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Research Article

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Phytochemical studies on *Leucas aspera*

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ABSTRACT

Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The alkaloids, tannins, flavonoids and phenol compounds play a major role in preventing a number of chronic diseases by a definite physiological action on the human body like anti-inflammatory, anti-thrombotic, anti-oxidant, hepatoprotective and anticarcinogenic activities.Leucas aspera (Family:Lamiaceae) commonly known as 'Thumbai' is one such medicinal plant which is being used traditionally as an antipyretic and insecticide. Parts of the plant is also being used for many disorders like rheumatism, psoriasis and chronic skin eruptions.The aim of the present study was to evaluate the invitro phytochemical analysis on aqueous ethanolic extract of whole plant of Leucas aspera. Phytochemical screening was carried out for chloroform,aqueous ethanol and water as per the standard methods. Plant contains sterols, alkaloids, flavonoids,galactose, oleanolic acid, ursolic acid and Beta-sitosterol . Aerial parts contain alpha -sitosterol, beta-sitosterol. The plant extracts were subjected to quantification of phytoconstituents such as total phenolic content and total antioxidant capacity by invitro methods. Preliminary phytochemical screening of the extract(chloroform and aqueous ethanol) revealed the presence of various bioactive components which include alkaloids, flavonoids, steroids, cardiacglycosides, saponins and tannins. The total antioxidant capacity and total phenolic contents of ethanol extract were found to be 190.00± 7.95 mg/g and 15.36 ± 0.512 GAE/g dry weight of extract respectively. From invitro studies it can be concluded that ethanol soluble fractions of L.aspera showed good antioxidant activity and thus scientifically proves the use of entire plant in traditional medicine for various ailments.

Key words: *Leucas aspera*, phytochemical, alkaloids, total antioxidant

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INTRODUCTION

The importance of plants is known to us well. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs [1], antimicrobial drugs [2], antihepatotoxic compounds. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency [3].

A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro [4-8]. Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds [9]. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [10-12].Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [13,14].These compounds are synthesized by primary or rather secondary metabolism of living

organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [15].

Secondary Plant Metabolites are biosynthetically derived from the primary metabolites and their distribution in plant Kingdom is restricted [16].The secondary compounds include alkaloids, flavonoids, terpenoids, steroids, anthraquinones and volatile oils. Attempts are being made on the research of producing phytochemicals and also to discover new compounds from plants for using as pharmaceuticals. As antioxidants, flavonoids have been reported to be able to interfere with the activities of enzymes involved in reactive oxygen species generation, quenching free radicals, chelating transition metals and rendering them redox inactive in the Fenton reaction [17].

Leucas aspera (Family:*Lamiaceae*) commonly known as 'Thumbai' is one such medicinal plant which is being used traditionally as an antipyretic and insecticide. Parts of the plant is also being used for many disorders like rheumatism, psoriasis and chronic skin eruptions. Plant contains sterols, alkaloids, galactose, oleanolic acid, ursolic acid and Beta-sitosterol . Aerial parts contain alpha -sitosterol, beta-sitosterol. Shoots contain long chain compounds -l-hydroxytetratriacontan-4-one and 32-methyl- tetratriacontan-8-ol, dotriacontanol. The present study attempts to investigate the qualitative phytochemical constituents from extracts of entire plant of L.aspera and to quantitate total phenolics and total antioxidant capacity.

EXPERIMENTAL SECTION

Collection and processing of plant samples
Leucas aspera were collected from Kottayam and authenticated. They were properly washed in tap water and rinsed in distilled water and dried in an oven at a temperature of 35 –40^0C for 3 days and pulverized using a mortar and pestle, to obtain a powdered form. The powdered form is stored in air tight glass containers, protected from sunlight until required for analysis.

Preparation of aqueous ethanol extract
The aqueous ethanol extract of L.aspera was prepared by soaking 10g of powdered samples in 100ml of (50:50) ethanol and water for 12 hrs. The extract was then filtered using filter paper , then concentrated to 50ml and stored in airtight container.

Preparation of chloroform extract
The chloroform extract of the plant was prepared by soaking 10g of powdered samples in 100ml of chloroform for the same 12 hrs. The extracts were then filtered using filter paper, then concentrated to 50ml and stored in airtight container.

