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Phytochemical screening, *In-vitro* Wound healing, Antifungal and Antimicrobial activity of *Leucas aspera* and *Leucas indica*

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Abstract

*Leucas **a**spera* commonly known as Thumbai is distributed throughout India from the Himalayas down to ceylon. The plant is used traditionally as an antipyretic and insecticide. Medicinally, it has been proven to possess various pharmacological actions like anti-fungal, antioxidant, antimicrobial, and cytotoxic activity. Further studies reveal the presence of various phytochemical constituents mainly triterpenoids, nicotine, sterols, glucoside, diterpenes, phenolic compounds (4-(24-hydroxy-1-oxo-5-propyltetracosanyl)-phenol). These studies reveal that *Leucas aspera* is a source of medicinally active compounds and have various pharmacological effects; hence this drug encourage finding its new therapeutic uses.

Leucas indica belongs to the family Lamiaceae also known as Guma, Tumba, Dandokalos. Traditionally it is a Garhwal region of Uttarakhand as a wound healer. The leaf of this plant are squeezed and placed on wounds to acquire wound healing. Leaves are also used as vermifuge, stomachic, sedation and in sores.A new phenylethanoid glycoside 2-(3,4-dihydroxyphenyl)-ethyl-*0*-L-rhamnopyranosyl-(1--3)-*0*-A-rhamnopyranosyl-(1--6)-4-*0*-E-feruloyl-B-D-glucopyranoside(3-*0*-methyl poliumoside,1)along with five known phenylethanoid glycosides (2-6)were isolated from the aerial parts of *Leucas indica linn.* The structure of component 1 has been elucidated on the basis of spectral data.

Keyword: Antimicrobial activity; *Leucas aspera;* Triterpinoids; *Leucas indica Linn;* Phytochemistry; Pharmacology; Wound healing

1. Introduction

Leucas aspera is also known as Thumbai is distributed throughout India from the Himalayas down to Ceylon. The plant is used traditionally as an antipyretic and expectorant, aperient, diaphoretic, insectide and emmenagogue. SLeaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. Bruised leaves are applied locally in snake bites.

1.1. Other names

- Telugu: Thumbai
- English: *Leucas aspera*
- Hindi: Goma madhupati
- Sanskrit: Dronapushpi, Chitrapathrika, Chitrak-shupa

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Figure 1 Fresh and dried leaves of *Leucas aspera*

1.2. Taxonomical classification

Table 1 Leucas aspera Taxonomy

Kingdom	**Plantae**
Sub-kingdom	Tracheobionta, Vascular plant
Super division	Spermatophyta, Seed plant
Division	Angiosperma
Class	Dicotyledon
Sub- class	Gamopetalae
Series	Bicarpellatae
Order	Tubiflorae
Family	Lamiaceae
Genus	*Leucas*
Species	*aspera*

1.3. Botanical description

Leucas aspera is an annual, branched, herb erecting to a height of 15-60 cm with stout and hispid actually quadrangular stem and branches. Leaves are sub-sessile or shortly petiolate, linear or linearly lanceolate ,obtuse, pubescent up to 8.0 cm long and 1.25 cm broad, with entire or crenate margin; petiole 2.5-6 mm long ;flowers white , sessile small, in dense terminal or axillary whorls; bracts 6 mm long, linear, acute, bristle-tipped, ciliate with long slender hairs; calyx variable, tubular, 8-13 mm long ;tube curved ,contracted above the nutlets ,the lower half usually glabrous and membranous, the upper half ribbed and hispid; mouth small , very oblique, not villous, the upper part produced forward; teeth small; triangular, bristle-tipped ,ciliate 1cm long; tube 5 cm and pubescent above , annulate in the middle ;upper lip 3mm long ;densely white -woolly ;lower lip about twice as long , the middle lobe obviate , rounded , the lateral lobes small , subacute .fruit nutlets ,2.5 mm long , along ,brown , smooth , inner face angular and outer face round.

Figure 2 Fresh leaves of *leucas aspera*

1.4. Microscopic description

TS of young stem is quadrangular in outline with four distinct collen chymatous ridges, covered by hairs. It shows narrow cortex and a ring with vascular tissue enclosed with the wide stele.

