Research Article

Preliminary Phytochemical Screening of 6 Members of *Leucas* (Lamiaceae)

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ABSTRACT

In the present study, six species of Leucas were assessed for phytochemical screening. Aqueous, methanol, ethanol and chloroform extracts of each plant were subjected to qualitative phytochemical screening. Ethanol extract were positive for proteins and amino acids for six species. Methanol extracts were positive for phenols, tannins, flavonoids, carbohydrates and glycosides. The total phenols, flavonoids and tannins, were quantified in the methanolic extracts by standard spectrophotometric methods. Gallic acid was used as standard for the determination of total phenol by Folin-ciocalteu method. Quercetin and tannic acids were used as the standards for flavonoids and tannins respectively. *L.eriostoma* shows higher concentration of total phenolics whereas *L. lavandulifolia* shows higher concentration of flavonoids and tannins. The study reveals that the presence or absences of particular phytochemicals are determnined by the polarity of solvents used for extraction.

Keywords: Aqueous extract, chloroform extract, ethanol extract, *Leucas*, methanol extract, phytochemicals, preliminary.

INTRODUCTION

The traditional system of herbal medicine are considered as the rich sources of lead compounds which are eco-friendly and quite safe for human use and has become a topic of global importance. According to World Health Organization, for their preliminary health care, 80% of world's population depends on traditional medicine.[1]Medicinal properties of several herbal plants and the herbal extracts used to cure variety of diseases are documented in ancient Indian literature.[2]Medicinal value of these plants lies in some bioactive substances that produce a definite physiological action on human body.

Phytochemicals are primary and secondary metabolites occurring naturally in different parts of plants posses, defense mechanism to protect them from various diseases. Primary metabolites are involved directly in growth and metabolism (carbohydrates, lipids and proteins) of plants, and secondary metabolites are considered as end products of primary metabolism and not involved in metabolic activity (alkaloids, phenolics, sterols, steroids, essential oils, lignins and tannins etc).They act as defense chemicals. Their absence does not cause bad effects in the plants. Correlation between the bio activity of plant and the phytoconstituents is desirable to know for the artificial synthesis of compounds with specific activities to treat various health ailments and chronic diseases as well.[3]

Lamiaceae, commonly called as Mint family, is one of the largest families among dicotyledons, being composed of more than 236 genera and 7173 species.[4] Many species belonging to the family are aromatic, due to the presence of external glandular structures that produce volatile oil. The family is of outstanding importance in its use in indigenous medicine used by people world over, particularly in Indian cultures and tradition. Throughout the world, hundreds of Lamiaceae species are used as medicinal and aromatic plants.[5]

Preliminary phytochemical screening of plants is the need of the hour in order to discover and develop novel therapeutic agents with improved efficacy. Throughout the world, numerous research groups have also reported such studies.[6-10] Thus, the present study deals with the screening based on phytochemical tests of six *Leucas* species viz., *L. lavandulifolia*, *L. biflora*, *L. angularis*, *L. zeylanica*, *L. stelligera*, *L. eriostoma* for identifying their chemical constituents. All these plants possess different bioactivities which were later correlated with the presence of some specific phytoconstituents.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *L. lavandulifolia, L. biflora*, *L. angularis*, *L. zeylanica*, *L. stelligera* and *L. eriostoma* were collected from different regions of Western Ghats and were identified. Voucher specimens have been deposited in Calicut University Herbarium.

Preparation of Crude extracts

The collected leaves were washed with distilled water, shade dried and powdered. These dried samples were powdered and stored at 4ºC until further use. Crude extracts (10% w/v) were made using 4 solvents i.e., water, methanol, ethanol and chloroform. The extracts were filtered through fine muslin cloth and the clear filtrate was evaporated to dryness to form the crude extract.

Phytochemical screening

The chemical tests were carried out with the crude extracts of each plant i.e., aqueous extract (AE), methanol extract (ME), ethanol extract (EE) and chloroform extract (CE).