PHYTOCHEMICAL SCREENING METHODS
Phytochemical screening was carried out using chloroform, aqueous ethanol and water (for qualitative) and aqueous ethanol extract for quantitative analysis as per the standard methods[18,19] .

1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer’s Test: Filtrates were treated with Mayer’s reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner’s Test: Filtrates were treated with Wagner’s reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff’s Test: Filtrates were treated with Dragendroff’s reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager’s Test: Filtrates were treated with Hager’s reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch’s Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

4. Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

5. Detection of saponins

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

6. Detection of phytosterols
Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

7. Detection of phenols
Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

8.Detection of tannins
Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

9. Detection of flavonoids
Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

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

10. Detection of proteins and aminoacids
Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Estimation of phytoconstituents
The plant extracts were subjected to quantification of phytoconstituents such as total phenolic content and total antioxidant capacity by invitro methods.

Exactly weighed sample powder was ground with a pestle and mortar in the measured volume of solvents chloroform, ethanol and water to prepare respective extracts. The extract was filtered through a Whatman Filter paper. Each extract was prepared just before the experiment so as to prevent any further degradation.

Determination of total antioxidant capacity: The antioxidant activity of the extracts of *L.aspera* was evaluated by the phospho- molybdenum method according to the procedure of Prieto *et al* [20]. 0.3 ml of extract was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95°C for 90 min and cooled to room temperature. Finally, absorbance was measured at 695 nm using a spectrophotometer against blank. A typical blank solution contains 3ml reagent solution and appropriate volume (0.3ml) of the same solvent in place of the extract. The total antioxidant capacity was expressed as the number of equivalents of ascorbic acid.

Determination of total phenolic contents: The content of total phenolic compounds in plant extracts was determined by Folin–Ciocalteu method [21].For the preparation of calibration curve 1 ml aliquots of 50, 100, 150 and 200 µg/ml ethanolic gallic acid solutions were mixed with 5 ml Folin–Ciocaleu reagent (diluted ten-fold) and 4 ml (75 g/l) sodium carbonate. The absorption was read after 30 min at 20°C at 765 nm and the calibration curve was drawn. One ml aqueous ethanol extract at 1mg/ml was mixed with the same reagents as described above, and after 1 hr the absorption was measured for the determination of plant phenolics. All determinations were performed in triplicate. Total content of phenolic compounds in ethanol extracts in gallic acid equivalents (GAE) was calculated by the following formula

$$C = c. V/m$$

Where:
C = total content of phenolic compounds, mg/g plant extract, in GAE;
c = the concentration of gallic acid established from the calibration curve, mg/ml;
V = the volume of extract, ml; m = the weight of pure plant ethanolic extract, g.

Statistical analysis

Results were expressed as Mean ± S.D and all statistical comparisons were made by means of one way ANOVA test followed by Tukey's post hoc analysis and p-values less than or equal to 0.05 were considered significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the extract (chloroform and aqueous ethanol) of flowers of L.aspera revealed the presence of various bioactive components which include alkaloids, flavonoids, steroids, cardiac glycosides, saponins and tannins.The results of phytochemical test(qualitative) has been summarized in Table 1

QUALITATIVE ANALYSIS

Table 1:Phytochemical qualitative analysis of Leucas aspera

Sl.no	Phytochemical test	Reagents used (test performed)	Inference	Result
1	Alkaloids test	Mayer's test Wagner's test Dragendoroff's test	Creamy white precipitate Reddish brown colour yellow precipitate	+ + +
2	Carbohydrate test	Molisch's test Benedict's test Fehling's test	Formation of violet ring Formation of orange red precipitate Formation of red precipitate	+ + +
3	Saponin test	Foam test	Froath formation	+
4	Glycosides test	Borntrager's test	Formation of pink colour	+
5	Steroid test	Salkowski's test	Formation of green colour	+
6	Flavonoid test	Lead Acetate test	Yellow precipitate	+
7	Proteins and Amino Acids	Xanthoproteic test	Formation of yellow colour	+
8	Tannins test	Ferric chloride test	Formation of green colour	+
9	Terpenoids test	Salkowski's test	No pink colour formation	-

+ sign indicates presence and – sign indicates absence.