Detailed TS shows an epidermis covered with thick cuticle , traversed occasionally with stomata and bears simple multicellular (three to four-called) uniseriate lignified trichomes and sessile , glandular trichomes with multicellular head; narrow parenchyma cortex , except under the ridges where it is collenchymatous,distinct endodermis and parenchymatous pericycle especially; stellar region consisting of a ring of vascular bundles co…nnected with interfascicular sclerenchymatous band ;very narrow parenchymatous phloem , and radially arranged xylem tissue.

In mature stem, trichomes are few, phloem tissue is wide and found on opposite side of the wide xylem band; pith is parenchymatous, wide and implanted with acicular crystals of calcium oxalate.

1.4.1. Leaf

TS of leaves passing through the midrib it is broadly convex on the lower side and slightly grooved or flat on the upper side, a centrally detected conjoint and collateral meristele associated with a parenchymatous pericycle layer on lower collenchymatous tissue underneath both the epidermis dorsiventeral lamina epidermis covered with thick cuticle traversed with stomata" bears simple and glandular trichomes of the same type as found on stem ,1to2layers palisade tissue occupying the major area of the section and spongy parenchyma .

1.5. Phytochemical studies

Preliminary chemical examination of *L.aspera* revealed presence of triterpenoids in entire plant.whole plant is reported to contains oleanolic acid,ursolic acid and 3-sitosterol.Aerial parts are reported to contain nicotine, sterols ,two new alkaloids (compound A m.p.183-4), reducing sugars (galactose), glucoside , diterpens isopimarane glycosides (leucasperosides A,B and C , together with other compounds like asperphenamate , maslinic acid ,(-)- isoliolide, linifolioside , nectandrin B , meso dihydroguaiaretic acid , macelignan , acacetin, apigenin , nectandrin B , mesodihydroguaiaretic acid , macelignan , acacetin apigenin 7-0-(6-0(p-coumaroyl)-3-D-glucoside),chrysoeriol apigenin , erythro-2-(4-ally-2-6-dimethoxyphenoxy)-1-(4-hydroxy -3- methoxyphenyl)propan-1-ol, myristargenol B , and machilin C ,(-)-chicanine , (7R,8R)- and (75,85)- licarin A .

1.5.1. Leucas indica

Medicinal plants have a great importance in human being as it shows diverse pharmacological properties and have attained a significant position in health systems all over the world an antimicrobial is a substance that kill or inhibit the growth of micro-organisms such as bacteria, fungi, protozoa or viruses. History of anti-microbials with the observation of Pasteur and Joubert,who discovered one type of bacteria could prevent the growth of another. phytochemicals may minimize the production of reactive oxygen species ROS or it may protect itself from deleterious effect of ROS by efficiently scavenging of ROS (Reactive oxygen species).

1.6. Taxonamical studies

Table 2 *Leucas indica* taxonomy

Kingdom	**Plantae**
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Lamiales
Family	Lamiaceae
Species	*indica*
Genus	*Leucas*

1.7. Botanical description

Leucas indica are diffuse, aromatic annual herb having length 16-60cm. Leaves of this plant contain 0.5-1.0 cm long petiole having lamina 6.0-9.0 *0.5-2.5 cm which is linear or narrowly oblong-lanceolate or elliptic-lanceolate .The shape of the leaves are acute having a narrow base, margin entire, more or less pubescent on both surfaces. Inflorescence whorls of this herb are terminal and axillary, often the whorls of the successive nodes join to form a cylindrical spike. Bract 0.6 cm long, almost equal to the calyx, tip spinulose, pubescent. Calyx 0.8 cm long, tubular, curved, constricted above the nutlets, usually smooth and glabrous below, ribbed and scabrid above, mouth very oblique, produced on the upper side, teeth dhort, triangular, spinulose, the upper one longest, pubescent within only above the nutlets. Nutlets 0.3*0.1 cm, oboviod-oblong, angular on the inner face, rounded on outer, smooth**,** brownish black. Flowering and fruiting is almost throughout the year, but especially during September to march.

