflammatory activity of *L. aspera* extracts. Aspirin was used as a standard drug. Linear regression analysis was used to calculate IC50 value

Sample	Conc. (µg/ml)	H_2O_2 scavenging activity		LOX inhibiting activity		Inhibition of albumin denaturation		Proteinase inhibition		hRBC stabilization	
		% inhibition	IC_{50} (µg/ml)	% inhibition	IC_{50} (µg/ml)	% inhibition	IC_{50} (µg/ml)	% inhibition	IC_{50} (µg/ml)	% stabilization	IC_{50} (µg/ml)
Ascorbic acid standard	100	18.4	227.6	20.22	328.2	34.2	255.5	34.7	216.0	39.1	206.2
	200	29.9		32.65		49.2		56.2		51.2	
	300	33.5		48.92		56.5		67.3		64.2	
	400	54.2		56.92		67.3		77.6		71.3	
L. aspera	100	26.0 ±0.7	244.6	18.6± 0.6	356.37	32.1± 1.03	249.62	16.6± 2.96	421.6	32.1±1.4	206.7
	200	45.2 ± 2.8		27.3± 0.8		46.4± 0.01		23.3±9.26		44.3±3.1	
	300	50.8 ± 4.3		39.6± 0.5		53.6 ±2.36		33.4± 6.51		59.6±2.4	
	400	81.7 ± 5.6		58.4± 2.2		69.2 ±5.09		50.4±5.39		64.1±3.5	

Results are mean±SEM (P<0.05), obtained from three replicates.

Antimicrobial activity

In our study, the antimicrobial activity of leaf extracts of *L. aspera* was evaluated by disc diffusion method (table 4). Three different doses (60, 70, 80 %) of aqueous extracts were used in the test and compared with the positive control ciprofloxacin. The antimicrobial potential of *L. aspera* leaf extracts was compared based on their zone of inhibition against the gram positive (*S. aureus*) and gram negative bacteria (*P. aeruginosa, K. pneumonia* and *E. coli*).

Among gram-negative bacteria, aqueous extract of *L. aspera* showed maximum inhibition towards *P. aeruginosa* (20 mm) and nil inhibition for *E. coli*. 70 % extract showed a zone of inhibition of 29 mm against *S. aureus* (table 4).

Table 4: Antibacterial activity of *L. aspera* extracts

Strains	*L. aspera* extract				Ciprofloxacin
	60%	70%	80%	UD	
	Mean zone of inhibition[a] (mm)[b]				
S. aureus	14±0.2	29±2.1	13±0.2	10±0.2	22±1.6
E. coli	NA	NA	NA	NA	18±1.0
K. pneumoniae	10±0.9	13±1.8	13±2.1	10±0.3	14±1.0
P. aeruginosa	13±2.6	20±1.4	13±0.9	13±1.1	16±1.2

[a]-diameter of the zone of inhibition (mm) including the disc diameter of 6 mm; [b]-mean of three assays; ±-standard deviation; NA–No activity, UD–undiluted extract

Dilution seemed to increase the antibacterial activity of *L. aspera* when compared to the undiluted extract. Nevertheless, the inhibition zone produced by the commercially available positive control was larger than those produced by both the extracts. This type of activity gives an indication of the presence of antimicrobial compounds with broad spectrum or simply general metabolic toxins [52]. Further work is necessary to isolate and purify compounds in the leaf extracts, which will allow the scientific community to recommend their utilization as an accessible alternative to synthetic antibiotics.

CONCLUSION

Results indicate that L. aspera possess anti-inflammatory properties due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins and steroids that serve as free radical inhibitors or scavenger. Compounds of the plant L. aspera may hence be used as lead compounds for designing potent anti-inflammatory drug which can be used for treatment of various diseases.

