

**PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACT
OF LEAVES OF *LEUCAS INDICA* (EELLI)****CHANDRASHEKAR. R* AND RAO S.N.***Dept of Pharmacology, Yenepoya Medical College, Derlakatte, Mangalore 575018.***ABSTRACT**

Plants of genus *Leucas* belongs to family Lamiaceae and have been widely employed by the traditional healers to cure many diseases. The objective of the study was to do preliminary phytochemical screening of Ethanolic Extract of Leaves of *Leucas indica* (EELLI). About 1000 gm of air dried powdered material of leaves of *Leucas indica* was extracted with 90% ethanol in a soxhlet extractor for 36 hours. It was concentrated to dryness under reduced pressure and controlled temperature (40-50°C) using rotary evaporator. The ethanolic extract yielded a dark brown Sticky mass weighing 125g. The ethanolic extract was concentrated by vacuum distillation to dryness; the yield obtained was 12.5% w/w with respect to dried leaf. The preliminary phytochemical screenings of EELLI for its phytochemical constituents were performed using generally accepted laboratory technique for qualitative determinations. Preliminary phytochemical screening of EELLI shows the presence of phytosterols, triterpenoids, flavinoids, lactones, glycosides, fatty acids, phenolic compounds and tannins.

KEYWORDS: *Leucas indica*, Phytochemicals**CHANDRASHEKAR. R**

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INTRODUCTION

Naturally occurring substances are of plants, animals and mineral origin. They are organic substances and could be obtained in both primary and secondary metabolic process and they also provide a source of medicine since the ancient times. The plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the pharmaceuticals in the world. Most important bioactive constituents of these plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. Plants in all facets of life have served a valuable starting material for drug development.¹ Phytochemicals are used as templates for lead optimization programs, to make safe and effective drugs.² *Leucas indica* is a species in the *Leucas* genus belonging to Lamiaceae / Labiatae family. Although the species has different names depending on the region in which it is located, it is most commonly known as Thumbai.³ Plants of genus *Leucas* have been widely employed by the traditional healers to cure many diseased conditions which insinuated that this genus have immense potential for the discovery of new drugs or lead molecules. The genus *Leucas* comprises of about 80 species.⁴ The highest species diversity has been found in East Africa.⁵ In India, 43 species are available.⁶ Variety of phytoconstituents has been isolated from the different species of *Leucas* which include lignans, flavonoids, coumarins, steroids, terpenes, fatty acids and aliphatic long chain compounds. Anti-inflammatory, analgesic, antidiarrhoeal, antimicrobial, antioxidant and insecticidal activities have been reported in the extracts of these plants and their phytoconstituents.⁷ The

Phytochemicals analysis

Phytochemical analysis of the test solution was done according to published methods.^{9, 10, 11}

paste of leaves of *Leucas indica* are widely used in traditional medicine to cure many diseases.⁸ However the phytoconstituents of species of *Leucas indica* has not been studied so far and hence the rationale of the present study was to carry out preliminary phytochemical screening of EELLI.

MATERIALS AND METHODS

Plant Material

The whole plant was collected from rural region of manjanady, in Mangalore region in the month of June – August 2010. It was authenticated by Dr. Krishna Kumar, Chairman, Dept of Applied Botany, Mangalore University, Mangalore. The herbarium of the plant (voucher specimen no YU/LI/2010) has been deposited at Yenepoya University, Mangalore.

Extraction

Leaves of *Leucas indica* were carefully separated, cleaned, shade dried, mechanically grinded and coarsely powdered. About 1000 gm of air dried powdered material was extracted with 90% ethanol in a soxhlet extractor for 36 hours. It was concentrated to dryness under reduced pressure and controlled temperature (40-50°C) using rotary evaporator. The ethanolic extract yielded a dark brown Sticky mass weighing 125g. The ethanolic extract was concentrated by vacuum distillation to dryness; the yield obtained was 12.5% w/w with respect to dried leaf. The collected leaf extract was stored in a desiccator.

TEST FOR PHYTOSTEROLS

Salkowski reaction

To 0.5 ml chloroform extract in a test tube add 1ml of Conc. H₂SO₄ from the sides of the test tubes. Appearance of reddish brown colour in

chloroform layer indicates presence of phytosterols

TEST FOR TRITERPENOIDS

Tschugajeu test

To chloroform extract, add excess of acetyl chloride and a pinch of zinc chloride. Keep aside for reaction to subside and then warmed on water bath. Appearance of eosin red colour indicates presence of triterpenoids.

TEST FOR SAPONINS

Foam test

A small amount of extract in a test tube with little quantity of water. Shake vigorously. Appearance of foam persisting for 10 minutes indicates presence of saponins.