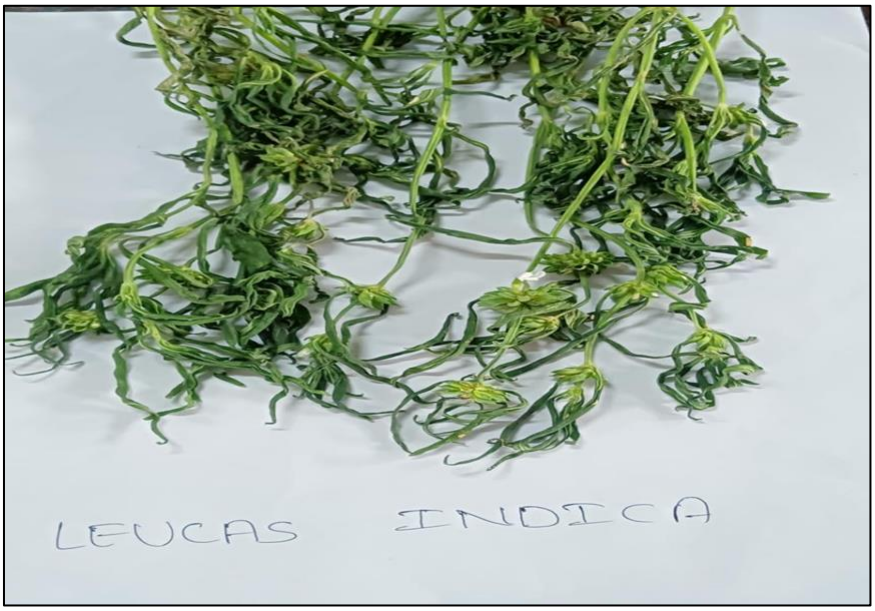

Figure 3 *Leucas indica*

1.8. Morphological effect

It belongs to the genus kshava. It means less height. In this the small thummi almost touches the ground and the big thummi grows up to one foot in height and has the same shape grows abundantly in the monsoons and in the sesame fields. The leaves of this plant have a slight smell and pungent fragrance and each node of plant has round cluster like a small flower ball and small white flowers are covered in it and these flowers are used to worship lord Shiva.

1.9. Materials required

The aerial parts, leaves and flowers of *Leucas aspera* and *Leucas indica Linn* were collected from venkatachalam, Nellore district, Andhra Pradesh, India, in the month of September to November, 2024. Plant materials were authenticated by faculty of department of pharmacognosy.

Leaves, flowers and other parts of plants are cleaned, shade dried, mechanically grinded. The coarse powder were taken separately by sieving and can be stored in an air tight container for use.

A	B

Figure 4 A *Leucas Aspera*; B *Leucas indica*

2. Methodology of leucas aspera and leucas indica

The dried extract is dissolved in DMSO (dimethyl sulfoxide) to acquire sample solution of 1mg/ml concentration. Aqueous extract is dissolved in distilled water at 1mg/ml of concentration.

2.1. Qualitative phytochemical analysis

Qualitative analysis to detect various phytochemical constituents were performed using standard methods.

2.1.1. Quantitative phytochemical analysis

The phenol content (TPC) of the sample extracts was estimated following the method described by Malik and Singh, (1980). The aluminum chloride method was used for determination of total flavonoid content of the sample extracts as described by Mervat and Hanan (2009).

2.1.2. Antioxidant activity study

DPPH radical seavenging activity was determined by the method described by Anti-Stanojevic et al ;(2009).

2.1.3. Antimicrobial activity study

Antimicrobial activity of bacterial strains are carried by agar well diffusion method as defined by Nair et al (2005) using 6mm borer.

Gram positive and gram-negative bacterial strains and fungal strains are used in this experiment to know the antimicrobial activity of the sample extracts.

2.1.4. GC-MS analysis

50 grams of methanol extracts was taken in a conical flask and broad fractionation was done using magnetic stir with the simultaneous use of solvents - hexane, chloroform, acetone, ethyl acetate and methanol these fractions were kept separately for further work.

The hexane broad fraction shows clear bands in TLC it is selected for represented by GC-MS analysis. The analysis was done Bombay, IIT, SAIF.

