

## Pharmacognostic and physicochemical evaluation of *Cymbopogon citratus* (DC) Stapf leaves

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### Abstract

Plants have been utilized as a source of nutrition and healthcare products since ancient times. The present study deals with various standardization parameters like morphological characters, microscopic evaluation, fluorescence analysis, physicochemical evaluations-proximate analysis (Moisture, ash content, crude protein, crude lipid and crude fiber), solubility value (alcohol soluble, water soluble and ether soluble) and mineral analysis (macro and micro nutrients). In the present investigation, various sensory parameters of the plant material (such as colour, odour, size, shape, and taste) were also studied by organoleptic evaluation method. In fluorescence analysis, the leaf sample was subjected to different acid and basic solvents and the fluorescent nature was observed in visible light, short UV and long UV. The proximate analysis showed the moisture content of lemongrass leaves was 5.36% dry weight and crude protein 14.64% dry weight. It was observed that alcohol soluble extractives value is higher than water soluble and ether soluble extractive value. The content of vitamin E was found high (216.67 mg/gm of extract) followed by Vitamin C content (155.07 mg/gm of extract). The mineral analysis revealed various major and trace elements like calcium, sodium, potassium, zinc, manganese and iron present in lemongrass leaves in various levels.

**Key words:** *Cymbopogon citratus* (DC) Stapf, pharmacognostic study, physicochemical properties

### 1. Introduction

India has a great wealth of traditional knowledge and wisdom. The classical Indian text include Rigveda, Atharveda, Charak Samhita and Sushruta Samhita. Ayurveda is one of the traditional systems of medicines practiced in India and Sri Lanka and its origin traced back to 6000 BC. Ayurvedic medicines are largely based on herbal and herbomineral preparations and have specific therapeutic principles (Patwardhan and Hopper, 1992).

Traditional healers used different parts of medicinal plants as medicine. Among the different parts, leaves were more frequently used apart from whole plant parts, fruits, stems, roots, flowers and latex. The methods of preparation fall into four categories, namely plant parts applied as paste (38%), juice from fresh plant parts (20%), powder from dried plant parts (20%), some fresh plant parts (6%) and decoction (12%). Both external applications (for skin diseases, snake bites and wounds) and internal consumption of the preparations were involved in the management of diseases (Mukherjee, 2002).

Today, several medicinal plants and their products are in use, being employed as home remedies, over the counter drugs as well as raw materials for the pharmaceutical industry and they represent a substantial proportion of the global drug market. The objectives of

producing inexpensive, potent and safer drugs of plant origin can be met to some extent by promoting compound formulations of plant medicines in their natural or semi processed form (powder or extract) as used in traditional medicine for common disorder (Kaur *et al.*, 2011). It becomes extremely important to make an effort towards standardization of the plant material used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy.

Global interest in the application of herbal medicine to attain health for all, the WHO, recommend taxonomic and pharmacognostical techniques like morphological (macro and microscopic), anatomical, palynological, qualitative and quantitative phytochemical screening, fluorescence (UV& IR analysis) and physico-chemical analysis for standardization of botanicals. Such type of screening programmes on permanent basis can prove useful in improving the standards and efficacy of herbal medicines for commercial preparations. In this way, the authentication methodologies shall assist in avoiding adulteration and other problems by herb traders, manufacturers and layman user. In the process of taxonomic and pharmacognostic treatment, this sort of analyses, and their observations may help to remove confusion about the identity of genuine source of herbal drug (Kumar *et al.*, 2012).

Proper standardization and dosage formulation in due consideration of the therapeutic and toxicity ratio will, however, be necessary for controlled clinical trials to prove their efficacy. Development of a single drug based on the active principles can also be taken up to

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rationalize drug therapy as well as to develop synthetic analogues on their model for specific activity. Research on medicinal plants in each geographical region should be conducted for proper integration of these remedies in the modern system of medicine for providing benefit of effective and cheaper drugs from the right plant resources available in our vast country (Kaur *et al.*, 2011).