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CONFLICTS OF INTERESTS

Declared none

REFERENCES

1. Chopra RN, Nayar SL, Chopra IC. In: Glossary of Indian medicinal plants. Vol. I. Council of Scientific and Industrial Research, New Delhi; 1956. p. 197.
2. Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. Life Sci 2005;78:431-41.
3. Tambe VD, Tambe VB, Jadhav RS. Evaluation of *Leucas aspera* whole plant extracts for the diuretic and laxative property. Asian J Pharm Clin Res 2016;9:118-21.
4. Hedge IC. Labiatae. In: Ali SI, Nasir YJ. editors. Flora of Pakistan. Karachi: University of Karachi Department of Botany; 1990. p. 192.
5. Santosh KM, Anil KS, Ankit S. Potential medicinal plants for lymphatic filariasis: a review. J Crit Rev 2015;2:1-6.
6. Girach RD, Aminuddin, Siddioui PA, Khan SA. Traditional plant remedies among the Kondh of district dhenkanal (Orissa). Int J Pharm 1994;32:274–83.
7. Bhandary MJ, Chandrasekhar KR, Kaveriappa KM. Medical ethnobotany of the siddis of Uttara Kannada district, Karnataka. India J Ethnopharmacol 1995;47:149–58.
8. Selvanayahgam ZE, Gnanevendhan SG, Balakrishna K, Rao RB. Antisnake venom botanicals from ethnomedicine. J Herbs Spices Med Plants Plants 1994;2:45–100.
9. Khaleque A, Huq ME, Huq MS, Mansoor MH. Chemical investigations on leucas aspera. I. Isolation of compound a, beta-sitosterol and alpha-sitosterol from the aerial parts. Sci Res 1970;7:125–7.
10. Kapur SK, Sarin YK. Medico-botanic survey of medicinal and aromatic plants of Katra valley (J. and K. state) India. Indian Drugs 1984;2:4–10.
11. John D. One hundred useful raw drugs of the kani tribes of Trivandrum forest division, Kerala, India. Int J Crude Drug Res 1984;22:17–39.

