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Phytochemical Screening, Quantitative Analysis of Primary and Secondary Metabolites of *Cymbopogan citratus* (DC) stapf. leaves from Kodaikanal hills, Tamilnadu

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Abstract: The study includes phytochemical screening and quantification of primary and secondary metabolites like chlorophyll, carbohydrates, protein, lipids, phenol, tannin and flavonoids from *Cymbopogan citratus* (DC) Stapf. leaves.
Keywords : Phytochemical screening, Primary and Secondary metabolites, *Cymbopogan citrates.*

Introduction:

Since ancient times, people have been exploring the nature particularly medicinal plants in search of new drugs. Medicinal plants are used by 80% of the world population for their basic health needs. India is the birth place of renewed system of indigenous medicines such as Siddha, Ayurveda and Unani. Traditional systems of medicines are prepared from a single plant or combinations of more than one plant. These efficacy depends upon the current knowledge about taxonomic features of plant species, plant parts and biological property of medicinal plants which in turn depends upon the occurrence of primary and secondary metabolites [1].

Plant synthesize a wide range of chemical compounds which are classified based on their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites. Primary metabolites directly involved in growth and development while secondary metabolites are not involved directly and they have been worked as biocatalysts. Primary metabolites are widely distributed in nature, occurring in one form or another in virtually all organisms. They are like chlorophyll, amino acids, nucleotides, carbohydrates etc., which have a key role in metabolic processes such as photosynthesis, respiration and nutrient assimilation. They are used as industrial raw materials and food additives. Secondary metabolites are synthesized during secondary metabolism of plants. They are the basic source for the establishment of several pharmaceutical industries since they have enormous medicinal properties [2]. The most important secondary metabolites are alkaloids, tannins, flavonoids, phloblatannins, saponin and cardiac glycosides. All secondary metabolites have specific function as like saponins have antifungal activity [3], some alkaloid may be useful against HIV infection [4], flavonoids have strong anticancer activity [5] and tannin have antimicrobial activity.

Previously the crude drugs/extracts prepared from plants were identified by comparison only with the standard descriptions available in the literature, but recently due to advancement in the field of pharmacognosy, various techniques have been followed for the standardization of crude drugs. Phytochemical screening is one of the techniques to identify new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenolics, steroids, tannins, saponins etc. present in the plant extracts. These compounds can be derived from any part of the plants like bark, leaves, flowers, seeds, etc. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of new bioactive compound/s for treating the specific disease. Such phytochemical screening in various plants is reported by many workers. Many plants such as *Nerium indicum, Gloriosa superba*[6], *Ricinus communis* and *Euphorbia hitra*[7] , *Pongamia pinnata* [8] , *Moringa oleifera*[9], *Svensonia hyderobadensis*[10] and *Melothria maderaspatana* [11] have been evaluated for their composition of primary metabolites. Likewise number of plants were screened for secondary metabolites for their medicinal values in *Cichorium intybus, Eclipta alba, Morinda citrifolia, Mangifera indica, Cissus populnea* and *Bauhinia tomentosa*[12-16].

Number of environmental factors such as climate, altitude, rainfall and other conditions may affect growth of plants which in turn affect the quality of herbal ingredients present in a particular species even when it is produced in the same country. These conditions may produce major variations in the bioactive compounds present in the plants[17]. HPLC chromatogram of *Rauwolfia* samples collected from different locations of South India showed significant variations in the content of reserpine. Qualitative phytochemical screening will help to understand a variety of chemical compounds produced by plants and quantification of those metabolites will help to extract, purify and identify the bioactive compounds.

The genus *Cymbopogan* comprises 140 species that are widely distributed in the world. Approximately 45 species have been reported to occur in India. *Cymbopogan citratus* (DC) Stapf. known as West Indian lemon grass is an important species of Poaceae family commonly found in Southeast Asia, which its origin can be tracked from India. It is a tall, clumped perennial grass growing to a height of 1 m and it is commonly growing grass in Kodaikanal hills, Tamil Nadu (Fig.1). The leaf blade is linear, tapered at both ends and can grow to a length of 50 cm and width of 1.5 cm[18]. The plant is commonly used in folk medicine in many countries since it exhibit antioxidant properties which in turn inhibits the propagation of free radical reactions and protects the human body from disease. The isolated and identified substances from the leaves are mainly aldehydes, alkaloids, saponin, terpenes, alcohols, ketone, flavonoids and these components have various medicinal properties. The chemical composition of the essential oil and aqueous extracts of *C. citratus* varies according to the geographical origin[19]. Kodaikanal is situated on top of palani hills and always cool (18^{o}-22^{o}C) throughout the year due to high elevation of the city (2,200m). It comprises unique jungle, grassland and shola. In the present investigation qualitative and quantitative phytochemical analyses were carried out using lemongrass leaves grown in Kodaikanal.