Based on the foregoing details, this present study investigates the pharmacognostic and physico-chemical properties and nutrient composition of leaves of *C. citratus* (DC) Stapf with a view to provide pertinent information on its identification, chemical elaboration and pharmacological potentials. Findings from this study would be useful as standards as well as source of reference for further scientific investigation of this species.

## 2. Materials and Methods

### 2.1 Collection of plant material

The aerial parts of the plant *Cymbopogon citratus* were collected from Department of Biotechnology 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 (PARC/2014/2049) has been deposited in our department herbarium for future reference.

### 2.2 Plant characterization

#### 2.2.1 Organoleptic evaluation

Organoleptic features of the plant were evaluated by observing colour, odour, taste, size, shape of morphology and special features like texture.

#### 2.2.2 Microscopic evaluation

In microscopic evaluation, the required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin-5ml+ Acetic acid-5 ml + 70% Ethyl alcohol-90ml). After 24 h. of fixing, the specimens were dehydrated with graded series of tertiary –Butyl alcohol as per the methodology standardized by SASS (1958).

#### 2.2.3 Fluorescence analysis

The leaf powder of *C. citratus* was treated with various acidic and basic solvents like methanol, ammonia, potassium hydroxide, picric acid, distilled water, petroleum ether, 50% sulphuric acid, 50% nitric acid and 50% hydrochloric acid for fluorescence analysis and were then observed under UV/ visible chamber under visible, short wave and long wave regions simultaneously and then observed under visible light, short ultra violet light, long ultra violet light.

### 2.3 Physicochemical evaluations

#### 2.3.1 Proximate analysis

Proximate analysis of extract was done using the method of Association of official analytical chemist (AOAC,2000) and Pearson composition and analysis of food.

#### 2.3.2 Moisture content

About 2 g of the extract was weighed and placed in a crucible of constant weight. This was placed in an oven at 105°C then dried; the weight was measured carefully to get a constant weight. The loss in weight indicates the moisture content.

#### 2.3.3 Ash content determination

Crucible used for ash content determination was weighed and dried in an hot air oven at 110°C to a constant weight. About 2 g of each extract was weighed and placed in the crucible and weight of the crucible and extract was taken. This was placed in a furnace and ignited for 3 h. at 55°C till the samples have a cotton wool like texture and then it was cooled in a dessicator and weighed using balance.

#### 2.3.4 Crude protein

About 1 g of the sample was weighed into the Kjeldahl flask. About 0.1 gm of  $\text{Ca}_2\text{SO}_4$  were added into the flask with 20 ml Conc. $\text{H}_2\text{SO}_4$ . The flask was then placed in a slanting position on Kjeldahl heating mantle in the fume cupboard. Digestion continued until there was a color change from black to bluish green which indicating that digestion has ended. It was set up against blank, the digest were removed and allowed to cool and was then diluted with water and made upto 200 ml on ice.

About 50 ml of aliquot of each digest were poured into a distillation flask. About 30 ml of NaOH were carefully layered into solution in order to make it a strong alkaline and 50 ml of 0.1 N  $\text{H}_2\text{SO}_4$  measured and kept in a beaker with 2 drops of methyl red as an indicator. The  $\text{H}_2\text{SO}_4$  acted as a receiving flask. About 150 ml was distilled over heat was put off to avoid drop in pressure. The distillate was titrated with 0.1M NaOH in the burette. This was done for extract and blank and % of nitrogen was calculated.

#### 2.3.5 Lipid content

About 1 g of sample was weighed into a thimble of known weight. About 150 ml of petroleum ether (60-80°C) was poured into 250 ml conical flask using measuring cylinder. The soxhlet extractor were the sack and its content had been introduced was fitted and solvent boiled under reflux. The extraction process lasted for 8 h. and sack with its content were removed dried in an oven for 2 h. and then weighed with a balance.

#### 2.3.6 Crude fibre

This is organic residue which remains after the materials have been treated with standardized conditions with light petroleum, boiled diluted  $\text{H}_2\text{SO}_4$  boiled diluted HCl, alcohol and ether. The crude fibre consists largely of cellulose together with little lignin and it can extrapolated as :  $100 - (\text{Moisture \%} + \text{ash \%} + \text{lipid \%} + \text{protein\%})$ .

