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A Comprehensive Review of Traditional Uses, Phytochemistry, Pharmacological Activities, Toxicity and Research Priorities of *Leucas Aspera* (Thumbai)

Mr. M. Ponnivalavan, Dr. D. Dhachanmoorthi, K. Esakki Muthu*, Shalna Shafeek, V. Muhammed Shameem, M.S. Ajay Siddharthan

Sree Abirami College of Pharmacy, Affiliated to the Tamil Nadu Dr.M.G.R. Medical University, Coimbatore, Tamil Nadu, India

*Address for correspondence: **K. Esakki Muthu,**
Email Id: esakkimuthu191223@gamil.com

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Abstract:
Leucas aspera (Willd.) Link, belonging to the family Lamiaceae, is a widely distributed annual herb commonly known as “Thumbai” or “Thumba” in India and other parts of South and Southeast Asia. The plant has a long history of traditional medicinal use for the treatment of fever, cough, cold, asthma, respiratory disorders, inflammation, wounds, skin diseases and insect bites. Leaves, flowers and aerial parts are commonly used in traditional medicine because of their diverse therapeutic properties. Phytochemical studies have revealed the presence of several important secondary metabolites, including triterpenoids, steroids, diterpenes, flavonoids, phenolic compounds, alkaloids, tannins and essential oils. Important constituents reported from the plant include oleanolic acid, unsolid acid and β-sitosterol. Characteristic compounds such as leucasperones and leucasperols have also been identified, indicating significant chemical diversity. These constituents may contribute to the pharmacological properties of the plant. Preclinical investigations using in-vitro, in-vivo and in-silico models have demonstrated several pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antifungal, anticancer, cytotoxic, antinociceptive, antipyretic, hepatoprotective, antivenom and insecticidal effects. The antioxidant activity is mainly associated with phenolic and flavonoid compounds that can scavenge free radicals and reduce oxidative stress. Antimicrobial studies have also demonstrated inhibitory effects against various pathogenic microorganisms, supporting some of its traditional applications. Toxicological studies generally indicate relatively low toxicity at commonly investigated experimental doses; however, comprehensive human clinical evidence remains limited. Further research is therefore required to standardize extracts, identify active phytoconstituents, clarify molecular mechanisms, assess long-term toxicity and conduct well-designed clinical trials. Overall, *L. aspera* represents a promising medicinal plant with considerable phytochemical diversity and broad pharmacological potential.

Key words: *Leucas aspera,* Lamiaceae, Thumbai, Phytochemical constituents, Flavonoids and phenolic compounds Antioxidant activity, antimicrobial activity, Pharmacological potential

Introduction:

Leucas aspera is a widely available medicinal weed of the mint family (Lamiaceae), used in Ayurveda, Siddha and folk systems across India, Sri Lanka, the Philippines and other tropical regions. Its simple habit, rapid growth and abundance have made it a common component of ethnomedicinal pharmacopeia's where it is used for fever, coughs, bites, skin problems and as an insect repellent. Renewed phytochemical and pharmacological interest over the last two decades including metabolomics, nano formulation and in-silico approaches has expanded the evidence base supporting several bioactivities, while also identifying molecules amenable to further drug-discovery efforts.

Leucas Aspera Plant

Botanical description & identification:

Leaves: Opposite, sessile to short-petiole, linear to narrowly oblong-lanceolate, entire or crenate margins; up to ~8 cm long.

Biological source & family:

Botanical name: Leucas aspera (Willd.) Link.

Family: Lamiaceae (formerly Labiatae).

Distribution: Widespread in India, Sri Lanka, Philippines, parts of Southeast Asia and some Indian Ocean islands; typically, in dry, open, sandy soils and disturbed sites.

Chemical constitution:

Phytochemical investigations (chromatography, LC–MS, GC–MS, isolation and structure elucidation) have reported

Triterpenoids: oleanolic acid, unsolid acid.

Steroids: β-sitosterol.

Diterpenes & isopalmitate derivatives.

Phenolics & flavonoids: various flavanols, phenolic acids responsible for antioxidant activity.

Unique isolated constituents: leucasperones A & B, leucasperols A & B and other named metabolites from leaf/whole-plant extracts.

Essential oils: volatile terpenoids contributing to insecticidal/insect repellent actions.

Recent genus-level reviews and targeted phytochemical studies (HR-LC/MS profiling) continue to expand the compound list and enable structure-activity predictions.