Materials and Methods:

Collection of Plant Material

Leaves of *Cymbopogan citratus* were collected from Department of Biotechnology, herbal garden at Mother Teresa Women's University, Kodaikanal. The plant was authenticated by Dr. N.Jayaraman, Director, National Institute of Herbal Science, Plant Anatomy Research Center, Tambaram, Chennai and the voucher specimen has been deposited in biotechnology department as herbarium for future reference.

Preparation of the plant extracts

The leaves were washed under running tap water to remove the surface pollutants and the leaves were air dried under shade. The powdered leaf samples were subjected to successive extraction with chloroform, methanol and acetone using soxhlet apparatus. Fresh leaf material was ground using distilled water and filtered and used as an aqueous extract. The extracts obtained using solvents were concentrated using rotary vacuum evaporator and then dried. The extract thus obtained was used for various analyses.

Phytochemical Screening of extracts

Chloroform, methanol, aqueous and acetone extracts were used for preliminary phytochemical analyses using standard procedures[20,21]. The following qualitative tests for both the metabolites were done as follows:

a)Test for alkaloids

Wagner's test: About ten mg of extract was taken and few drops of Wagner's reagent was added and the formation of a reddish brown precipitate indicates the presence of alkaloids.

b)Test for Flavanoids

Shinoda Test: Ten mg of extract was added to pinch of magnesium turnings and 1-2 drops of concentrated hydrochloric acid was added. Formation of pink color indicates the presence of Flavanoids.

Lead acetate test : Ten mg of extract was taken and few drops of 10% lead acetate solution was added. Appearance of yellow colour precipitate indicates the presence of flavonoids.

c) Test for Phenols and Tannins

Lead acetate test: Ten mg of extract was taken and 0.5 ml of 1% lead acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolic compounds.

Ferric chloride test: Five mg of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish black color indicates the presence of tannins.

Sodium hydroxide test: Five mg of extract was dissolved in 0.5 ml of 20% sulphuric acid solution. Followed by addition of few drops of aqueous sodium hydroxide solution, it turns blue which indicates the presence of phenols.

d) Test for steroids and sterols

Salkowski's test: Five mg of extract was dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterols compound, in the extract.

e) Test for Carbohydrates

Fehling's test : Five ml of Fehling's solution was added to 0.5 mg of extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing power.

Benedict's test: Five ml of Benedict's solution was added to 0.5 mg of extract and boiled in water bath. The appearance of red or yellow or green precipitate indicates the presence of reducing sugars.

f) Test for Saponins

Honey comb test: 0.5 mg of extract was taken in a test tube and few drops of 5% sodium bicarbonate solution was added. The mixture was shaken vigorously and kept for 3 minutes. Formation of honey comb like froth shows the presence of saponins.

Foam test: 0.5 mg of extract was diluted with 20 ml distilled water and shaken well in a graduated cylinder for 15 min. The formation of foam to a length of 1cm indicated the presence of saponins and steroids.

g) Test for Glycosides

Glycoside test: 0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

h) Test for Protein & amino acids

Biuret test: To 0.5 mg of extract equal volume of 40% NaOH solution and two drops of 1% copper sulphate solution was added. The appearance of violet colour indicates the presence of protein.

Ninhydrin test: About 0.5 mg of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

i) Test for Anthraquinone

Borntragers test : About 0.5 gm of the extract was taken into a dry test tube and 5 ml of chloroform was added and shaken for 5 minutes. The extract was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red colour in the lower layer indicates the presence of anthraquinone.

Quantitative determination of primary metabolites

Determination of carbohydrate

100 mg of sample was hydrolysed in a boiling tube with 5 ml of 2.5 N HCl in a boiling water bath for a period of 3 hours. It was cooled to room temperature and solid sodium carbonate was added until effervescence ceases. The contents were centrifuged and the supernatant was made to 100 ml using distilled water. From this 0.2 ml of sample was pipetted out and made up the volume to 1 ml with distilled water. Then 1.0 ml of phenol reagent was added followed by 5.0 ml of sulphuric acid. The tubes were kept at 25-30˚C for 20 min. The absorbance was read at 490 nm [22].