Qualitative analysis

Qualitative analysis was done to identify the presence of the following phytoconstituents; alkaloids, flavonoids, phenols, tannins, terpenoids, saponins, carbohydrates, glycosides, proteins and amino acids using standard procedures.[11, 12]

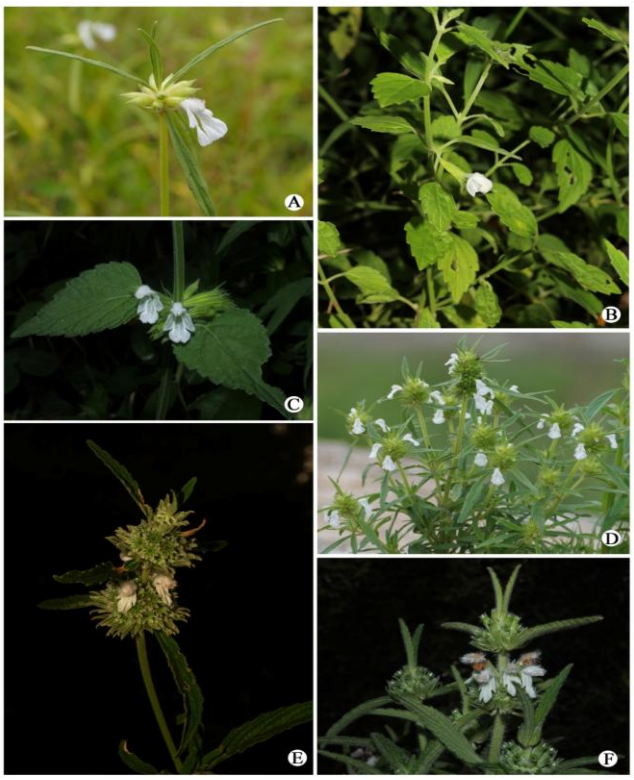

Figure 1: (A) *L. lavandulifolia*, (B) *L. biflora*, (C) *L. angularis*, (D) *L.zeylanica*, (E) *L. stelligera*, (F) *L. eriostoma*

Detection of Alkaloids

a) Hager's test: To one ml of extract, two drops of Hager's reagent was added. Formation of yellow colored precipitate indicates positive test.

b) Dragendroff's test: To one ml of extract, two drops of Dragendroff's reagent was added. Formation of reddish orange precipitate indicates positive test.

c) Wagner's Test: To 1 ml of extract, two drops of Wagner's reagent was added. Formation of reddish brown precipitate indicates the presence of alkaloids.

d) Mayer's Test: To one ml of extract, two drops of Mayer's reagent was added along the sides of the test tube. Appearance of whitish yellow precipitate indicates the presence of alkaloids.

Detection of Flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of NaOH solution. Formation of an intense yellow color which becomes colorless on addition of few drops of dilute acid indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colored precipitate indicates the presence of flavonoids.

Detection of Phenols

a) Ferric chloride test: One ml of extract was treated with 5% Ferric chloride solution. A dark bluish green color indicated the presence of phenolic compounds.

Detection of Tannins

a) Gelatin Test: To the extract 1% gelatin solution containing 10% sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

b) About 1 g of extract was dissolved into 10 ml of 10% KOH in a beaker and shaken to dissolve. A dirty precipitate indicates the presence of tannins.

Detection of Terpenoids

a) Salkowski's Test: 2 ml of extracts were dissolved in 3ml chloroform and filtered and two drops of concentrated sulphuric acid was added along the sides of test tube. The formation of reddish brown ring at the interface indicated the presence of terpenoids.

Detection of Saponins

a) Foam test: 100 mg of extract was diluted with distilled water to 20 ml. The suspension was shaken in a graduated cylinder for 15 minutes. Formation of 2 cm layer foam indicated the presence of saponins.

Detection of Carbohydrates

a) Molish's test: Two ml of extract was added with two drops of alcoholic solution of α-naphthol. The mixture was shaken well and one ml of concentrated sulphuric acid was added along the sides of the test tube and allowed to stand. A violet or purple ring indicated the presence of carbohydrates.

b) Fehling's test: One ml of extract was boiled on the water bath with one ml of each of Fehling's solution A and B. A red precipitate indicated the presence of carbohydrates.

Detection of Proteins and Amino acids

a) Biuret test: An aliquot of two ml of extract was treated with one drop of 2% copper sulphate solution. To this one ml of (95%) ethanol was added, followed by excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicated the presence of proteins.

b) Ninhydrin test: Two drops of Ninhydrin solution was added to two ml of aqueous extract. A characteristic purple color indicated the presence of amino acids.

c) Xanthoproteic test: Extracts were treated with few drops of concentrated HNO_3. Formation of yellow color indicates the presence of proteins.

Detection of Glycosides

Keller Killiani Test: 0.5 g of the extract was treated with 2 ml of glacial acetic acid and a drop of 5% (w/v) $FeCl_3$ was added to it. This was underplayed with 1 ml of concentrated H_2SO_4. Presence of brown ring at interface indicates the presence of glycosides.