Total Antioxidant Activity: The antioxidant activity of plant extract at 5, 50, 100, 200 and 500 µg/ml concentrations was measured by the phospho-molybdenum method. Total antioxidant capacity of extracts of *L.aspera* was calculated using the standard curve of ascorbic acid and is expressed as number of equivalent of ascorbic acid per gram of plant extract (AAE). The total antioxidant capacity of ethanol extract was found to be 195

±8.05, mg/g of plant extract respectively (expressed as ascorbic acid equivalents) as shown in **table 2**. The phospho-molybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm.

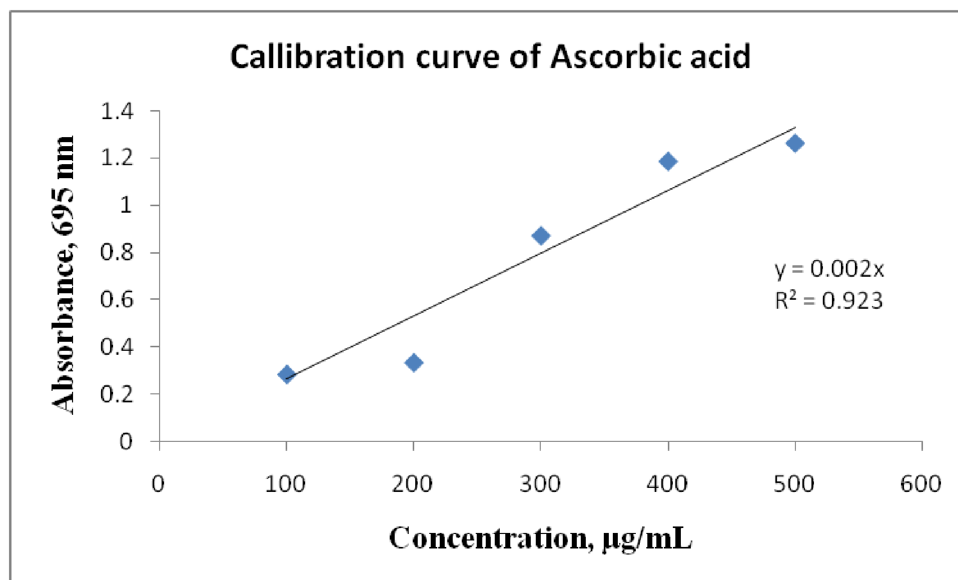

Fig.1:Callibration curve of Ascorbic acid

Table 2: Total antioxidant capacity of the aqueous ethanol extract

Extract	Total antioxidant capacity, mg/g plant extract in AAE±SD
Aqueous ethanol	195.00± 8.056

Values are Mean ± S.E.M, n=3

Total Phenolic Content: Phenolic compounds are commonly found in both edible and inedible plants and plant parts. They have been reported to have multiple biological effects, including antioxidant activity. The content of phenolic compounds (mg/100g DW) in ethanolic extract was determined and expressed in gallic acid equivalents (GAE). The total phenolic contents of *L.aspera* ethanol extract was found to be promising with a value of 15.36 ± 0.512 GAE/g dry weight of extract (table 3).Total phenol content of aq.ethanol extract of L.aspera was calculated using the standard curve of Gallic acid ($y = 0.012x + 0.1276$; $R^2 = 0.987$) and is expressed as Gallic acid equivalent (GAE) per gram of plant extract (fig. 2).