Estimation of total chlorophyll content

100 mg leaf tissues were soaked in 10 ml of DMSO: acetone mixture (1:1) for overnight incubation (in the dark) and absorbance read at 663 and 645 nm and total chlorophyll content was calculated using the following equations.

Chlorophyll a (Ca) = (12.25 × OD at 663) - (2.79× OD at 645) ×10/ (1000×wt)

Chlorophyll b (Cb) = (21.50 × OD at 645) - (5.10× OD at 663) ×10/ (1000×wt)

Total Chlorophyll (C) = (7.15 × OD at 663) + (18.71× OD at 645) ×10/ (1000×wt)

Determination of protein

The dried and powdered samples was extracted by stirring with 50 ml of 50% methanol (1:5 w/v) at 25 ℃ for 24 h and centrifuged at 7,000 rpm for 10 min .0.2 ml of extract was pipette out and the volume was made to 1.0 ml with distilled water. 5.0 ml of alkaline copper reagent was added to all the tubes and allowed it to stand for 10 min. Then 0.5 ml of Folin's Ciocalteau reagent was added and incubated in dark for 30 min. The intensity of the colour developed was read at 660 nm [23].

Estimation of total lipid content

10 gm sample was used to extract lipids with 150 ml of petroleum ether for 16 hr, at a solvent condensation rate of 2–3 drops/sec according to AACC Approved Method 30-25 with minor modifications of sample size and extraction time. The obtained extract was concentrated and evaporated at room temperature to dryness. The weight of extract gives the total lipid content which was expressed as mg/g dry matter [24].

Quantitative determination of secondary metabolites

Determination of total phenolics and tannins

The total phenolic content was determined according to the method described by[25]. Ten microlitre aliquots of the extracts (2 mg/2 ml) was taken in test tubes and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as tannic acid equivalents.

Using the same extracts the tannins was estimated after treatment with polyvinyl polypyrrolidone (PVPP). One hundred milligrams of PVPP was placed in a test tube and to this 1 ml distilled water and then 1 ml of the sample extracts were added. The contents were vortexed and kept in the test tube at 4°C for 4h. Then it was centrifuged (3000 rpm for 10 min at room temperature) and the supernatant was collected. This supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was measured as mentioned by[25] and expressed as the content of non-tannin phenolics (tannic acid equivalents) on a dry matter basis. From the above results, the tannin content of the sample was calculated as follows:

Tannin (%) = Total phenolics (%) – Non-tannin phenolics (%)

Determination of total flavonoid content

The flavonoid content was determined by the use of a slightly modified colorimetry method described previously by[26]. A 0.5 ml aliquot of appropriately (2 mg/2 ml) diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5 % $NaNO_2$ solution. After 6 min, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, and then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus water blank. The analysis was performed in triplicate and the results were expressed as rutin equivalent.

Statistical Analysis

All the analyses were performed in triplicate and the results were statistically analyzed and expressed as mean (n=3) ± standard deviation (SD).

Results and Discussion:

Phytochemical analysis is of paramount importance in identifying new source of therapeutically and industrially valuable compounds having medicinal plants have been chemically investigated[27]. In the present investigation primary and secondary metabolites were qualitatively and quantitatively analysed using lemon grass leaves. The results are presented in Table 1& 2 and figure 2.

In the present study lemon grass leaf extracts such as chloroform, methanol, acetone and aqueous were used. Out of these four extracts methanolic extract showed maximum number of plant constitutents such as flavonoids, phenol, tannins, steroids, sterols, carbohydrate, glycosides, protein and amino acids and anthraquinone (Table-1).Our results were in agreement with findings of [28,29] and disagreement with reports of [30]. The medicinal value of plants lies in some chemical substances that have a definite physiological functions

in the human body. Different phytochemicals have been found to possess a wide range of medicinal properties, which may help in protection against various diseases. For example, alkaloids protect against chronic diseases; saponins protect against hypercholesterolemia and steroids and triterpenoids show the analgesic properties.