TEST FOR ALKALOIDS

Dragendroff's test

Dissolve various extract of the herbal drug in chloroform. Evaporate chloroform and acidify the residue by adding few drops of Dragendroff's reagent (Potassium Bismuth Iodide). Appearance of orange red precipitate indicates presence of alkaloids.

TEST FOR CARBOHYDRATES

Fehling's test

Few drops of extract heated with Fehling's A and B solution. Appearance of orange red precipitate indicates presence of carbohydrates.

TEST FOR FLAVANOIDS

Ferric chloride test

To the alcoholic solution of the extract add few drops of neutral ferric chloride solution. Appearance of green colour indicates presence of flavanoids.

TEST FOR LACTONES

Baljets test

Treat extract with sodium picrate solution. Appearance of yellow to orange colour indicates presence of lactone ring.

TEST FOR PHENOLIC COMPOUNDS AND TANNINS

Ferric chloride test

Take 2 ml of extract in a test tube and add ferric chloride solution drop by drop. Appearance of bluish black precipitate indicates presence of tannins and phenolic compounds.

TEST FOR PROTEINS

Biuret test

Add 2ml of Biuret reagent to 2ml of extract. Shake well and warm it on water bath. Appearance of red or violet colour indicates presence of proteins.

TEST FOR GLYCOSIDES

Baljets test

Treat the extract with sodium picrate solution. Appearance of yellow to orange colour indicates presence of glycoside with lactone ring.

FIXED OILS AND FATTY ACID

Spot test

Prepared spot on the filter paper with the test solution and oil staining on the filter paper indicated the presence of fixed oil & fats.

RESULTS

In this study, we found that EELLI possess phytosterols, triterpenoids, flavinoids, lactones, fats and fatty acid, glycoside, phenolic compound and tannins. (Table 1).

Table 1
phytochemical analysis of ethanolic extract of leaves of *Leucas indica* (EELLI).

Phytochemicals	Ethanolic Extract Of Leaves Of <i>Leucas</i> <i>Indica</i> (EELLI) + / -
Phytosterols	+
Triterpenoids	+
Saponins	-
Alkaloids	-
Carbohydrates	-
Flavanoids	+
Lactones	+
Tannins & Phenolic Compounds	+
Proteins	-
Glycosides	+
Fixed Oils And Fatty Acid	+

(+) *Presence*, (-) *Absence*.

DISCUSSION

Plants of genus *Leucas* is widely used in traditional medicine to cure many diseases such as cough, cold, diarrhoea and inflammatory skin disorders. Several phytoconstituents has been isolated from the *Leucas* species which include lignans, flavonoids, coumarins, steroids, terpenes, fatty acids and aliphatic long chain compounds. Anti-inflammatory, analgesic, antidiarrhoeal, antimicrobial, antioxidant and insecticidal activities have been reported in the extracts of these plants and their phytoconstituents.¹² The species of *Leucas indica* is abundantly found in Bangladesh and India^{13, 14} especially in the region of dakshin kannad district in southern part of India. The present phytochemical analysis is being reported for first time for the species of *Leucas indica*. Some data from cell culture and animal studies suggest that phytosterols containing plants may attenuate the inflammatory activity of immune cells, including macrophages and neutrophils.^{15, 16} Plant Sitosterol has been found to induce

apoptosis when added to cultured human prostate¹⁷, breast¹⁸, and colon cancer cells¹⁹. The plants containing triterpenoids shows antimicrobial activity.²⁰ Flavonoids are known to inhibit or kill many bacterial strains, inhibit important viral enzymes, such as reverse transcriptase and protease, and also destroy some pathogenic protozoans.²¹ Lactones are known to possess wide variety of biological and pharmacological activities such as antimicrobial, anticancer, anti-inflammatory, antiviral, antibacterial, antifungal activities, effects on the central nervous and cardiovascular systems as well as allergenic potency.²² Phenolic compounds possess biological properties such as antiapoptosis, antiaging, anticancer, anti-inflammatory, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds.²³ Tannin-containing plants and tannins could potentially be a natural anthelmintics.²⁴ Glycosides (Phenylethanoid) are

naturally occurring compounds of plant origin and are known to possess broad array of biological activities including antibacterial, antitumor, antiviral, anti-inflammatory, neuro-protective, antioxidant, hepatoprotective, immunomodulatory, and tyrosinase inhibitory actions.²⁵ Plants with fixed oils has shown antiviral and antibacterial activity.²⁶ In our preliminary phytochemical screening, we found that the EELLI possess phytosterols, triterpenoids, flavinoids, lactones, fats and fatty acid, phenolic compound and tannins,

glycoside. This suggests that leaves of *Leucas indica* may possess Anti-inflammatory, analgesic, antidiarrhoeal, antimicrobial, antioxidant and insecticidal activities. However further studies are required to study its comprehensive analysis including quantitative / semi quantitative analysis, characterize its chemical structure and access its biological activities.

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