Quantitative analysis of phytochemicals

Total phenolic content

The total phenolic content was estimated by Folin-Ciocalteu method.[13] An aliquot (1 ml) of extracts or standard solution of gallic acid (20, 40, 60, 80 and 100 µg/ml) was added to a 25 ml volumetric flask, containing 9 ml of distilled water. A reagent blank was prepared using distilled water. One milliliter of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. The volume was then made up to the mark. After incubation for 90 min at room temperature, the absorbance against the reagent blank was determined at 550 nm with an UV/Vis spectrophotometer. Total phenolics content was expressed as mg gallic acid equivalents (GAE).

Total flavanoid

Total flavonoid content was measured by the aluminium chloride colorimetric assay.[14] An aliquot (1 ml) of the extracts or standard solutions of quercetin (50, 100, 150, 200 and 250 µg/ml) was added to a 10 ml volumetric flask containing a 4 ml of distilled water. To the flask, 0.30 ml of 5% $NaNO_2$ was added and after 5 min, 0.3 ml of 10 % $AlCl_3$ was added. After 5 min, 2 ml of 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm. The total flavonoid content was expressed as mg quercetin equivalents (QE).

Determination of Tannins

In this method, an aliquot (1 ml) of the extracts or standard solutions of tannic acid (1, 2.5, 5.0, 10 µg/ml) was added to a 10 ml volumetric flask containing a 4 ml of distilled water. A reagent blank was prepared using distilled water. 500 µl of Folin-Denis reagent was added to the mixture and shaken. After 5 min, 1 ml of 7 % Na_2CO_3 was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 775 nm. The concentration of tannins was expressed as tannic acid equivalents in milligram per gram (TAE mg/g) of crude extract.

Analysis

The samples were analyzed in triplicates (n=3) and the results were expressed as mean ± standard deviation values.

RESULTS AND DISCUSSION

Preliminary screening for phytochemicals

The data shown in Table 1 shows screening of four different extracts, i.e., aqueous extract (AE), methanol extract (ME), ethanol extract (EE) and chloroform extract (CE) of leaves of 6 *Leucas* species viz., *L. lavandulifolia*, *L. biflora*, *L. angularis*, *L. zeylanica*, *L. stelligera* and *L. eriostoma* based on phytochemical tests. These tests reveal the presence of various bioactive secondary metabolites which might be responsible for their medicinal attributes. The observations and inferences made in the phytochemical tests are presented as follows:

The secondary metabolites contribute significantly towards the biological activities of medicinal plants such as antioxidant, antidiabetic, hypoglycemic, antimicrobial, anticarcinogenic, antimalarial, anti-inflammatory, anticholinergic, antileprosy activities etc.[15]

All the six *Leucas* members screened were found to possess high amount of phenolics. They are rich in phenolics like flavonoids and all shows presence of a minimum quantity of tannins in methanolic extracts only. The result shows that *Leucas* species are poor in alkaloid composition and are present only in *L. stelligera* and *L. lavandulifolia*. Methanol extracts showed the presence of most of the phytochemicals analysed i.e., alkaloids, flavonoids, tannins, phenolics, terpenoids, proteins, carbohydrates and glycosides, with the exception of saponins, proteins and amino acids. Saponins were present only in *L. stelligera.* The presence of proteins and amino acids were clearly distinguished in ethanolic extracts. All six species show a high degree of composition of carbohydrates in all four extracts. Glycosides and Terpenoids are altogether absent in *L. lavandulifolia* whereas absent in all other species. The four different solvents used for extraction has revealed that the phytochemical composition of the extract varies with the solvent used.

Quantitative analysis

The results of the quantitative estimation of the chemical constituents like total phenols, flavonoids and tannins of methanolic extract by spectrophotometric method is summarized in table 2.

Among all the six extracts studied, *L. eriostoma* had the highest concentration and *L. biflora* had the lowest concentration of total phenolics i.e., 19.75 ± 2.47% and 8.00 ± 0.71 % respectively. *L. angularis* and *L. zeylanica* had almost equal quantity of phenolics but slightly differs in concentration of flavonoids and tannins. The higher solubility of phenols in polar solvents provides a higher concentration of these compounds in the extracts obtained using polar solvents for the extraction.[16-17]

Table 1: Phytochemical composition of the leaves of *L. lavandulifolia, L. biflora, L. angularis, L.zeylanica, L. stelligera, L. eriostoma* in aqueous, methanol, ethanol and chloroform extracts.