Table 1: Preliminary phytochemical screening of Lemon grass leaves

Plant Constituent	Extracts				
	Chloroform Extract	Methanol Extract	Aqueous Extract	Acetone Extract	Name of the test
1) Alkaloids	-	-	-	-	Wagners Test
2) Flavonoids	-	+	-	+	Shimoda, Lead Acetate Test
3)Phenolics & Tannins	-	+	+	-	Lead Acetate Test, Ferric Chloride Test
4)Steriods and Sterols	+	+	-	+	Salkowski Test
5) Carbohydrates	-	+	-	-	Fehlings Test, Benedicts Test
6)Saponin Test	-	-	+	+	Honey Comb Test, Foam Test
7) Glycosides	-	+	-	-	Glycosides Test
8)Protein & amino acids	+	+	+	-	Biuret test, Ninhydrin test
9)Anthraquinone test	+	+	+	-	Borntragers test

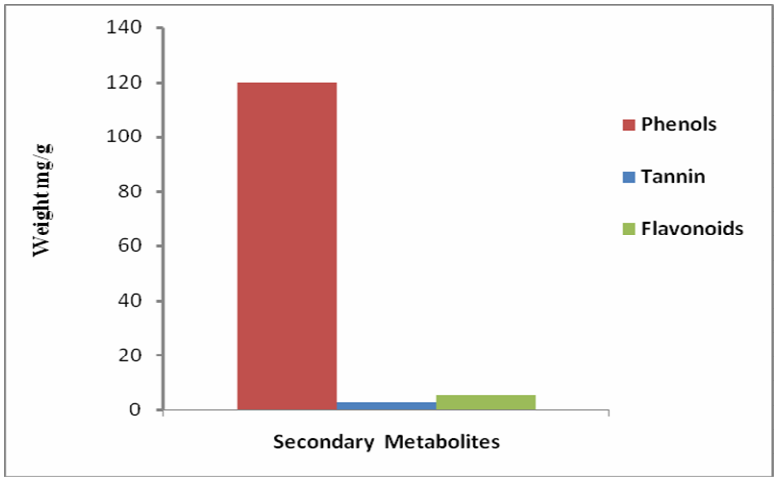
Table 2: Quantification of primary metabolites of Lemon grass leaves

S.NO	Primary Metabolites	Weight (mg/g dw)
1	Carbohydrates	150.63 ± 26.83
2	Chlorophyll	2.03 ± 0.02
3	Protein	105.4 ± 2.78
4	Lipids	0.03 ± 0.001

Values are means of three independent analyses ± SD (n=3)

Figure -1 : Lemon Grass Plant

Figure- 2: Quantification of Secondary Metabolites of Lemon grass leaves



The quantitative estimation of primary metabolites reveals various chemical constituents present in the plant (Table-2). The chlorophyll content of lemon grass is 2.03 mg / g dw. Carbohydrate content was found high (150.63 mg /g dw) followed by protein (105.4 mg / g dw) and lipid content was found very low i.e., 0.003 mg / g dw. Chlorophyll is the most indispensable class of primary compounds as they are the only substances that capture sunlight and make it available to plant system for its cultivation on photosynthesis. Earlier workers observed the leaves of lemon grass extract has high carbohydrate content and concluded that *Cymbopogon citratus* is a very good source of energy[31,32]. The presence of higher protein level in the plant parts towards their possible increase food value or that a protein base bioactive compound could also be isolated in future. The same result was noted by [33]. Lemon grass has a low level of lipid an indication that it would have little or no cholesterol. Our result was in agreement with [33].

Secondary metabolite analysis is necessary for extraction, purification, separation, crystallization, identification of various phytocompounds. The methanolic extract showed higher level of phenols (119.80 mg/g dw) than the other secondary metabolites. The higher amount of phenol is important in regulation of plant growth, development and disease resistance. Usually lemon grass plants contain highest amount of phenolic contents as reported by[34]. The level of flavonoid content was 5.28 mg/g dw extract. Earlier reports revealed that plant phenolic compounds including flavonoids are potent antioxidants with reported antimutageneic and anticarcinogenic effects [35]**.** The level of tannin content was 3.00 mg/g dw. The presence of tannin in lemon grass leaves was also confirmed by [29].Tannin contribute various medicinal properties such as antimicrobial, anti-inflammatory and astringent activity [36].

The same type of phytochemical screening of primary and secondary metabolites were reported earlier by many workers. The lemon grass samples collected from different locations of India showed significant variations in the contents of phenols, flavonoids and tannins [29,37] . The present investigation showed significant variation in the contents like phenol, flavonoids, and tannin when compared to above mentioned reports. These variations are due to number of environmental factors such as climate, altitude, rainfall etc. as mentioned by[17].

Conclusion:

Thus, the results obtained in the present study indicates lemon grass leaves have the potential to act as a source of useful drugs because of presence of various phytochemical components such as carbohydrate, protein, lipids, phenols, flavonoids and tannin. The results are very much encouraging but scientific validation is necessary before being put into practice.

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