12. Alby AB, Regi RK. Evaluation of the folk claim and identification of the active pharmacological principles in *Bauhinia phoenicea* leaves. Asian J Pharm Clin Res 2016;9:1-4.
13. Mukherjee PK, Saha K, Murugesan T, Mandal SC, Pal M, Saha BP. Screening of anti-diarrhoeal profile of some plant extracts of a specific region of west Bengal, India. J Ethnopharmacol 1998;60:85–9.
14. Saha K, Mukherjee PK, Das J, Mandal SC, Pal M, Saha BP. Hypoglycemic activity of *Leucas lavandulaefolia* Rees. In streptozotocin-induced diabetic rats. Phytother Res 1998;11:463–6.
15. Mangathayaru K, Grace FX, Bhavani M, Meignanam E, Karna RS, Kumar PD. Effect of *Leucas aspera* on hepatotoxicity in rats. Indian J Pharmacol 2005;37:329–30.
16. Krishnaraju AV, Rao TV, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. Int J Appl Sci Eng 2005;3:125–34.
17. Muthukrishnan J, Puphlantha H, Kasthuribhai K. Biological effects of four plants extracts on *Culex quinquefasciatus* Say. Larval stages. Insect Sci Its Appl 1997;17:389–94.
18. Okeke IN, Klugman KP, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF. Antimicrobial resistance in developing countries. Part II: strategies for containment. Lancet Infect Dis 2005;5:568–80.
19. Abiramasundari P, Priya V, Jeyanthi GP, Gayathri DS. Evaluation of the antibacterial activity of cocculus hirsutus. Hygeia: J Drugs Med 2011;2:26-31.
20. Sandosskumar R, Karthikeyan M, Mathiyazhagan S, Mohankumar M, Chandrasekar G, Velazhahan R. Inhibition of *Aspergillus flavus* growth and detoxification of aflatoxin B1 by the medicinal plant zimmu (*Allium sativum* L. x *Allium cepa* L.). World J Microbiol Biotechnol 2007;23:1007-14.
21. Gupta SK, Banerjee AB. Screening of selected West Bengal plants for antifungal activity. Ecol Bot 1972;26:255-9.
22. Sofowara A. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd. Ibadan: Nigeria; 1993. p. 289-300.
23. Harborne JB. Phytochemical Methods. Chapman and hall Ltd, London: UK; 1973. p. 49-188, 11.
24. Ogbuewu IP. Physiological responses of rabbits fed graded levels of neem (*Azadirachta indica*) leaf meal. Federal University of Technology: Owerri; 2008.
25. Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. Am J Enol Vitic 1977;28:49-55.
26. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002;10:178-82.
27. Braca A, Sortino C, Politi M. Antioxidant activity of flavonoids from *Licania licaniaeflora*. J Ethnopharmacol 2002;79:379-81.
28. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 1989;10:1003-8.
29. Axelrod BC, Cheesbrough TM, Laasko SL. Lipoxygenase from soybean. Methods Enzymol 1981;71:441-51.
30. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J Pharm Pharmacol 1968;20:169-73.
31. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharm Pharmacol Sci 2010;2:146-55.
32. Oyedepo OO, Femurewa AJ. Anti-protease and membrane stabilizing activities of extracts of Fagra zanthoxiloides, Olax subscorpioides and *Tetrapleura tetraptera*. Int J Pharmacogn 1995;33:65-9.
33. Azeem AK, Dilip C, Prasanth SS, Junise V, Hanan S. Anti-inflammatory activity of the glandular extracts of *Thunnus alalunga*. Asia Pac J Med 2010;3:412-20.
34. Negi JS, Singh P, Rawat B. Chemical constituents and biological importance of swertia: a review. Curr Res Chem 2011;3:1-15.
35. Shwetha RJ, Tahareen S, Myrene RD. Antioxidant and anti-inflammatory activity of *Tinospora cordifolia* using *in vitro* models. J Chem Biol Phys Sci 2006;6:497-512.
36. Rio DA, Obdululio BG, Casfillo J, Marin FG, Ortuno A. Uses and properties of citrus flavonoids. J Agric Food Chem 1997;45:4505-15.
37. Okwu DE. Phytochemicals and vitamin content of indigenous spices of southeastern Nigeria. J Sustain Agric Environ 2004;6:30-7.
38. Shah BA, Qazi GN, Taneja SC. Boswellic acids: a group of medicinally important compounds. Nat Prod Rep 2009;26:72-89.
39. The United States Department of Agriculture. Center for Nutrition Policy and Promotion. Dietary Guidelines for Americans. National Academy Press: Washington DC: USA; 2010.
40. Ikyenge BA, Odiniya EO, Okhale SE. Preliminary phytochemical and pharmacognostic investigation of laggerapter odonta (DC) Sch. Bip. Int J Tradit Nat Med 2010;1:1-7.
41. Amarowicz R. Tannins: the new natural antioxidants? Eur J Lipid Sci Technol 2007;109:549–51.
42. Veitch NC. Isoflavonoids of the leguminosae. Nat Prod Rep 2007;24:417-64.
43. Jiang H, Zhan WQ, Liu X, Jiang X. Antioxidant activities of extracts and flavonoid compounds from *Oxytropis falcate* Bunge. Nat Prod Res 2008;22:1650-6.
44. Kim HP, Son HK, Chang HW, Kang SS. Anti-inflammatory plant flavonoids and cellular action mechanisms. J Phamacol Sci 2004;96:229-45.
45. Thabrew MI, Hughes RD, McFarlane IG. Antioxidant activity of *Osbeckia aspera.* Phytother Res 1998;12:288–90.
46. Gardner HW. Recent investigations into the lipoxygenase pathway of plants. Biochim Biophys Acta 1991;1084:221–39.
47. Abad MJ, Bermejo P, Villar A. The activity of flavonoids extracted from *Tanacetum microphyllum* DC. (Compositae) on soybean lipoxygenase and prostaglandin synthetase. Gen Pharmacol 1995;26:815–9.
48. Das SN, Chatterjee S. Long-term toxicity study of ART-400. Indian Indg Med 1995;16:117-23.
49. Shenoy S, Shwetha K, Prabhu K, Maradi R, Bairy KL, Shanbhag T. Evaluation of anti-inflammatory activity of *Tephrosia purpurea* in rats. Asian Pac J Trop Med 2010;3:193-5.
50. El-Shabrany OA, El-Gindi OD, Melek FR, Abdel-Khalk SM, Haggig MM. Biological properties of saponin mixtures of *Fagonia cretica* and *Fagonia mollis*. Fitoterapia LXVIII 1997;68:219-22.
51. Saad S, Taher M, Susanti D, Qaralleh H, Awang AF. *In vitro* antimicrobial activity of mangrove plant *Sonneratia alba*. Asian Pac J Trop Biomed 2012;2:427–9.
52. Mohammed AA, Khalil AA, El-Beltagi HE. Antioxidant and antimicrobial properties of kaff maryam (*Anastatica hierochuntica*) and doum palm (*Hyphaene thebaica*). Grases Y Acites 2010;61:67–75.

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