Plant Name	Crude extract	Phyto-constituents								
		Alkaloids	Flavonoids	Phenols	Tannins	Terpenoids	Saponins	Carbohydrates	Proteins & Amino acids	Glycosides
Leucas lavandulifolia	AE	+++	+++	+++	-	-	-	+++	+	-
	ME	++	+	+++	+	-	-	+++	-	-
	EE	-	-	+	-	-	-	+	+++	-
	CE	-	-	-	-	-	-	-	-	-
Leucas biflora	AE	-	+++	+++	-	+++	-	+++	+++	+++
	ME	+	-	+++	+	-	-	+++	-	-
	EE	-	-	-	-	+	-	+	-	+
	CE	-	-	-	-	-	-	+++	-	-
Leucas angularis	AE	-	+	+++	-	+++	-	+++	+++	+++
	ME	+	+	+++	+	+++	-	+++	-	+
	EE	-	-	-	-	+++	-	+++	+++	-
	CE	-	-	-	-	-	-	-	-	-
Leucas zeylanica	AE	-	+++	+++	-	+++	-	+++	+++	+++
	ME	+	-	+++	+	+++	-	+++	-	+++
	EE	-	-	-	-	+++	-	+++	+++	+++
	CE	-	-	-	-	+	-	+++	-	+++
Leucas stelligera	AE	-	+++	+++	-	+++	+	+++	+++	+++
	ME	+++	+++	+++	+	+++	+	+++	-	+++
	EE	-	+	-	-	+++	+	+++	+++	+++
	CE	-	-	-	-	+++	+	+++	-	+++
Leucas eriostoma	AE	-	+++	+++	-	+	-	+++	+++	+++
	ME	-	++	+++	+	+++	-	+++	-	+++
	EE	-	++	+++	-	+++	-	+++	+++	+++
	CE	-	-	-	-	++	-	+++	+	+++

Table 2. Quantitative analysis for total phenolics, flavonoids and tannins.

Plants	Phytoconstituents		
	Total phenolics	Flavonoids	Tannins
L. lavandulifolia	17.13 ± 0.53	42.75 ±5.66	2.18 ± 0.18
L. biflora	8.00 ± 0.71	16.88 ± 0.53	1.18 ± 0.04
L. angularis	9.75 ± 1.06	30.13 ± 0.18	1.35 ± 0.00
L. zeylanica	9.75 ± 0.35	25.13 ± 0.53	1.30 ± 0.07
L. stelligera	13.75 ± 0.35	36.50 ± 3.18	1.63 ± 0.11
L. eriostoma	19.75 ± 2.47	32.13 ±2.65	2. 03 ±0.11

L. lavandulifolia exhibits the higher flavonoid and tannin concentration i.e., 42.75 ±5.66 and 2.18 ± 0.18 respectively whereas *L. biflora* exhibits lower i.e., 16.88 ± 0.53 and 1.18 ± 0.04 respectively. The concentration of tannins is very less compared to total phenolics and flavanoids. The polarity of solvents used in the extract preparation determines the concentration of flavonoids.[18] All the standard graphs showed that strong positive linear correlation (r) which is close to +1. These graphs indicate that as the value of concentration increases, values for absorbance also increase.

As a result of the presence of various compounds which are vital for good health, phenols and flavonoids improve the health status of the consumers and are seemed to have the potential to act as a source of useful drug.[19]Flavonoids inhibits the promotion of growth and progression of tumors so that it have been used against the cancer causing tumors.[20] Flavonoids are essential in human diet and are present in plant extracts that have been used for medicinal purpose.[21]The antioxidant properties, cell function modulation and reactive oxygen species scavenging and of flavonoids could account for the large part of their pharmacological activity.[22]

CONCLUSION

In this study, the preliminary qualitative estimation of phytoconstituents of four extracts (AE, ME, EE and CE) and total phenol, flavonoid and tannin contents from methanolic extracts of 6 species of *Leucas* were investigated. The results revealed that the phytochemical composition varies with the solvent used. The phytochemicals identified in the present study may be used as tools for quality control of drugs prepared with *Leucas* species in the future, for the treatment of a variety of disease conditions. Further studies on the mechanism of actions of this plant and identification of the constituent compounds responsible for the pharmacological activities should be carried out in future.

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