### 2.4 Extractive value

Solubility values of crude drugs are useful for their evaluation especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug. The raw materials were dried and powdered and the powdered materials were used for analyzing different parameters.

#### 2.4.1 Alcohol soluble extractive (ASE)

Four gram of air dried and coarsely powdered tissue was macerated and placed in a glass stopper flask with 100 ml of 90% ethanol for 24 h. The contents were frequently shaken for the first 6 h. and allowing to stand for 18 h. in 90% ethanol. Then the contents were

filtered rapidly with taking precautions against loss of ethanol. 25 ml of the filtrate was allowed to dry on a water bath using tared flat bottomed petriplate/shallow dish. The petriplate was dried at 105°C for 1h. in a hot air oven and removed and cooled in a dessicator and weighed. The process was repeated till the concordant weight was obtained and the percentage of ethanol-soluble extractive value was calculated using the following formula (Kokate *et al.*, 2008).

$$\% \text{ of alcohol soluble extractive value} = \frac{B - A \times 4 \times 100}{W}$$

where, A= empty wt. of the dish (g)

B = wt. of dish + residue (g)

W= wt. of plant material taken (g)

#### 2.4.2 Water soluble extractive (WSE)

Four gram of the air dried and coarsely, powdered tissue was macerated with 100 ml of 5% chloroform water in a glass stopper conical flask for 24 h. the contents were shaken frequently during the first 6 h. Thereafter the contents were filtered rapidly by decanting the water extract. 25 ml of the filtrate was evaporated to dryness on a water bath in tarred flat bottomed petriplate/shallow dish. 2 ml of alcohol was added to the dry residue and the contents were shaken and dried again on water bath. It was then dried at 105°C for 1 h. in the hot air oven and cooled in a desiccator for 30 mins and weighed. The process was repeated till the concordant weight is obtained. The % of WSE was calculated using the formula mentioned for calculation of alcohol soluble extractive value (Kokate *et al.*, 2008).

#### 2.4.3 Ether- soluble extractive (ESE)

1 g of air dried drug, coarsely powdered was macerated with 100 ml of ether in a closed flask for 24 h. with frequent shaking. It was filtered rapidly with taking precautions against loss of ether. 25 ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100°C and weighed. The percentage of ether soluble extractive was calculated using the formula mentioned for calculation of alcohol soluble extractive value (Kokate *et al.*, 2008).

### 2.5 Nutrient analysis

#### 2.5.1 Vitamins

##### 2.5.1.1 Determination of vitamin E

Vitamin E was evaluated in the methanol extract following the method given by Prieto *et al.*, 1999. An aliquot of 0.1 ml of extract (10 mg/ 2 ml) was mixed with 1 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 37°C for 90 min with vigorous shaking. Absorbance of the aqueous phase at 695 nm was measured against the appropriate blank. A typical blank contained 1 ml of reagent solution and 0.1 ml of the respective solvent, incubated under the same conditions as the samples. The analysis was performed in triplicate and the vitamin E content was expressed as  $\alpha$ -tocopherol equivalents.

##### 2.5.1.2 Determination of vitamin C

The ascorbic acid content was determined using 2, 6, dichloroindophenol (DIP) method with a modification of Yen and

Chen, (1996). One mg of sample extract was dissolved in 10 ml of 1% metaphosphoric acid and filtered using Whatmann No.1 filter paper. 1 ml of this filtrate was added with 9 ml of 50  $\mu$ M DIP and incubated at room temperature for 15 s. The developed color was measured at 515 nm. The analysis was performed in triplicate and the results were expressed as ascorbic acid equivalents.