Traditional / ethnomedicinal uses:

Ethnobotanical records and traditional medicine texts report uses including

- Antipyretic (fever reducer).
- Treatment of cough, cold and respiratory complaints (inhalation/steam of crushed plant).
- Topical application for bites, stings and skin ailments.
- Insecticidal/insect repellent (leaves burned or rubbed on skin).
- Remedy for headaches, rheumatism and body aches.
- Use as a green leafy vegetable in some regions (nutritional use).
- These uses motivated many pharmacological studies seeking mechanistic validation.

Common extraction methods:

1. Cold maceration:

Best for: broad crude extracts (phenolics, flavonoids, tannins). Widely used in Leucas studies. Typical solvents: methanol, ethanol, water, acetone, petroleum ether.

2. Reflux / boiling (decoction): for water-soluble constituents Best for traditional aqueous extracts, polysaccharides, some glycosides.

Practical step-by-step protocols:

These are standard lab templates used in studies of Leucas aspera; adjust scale, solvent grade, and safety controls to your lab.

A. Cold maceration:

Dry plant (shade-dry), grind to fine powder (40–60 mesh). Weigh sample (e.g., 50 g), transfer to glass bottle. Add solvent (1:10 w/v typical 50 g: 500 mL). Common solvents: methanol, ethanol, water, acetone, petroleum ether. Agitate occasionally; keep at room temperature for 24–72 h (longer for less polar solvents). Filter (Whatman #1 or similar), repeat extraction 1–2× on residue for better yield.
Pool filtrates, concentrate under reduced pressure (rotary evaporator) to dryness; store extract at 4 °C. (Used widely in L. aspera phytochemical studies.)

B. Reflux/Boiling Decoction Protocol

- Prepare Plant Material:
  - Weigh and record the plant material (e.g., 50g).
  - Grind or chop the material into small pieces.
- Prepare Solvent:
  - Measure and record the solvent volume (e.g., 500mL).
  - Mix solvent with plant material in the reflux apparatus**.**
- Set Up Reflux Apparatus:
  - Assemble the reflux apparatus.
  - Connect the condenser to a water source.

Heat and Reflux:

- Heat the mixture to boiling point.
- Maintain reflux for 30-60 minutes.
- Monitor temperature (e.g., 90-100°C).

Cool and Filter:

- Turn off heat and let the mixture cool.
- Filter the decoction through filter paper**.**

Concentrate (Optional):

- Concentrate the decoction using a rotary evaporator or by boiling off excess solvent.

Store:

- Store the decoction in a labelled container.

A. Hydro distillation (Clevenger):

Use fresh or air-dried aerial parts, coarsely chopped. Add water in flask (plant: water roughly 1:5–1:10 w/v), heat and collect hydro distillate for 3–4 h using Clevenger apparatus. Separate oil, dry over anhydrous sodium sulphate, store in amber vial at 4 °C until GC-MS. (Multiple L. aspera EO studies use this approach.)

Solvent choice: Water / hydroalcoholic: polar compounds glycosides, polysaccharides, some phenolics.

- Methanol / ethanol: wide polarity range phenolics, flavonoids, tannins; ethanol preferred when extracts are for food/ethnopharmacology (less toxic). Many L. aspera studies use methanol/ethanol.
- Acetone / ethyl acetate: intermediate polarity certain flavonoids, some terpenoids.
- Petroleum ether / hexane: non-polar lipids, waxes, non-polar terpenes/alkanes.
- Supercritical CO_2: non-polar volatiles/essential oils (selective, solvent-free).

Modern pharmacological activities:

Below are major activities supported by in-vitro and in-vivo research, short summary with representative evidence.

Antioxidant activity:

Multiple extracts (methanol, ethanol, aqueous) show strong radical scavenging (DPPH), reducing power (FRAP) and total phenolic correlations — phytochemicals (flavonoids, phenols) are the likely mediators. Antioxidant activity underpins hepatoprotective and cytoprotective finding.

Anti-inflammatory & antinociceptive activity:

Leaf and whole plant extracts inhibited inflammation markers and reduced nociceptive behaviours in rodent models, aligning with traditional uses for fever and rheumatism. Several studies used carrageenan-induced paw enema and formalin tests showing dose-dependent effects.

Antimicrobial:

Broad antibacterial and antifungal actions have been reported against gram-positive and gram-negative strains and dermatophytes; activity varies by solvent and plant part. Essential oils and phenolic fractions show notable inhibition.