2.1.5. Acute toxicity study

This study was carried out and suggested by Ganapaty et al. Swiss albino mice of either sex weighing between 25 and 30g were divided into different groups comprising 6 animals each. The control groups received normal saline (2ml/kg, post-operative the other groups received 100, 200, 300, 600, 800, 1000, 2000, 3000 and 4000mg/kg of the test extracts, respectively. Immediately after dosing, the animals were observed continuously for the first four hours for any behavioural changes.

2.2. Leucas aspera preparation

- Decoction: Boil 1-2 table spoons of dried *leucas aspera* in 400ml of water for 5-10 mins.
- Infusion: Steep 1 teaspoon of dried leaves or flowers in 200ml boiling water for 5-10mins.
- Powder: mix 1/2 teaspoon of powdered *leucas aspera* with water or honey.
- Oil: mix *leucas aspera* leaves with coconut or sesam oil for topical application.

2.3. Phytochemical analysis

Extract bio active compounds using solvents (methanol, etnanol or

2.3.1. Pharmacological evaluation

- *In vitro* studies: Assess anti-microbial, anti-oxidant and anti-inflammatory activities.
- *In vivo* studies: Evaluate analgesic, anti-pyrutic and anti-diarrheal effects.

2.3.2. Toxicity and safety evaluation:

- Acute toxicity studies: Assess LD50 values
- Sub-chronic toxicity studies: Evaluate hematological and biochemical parameters.

3. Pharmacological studies

3.1. Leucas aspera activities:

3.1.1. Antifungal activity:

In vitro study of chloroform and ether extracts of *Leucas aspera* reaveled its antifungal activity against Trichophyton and Microsporum gypseum. The minimum inhibitory concentration was found to be 5mg/ml. *Leucas aspera* as both anti fungistatic and fungicidal actions.

3.1.2. Prostaglandin inhibitory and Anti oxidant activities:

Leucas aspera showed both prostaglandin inhibitory and antioxidant activities at 3-4g/ml in guinea pig ielum against PGE-1 and PGE-2 induced concentration and 1,1diphenyl-2-picrylhydrazyl (DPPH) radical sceavenging effect.

3.1.3. Antimicrobial activity of Leucas aspera flowers and some essential oils:

Antibacterial activity was seen in the methanolic extract, its fractions alkaloidal residue and the flower juice of *Leucas aspera*, with maximum activity for alkaloidal residue.

In this study alkaloids, triterpenoids and flavonoid components present in the *Leucas aspera* showed that this can be used as antimicrobial agents and also as an ingredients in the different antioxidant formulations.

Bacteriostatic activity was showed by essential oils from *Leucas aspera* against Staphylococcus aureua, Pseudomonas pyocyanea, Escherichia coli, Vibrio cholerae, Proteus vulgaris, Dys. Flexneri, Salmonella typhi, and Klebsiella aerogenes.

3.1.4. Hepatoprotective activity

The methanol and petroleum ether extracts of *Leucas aspera* was evaluated for hepatoprotective potential by paracetamol and thoacetamide in induced hepato toxicity model. The results showed that the root extracts of *Leucas aspera* possess hepatoprotective potential.

3.1.5. Analgesic activity

The extract of *Leucas aspera* was evaluated for the antinociceptive activity in animal models. The study showed significant inhibition of writhing response in the acetic acid induced by model. The extract also produced importantly to increase in the latency in the hot plate test in a dose related manner.

3.1.6. Anti-diabetic activity

The study concluded that *Leucas aspera* leaf extracts as shown anti-diabetic potential in Streptozotocin-induced diabetic wistar albino rats invivo model.

3.1.7. Antihelmintic and cytotoxic activity

In this study the methanolic crude extract of *Leucas aspera* have shown significant cytotoxic activity and study aldo showed mild antihelmentic activity against the Phertima prosthuma in comparision with standard albendazole.

3.1.8. Anti-asthmatic activity

In this study the methanolic extract of whole plant of *Leucas aspera* showed signifiant anti histamine, branchodilatory, anti-inflammatory, mast cell stabilizing, anti-allergic and anti-spasmodic activity in various in-vivo and in-vitro and anti-asthmatic models and thus concluded significant anti-asthmatic activity.