#### 2.6 Mineral content by atomic absorption spectroscopy

Atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) with an air/acetylene flame and respective hollow-cathode lamps was used for absorbance measurements. Wavelengths, slits and lamp current used for the determination of six elements were 213.9 nm, 0.5 nm, 4.0 mA (zinc); 422.7 nm, 1.2 nm, 4.0 mA (calcium); 324.8 nm, 1.2 nm, 3.0 mA (manganese); 589.0 nm, 0.8 nm, 3.0 mA (sodium); 248.3 nm, 0.2 nm, 6.0 mA (iron) and 766.5 nm, 0.8 nm, 4.0 mA (potassium), respectively. The results for mineral contents were expressed as mg/100 g dry weight.

### 3. Results and Discussion

Medicinal plants are starting material for any herbal preparation such as herbal medicines, herbal tea, herbal oil, *etc.* 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. Documenting the indigenous knowledge through ethnobotanical studies is important for the conservation and utilization of biological resources. The present study was aimed to present the phytochemical and pharmacognostical properties of *C. citratus* by studying organoleptic features, proximate, nutrient and mineral contents of its leaves.

#### 3.1 Organoleptic features of leaves

Organoleptic authentication means the study of herbal medicines using various organs of senses which includes the analysis of color, odour, taste, shape, size, texture, weight, structure, *etc.* Obviously the initial visibility, odour, color, taste, sight and smell of the plant or plant extract are specific to identify itself. Organoleptic evaluation is simplest analysis but most common practice among the practitioners, herbalists, locals and herb sellers. After organoleptic evaluation, the lemongrass leaves are categorized as follows:

Type	- Simple leaf
Colour	- Upper surface: dark green and lower surface: light green
Odour	- lemon like smell
Taste	- bitter
Size	- 1-2 meter long and 5-10 mm wide
Shape	- leaf blade linear and tapered to both ends
Margin	- entire
Surface	- flat, very coarse
Venation	- parallel

#### 3.2 Microscopic evaluation of *C. citratus*

##### 3.2.1 Anatomy of leaf

The leaf exhibits isobilateral symmetry and thick circular midrib. The midrib is not projecting beyond the level of the lamina. The vascular bundle of the midrib is collateral and prominent. It consists of two large metaxylem elements and narrow protoxylem element.

The metaxylem elements are circular and thick walled. The diameter of the metaxylem element is 30  $\mu\text{m}$ . There is a large mass of phloem element located beneath the metaxylem elements. The vascular bundle is closed type. The phloem and xylem elements are surrounded by thick walled fibres. There are two thick masses of fibre caps on the adaxial and abaxial ends of the vascular bundle.

The lamina consists of quite large adaxial epidermal cells with thick smooth cuticle (Figure 1a). The abaxial epidermis is thin and squarish or cylindrical with thick cuticle on the outer tangential wall which bear long pointed prominent spines. The spongy mesophyll includes three or four layers of lobed less compact parenchyma cells. The lateral vein is similar to that of midrib. It is circular, collateral and closed. The bundle consists of two large elliptical metaxylem elements and a prominent mass of phloem element (Figure 1b). The lamina is 110  $\mu\text{m}$  thick.

The leaf margin is curved down and is slightly thin (Figure 1c). The extreme margin of the lamina consists of a single, circular large sclereid and a thick mass of fibre. The abaxial epidermal layer includes vertically oblong thin walled cells with thick outer tangential wall. Long, curved pointed spines are produced by the cuticle. The mesophyll tissue remains unchanged from that of lamina. The marginal part is 50  $\mu\text{m}$  thick.