Anticancer / cytotoxic effects:

In-vitro cytotoxicity (MTT, cell viability) reported against cell lines such as HepG2 (liver), MCF-7 (breast) and HeLa (cervical). Mechanistic hints include induction of apoptosis, ROS modulation and anti-angiogenic signals but detailed molecular pathways need further work. Nano formulations (e.g., encapsulation in zeolitic imidazole frameworks) have been tested to improve delivery and potency.

Hepatoprotective activity:

Animal studies (D-galactosamine or toxin-induced liver injury models) indicate hepatoprotective effects attributed to antioxidant and anti-inflammatory components; biochemical markers (ALT/AST) and histopathology improved in treated groups.

Antivenom/PLA2 inhibition:

Some reports show inhibition of venom phospholipase A2 and protective activity in envenomation models, supporting ethnomedicinal use against snakebite in certain regions these remain exploratory and not substitutes for clinical antivenom.

Insecticidal and larvicidal activity:

Essential oils and crude extracts display larvicidal activity against mosquito vectors and repellent/insecticidal effects; this explains traditional use as an insect deterrent.

In-silico antiviral leads:

Metabolite profiling combined with molecular docking has identified several in silico candidates with predicted binding to viral targets (e.g., SARS-CoV-2 protease); experimental validation is required.

Clinical studies & human data:

Systematic searches and reviews show no robust, well-powered randomized clinical trials establishing efficacy or safety for Leucas aspera in humans. Most "clinical" mentions are case reports, local traditional use records, or small observational studies. Therefore, translation into therapeutic recommendations for humans is premature; well-designed clinical trials are needed to confirm efficacy, dose and safety.

Toxicity studies & safety profile:

Acute & subacute toxicity (rodent studies): Oral administration of ethanolic extracts showed no mortality up to 2,000 mg/kg in acute testing and tolerated subacute doses (e.g., 100-400 mg/kg) with limited adverse findings in some studies; biochemical and histopathological parameters were within normal ranges in many reports.

Neurobehavioral and organ toxicology: Some subchronic studies assessed neurobehavioral profiles and organ histology; most reported no serious toxicity at commonly tested doses but variations in study design and reporting exist.

Conclusion on safety: Preclinical toxicology suggests relative safety at commonly studied doses, but absence of long-term, reproductive, genotoxicity and human safety trials mean caution is required before recommending high-dose or long-term human use.

Mechanisms of action:

Mechanistic proposals, derived from phytochemistry and preclinical assays, include, antioxidant/ROS modulation (phenolics/flavonoids), inhibition of inflammatory mediators (cyclooxygenase/prostaglandin pathways), membrane/enzymatic disruption for microbes, apoptosis induction in tumour cells, and direct inhibition of venom enzymes (PLA2). However, few studies provide definitive molecular pathway maps this remains a priority research gap.

Gaps, challenges & future directions:

Clinical trials: Phase I safety and small Phase II efficacy studies for prioritized indications (e.g., topical anti-inflammatory, hepatoprotective adjunct) are missing.

Standardization: Development of standardized extracts (marker compounds, dose ranges) for reproducibility.

Mechanistic studies: Molecular pharmacology (target identification, signalling studies) to move beyond descriptive bioassays.

Toxicology expansion: Chronic, reproductive and genotoxicity testing to fully characterize safety.

Formulation & delivery: Nanoencapsulation and bioavailability enhancement require systematic evaluation of safety and efficacy trade-offs.

Conclusion:

Leucas aspera is a highly promising medicinal herb with a solid ethnobotanical history and an expanding preclinical literature supporting antioxidant, anti-inflammatory, antimicrobial, anticancer and hepatoprotective activities. Its phytochemical richness (triterpenoids, flavonoids, unique named metabolites) offers multiple chemical leads. However, the translational gap to human therapeutics remains large: standardized extracts, rigorous mechanism studies and controlled clinical trials are needed before clinical recommendations can be made. When used traditionally (topically or as mild infusions), preclinical toxicity is low, but systematic human safety data are lacking. Overall, L. aspera merits prioritized, multidisciplinary research to validate and safely harness its pharmacological potential

Botanical family: Lamiaceae.

Main traditional uses: Antipyretic, insect repellent, respiratory ailments, topical for bites.

Key phytochemicals: Oleanolic acid, unsolid acid, β-sitosterol, leadspersons, leadspersons, flavonoids, essential oils.

Major proven preclinical activities: Antioxidant, anti-inflammatory, antimicrobial, cytotoxic/anticancer, hepatoprotective, insecticidal.

Toxicity: Low acute toxicity in rodents up to 2 g/kg (variable subacute data). Human clinical data: insufficient.

Research needs: Standardization, mechanism elucidation, chronic toxicology, human trials.

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