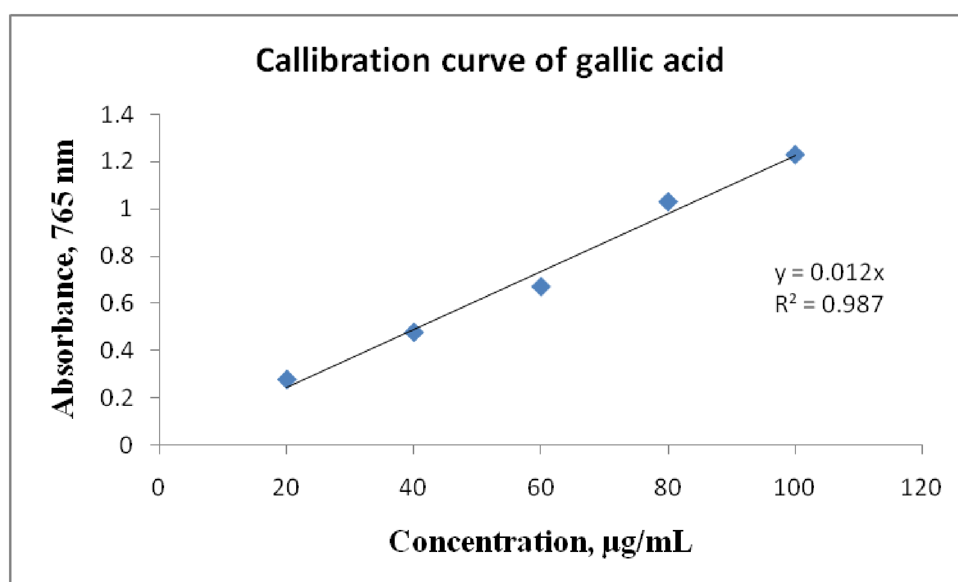

Fig.2: Callibration curve of gallic acid

Table 3: Total phenol content of the extract

Extract	Total phenol content, mg/g plant extract in GAE± SD
Aqueous ethanol	15.36 ± 0.512

Values are Mean ± S.E.M, n=3

DISCUSSION

Preliminary phytochemical screening revealed the presence of tannins, proteins, steroids, glycosides, carbohydrates, saponins, flavonoids and alkaloids in different extracts of L.aspera. These results show that L.aspera contain a number of chemical ingredients, which may be responsible for the various pharmacological actions although their specific roles remain to be investigated. It has been observed that most active principles present in the plant are alkaloids, flavonoids, phytosterols, tannins and glycosides. These phytoconstituents may be responsible for various pharmacological actions of this plant part, like antibacterial, antiulcer, anticancer, larvicidal and chemo protective activities.

Typical phenolics that possess antioxidant activity have been characterized as phenolic acids and flavonoids. Phenolic acids have repeatedly been implicated as natural antioxidants in fruits, vegetables, and other plants. In the present study the antioxidant activity test was found to be positive for each extract which can be attributed to the presence of alkaloids, phytosterols and flavonoids as shown in the phytochemical screening test. So the presence of both types of antioxidant compounds in chloroform and ethanol extracts can be concluded for higher antioxidant activity of these extracts.

Isolation of pure, pharmacologically active constituents from plants remains a long and tedious process. For this reason, it is necessary to have methods available which eliminate unnecessary separation procedures. Chemical screening is thus performed to allow localization and targeted isolation of new or useful constituents with potential activities. This procedure enables recognition of known metabolites in extracts or at the earliest stages of separation and is thus economically very important.

Phenols are very important plant constituents. There is a highly positive relationship between total phenols and antioxidant activity of many plant species, because of the scavenging ability of their hydroxyl groups. The phenolic contents of the extract can also scavenge hydrogen peroxide by donating electrons and thereby neutralizing it to water [22] It was also reported that phenolic compounds are effective hydrogen donors, making them very good antioxidants [23] .The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing super oxide anion (O2 -•), hydroxyl radical or peroxy radicals, quenching singlet and triplet oxygen or decomposing peroxides [24,25].Polyphenolic contents appear to function as good electron and hydrogen atom donors, and therefore, be able to terminate radical chain reaction involved in lipid peroxidation by converting free radicals and reactive oxygen species to more stable products. Thus, the antioxidant activity of L.aspera extracts might be attributed to these modes of activity due to their alkaloids, phytosterols and flavonoid contents.

CONCLUSION

Phytochemical analysis and antioxidant efficiency of Leucas aspera were performed. From our study it can be concluded that various phytochemicals including alkaloids, phytosterols, flavonoids, saponins, phenols and glycosides in chloroform and ethanol soluble fractions were responsible for its antioxidant property.

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