3.2. Leucas indica activities

3.2.1. Antimicrobial activity

The antimicrobial activity of the crude aerial plant extract of Leucas indica in chloroform and methanolic fraction showed positive results against Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, pseudomonas aeruginosa and Escherichia coli where as the aqueous fraction inhibited the growth of Staphylococcus aureus, Bacillus subtilis and salmonella typhi significantly, its anti bacterial property is due to the presence of flavonoids, total phenolic compounds, saponins and tannins.

Babu et al reported that ethanolic extract of aerial part of the plant gives very good antimicrobial activity Saphylococcus aureus and Pseudomonas sp.

3.2.2. Wound healing activity

Saha et ai studied the wound healing activity of *Leucas indica* (Leucas lavandulaefolia). They performed the experiment on Wister albino rats. They anasthetised 6 groups having 6 animals in each and wound was excised by cutting away a 500mm2 full thickness and opened in the full environment all the groups were administered with 0.2% nitrofurazone ointment, simple ointment, methanolic extract of plant at the dose of 200mg/ml and and 400mg/mi.they found that wound closure time of extract at the dose 400mg/ml was 16+/2 was better than nitrofurazone ointment,simple ointment,and extract at dose of 200 mg/ml which were 18+/2. in another experiment by Shankar et ai similar results were obtained which proves that this plant has very good wound healing capacity.

3.2.3. Anti-ulcer properties

Gastric ulcers were produced by the oral suspention of indomethacine in male wister albino rat and swiss albino mice. Both were grouped as control, standard extract 100mg/ml and extract 200mg/ml. The control group was treated with the ulcerogenic drug for three consecutive days and standard with ulcer-preventive drug misprostal. Similarly rest of the groups were treated with 100mg/ml methanolic extract of plant. Results showed that methanolic extract gives antiulcer activity and was dose-depandant as compared to standard.

3.2.4. Antioxidant activity

Vinayagam and sudha performed the experiment for the DPPH radical scavenging assay, nitric oxide scavenging assay and superoxide scavenging assay from the methanolic leaves extract of *Leucas indica* the results showed excellent antioxidant activity due to the presence of phenolic compounds.

3.2.5. Hepatoprotective activity

Ethyl extract acetate of aerial part of the *Leucas indica* with 5% gum acacia was used to study the hepatoprotective activity of plant on CCL4 treated albino male rats. It was observed that the extract decreased that the CCL4 induced elevated enzymes level which indicated the regeneration of the hepatic cells which were earliar damged by the CCL4 treatment.

4. Result and discussion

- Petroleum ether, ethanol and isopropyl alcohol extracts of alcohol extracts of Leucas aspera leaves showed better DPPH scaverging activity.
- Isopropyl alcohol, ethanol and petroleum ether exhibited better ABTS scavenging activity and total antioxidant activity.
- This in vitro antioxidant study indicates Leucas aspera(wild)Link is a significant natural source of antioxidants and may behelpful in preventing the oxidative stree and damage to cells.
- The pharmacological activity of Leucas aspera, also known a"Thumbai" and its use in traditional medicine.
- It also mentions the presence of phytochemical constituents in the plant, such as Triterpenoids, Oleanolic acid, and Ursolic acid and bisitosterol.
- The pharmacological activity value of Leucas aspera and its use in treating various disease it also mentions the use of plants leaves to treat Psoriasis and skin eruptions, and the use of bruised leaves to treat snake bites.
- The evaluation of leaves of Leucas aspera using swiss albino mice.

5. Conclusion

- Leucas indica and Leucas aspera are both plants with medicinal properties and are used in traditional medicine to treat a variety of conditions.
- In conclusion, the study demonstrates that methanol extracts from various parts of Leucas aspera are different in their antioxidant and antimicrobial effects.
- The results indicate that the root extract possess the highest antioxidant as well as antimicrobial activity.
- However, further pharmacological and toxicity studies are necessary to confirm this suggestion.
- Phytochemicals analysis should be carried out to characterize the compounds in Leucas aspera and Leucas indica.
- This work demonstrates that Leucas aspera and Leucas indica should be considered as a useful source of material for human health, as various infection agents.
- Both the plants contain many bio-active compounds, including alkaloids, steroids, terpenoids, phenol's, tannin's, proteins, flavonoid s and saponins.
- This include many pharmacological properties.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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