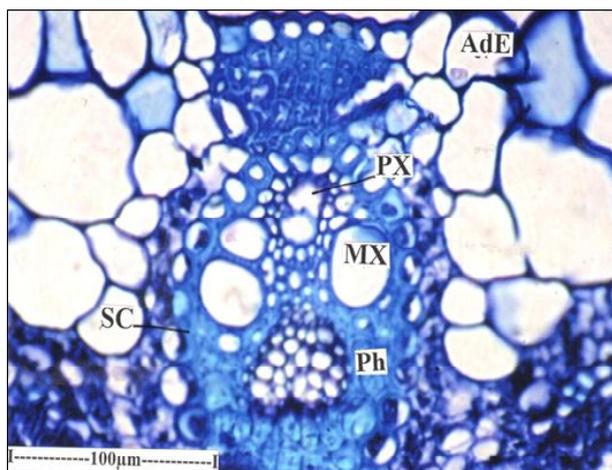


Figure 1(a): T.S of Midrib of the Lamina

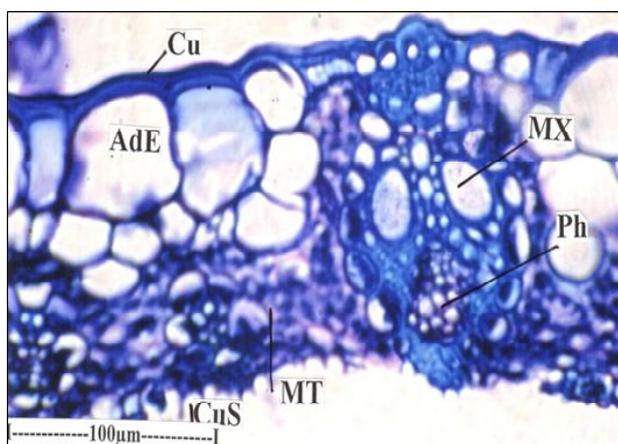


Figure 1(b): T.S of lamina through lateral vein

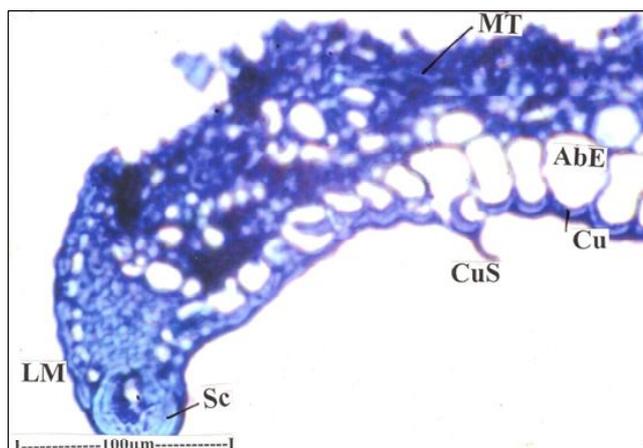


Figure 1(c): T.S of lamina through leaf margin

(AbE-Abaxial epidermis, AdE-Adaxial epidermis, Cu: cuticle, cuS : cuticular spine, LM:Leaf margin, MX: Metaxylem , MT: Mesophyll tissue, Ph : Phloem, Px: Protoxylem, Sc: Sclerenchyma)

### 3.3 Fluorescence analysis

The fluorescence analysis represented the behavioural changes of powdered leaf parts with different chemicals reagents under day light and UV (Kabata *et al.*, 2003; Badamir, 2002; Evana *et al.*, 1980) and the results are presented in Table 1.

Table 1: Fluorescence analysis of *C. citratus* (DC) Stapf leaf powder

Reagents	Visible	Short UV-254	Long UV-366
Powder	Pale green	Light green	Light green
Powder + Pet Ether	Pale Green	Green	Pale green
Powder + Ethyl acetate	No colour	Pale green	Pale green
Powder + Ethyl acetate : HCL (1:1)	No colour	Pale green	Pale green
Powder + Methanol	Dark green	Pale green	Dark green
Powder + Chloroform	White	Light brown	Red
Powder + Acetone	Pale green	Fluorescent green	Pale green
Powder + 50% $\text{H}_2\text{SO}_4$	Pale green	Fluorescent green	Fluorescent green
Powder + 50% $\text{HNO}_3$	Brown	Dark brown	Pale red
Powder + 50% HCl	Yellow	Orange	Red
Powder +10% NaOH	Yellow	Pale orange	Brown

### 3.4 Physicochemical evaluation

Physicochemical evaluation includes tests for moisture content, ash content, crude fibre, lipid content, crude fibre and solubility value of lemongrass leaves. In the present investigation, proximate composition of lemongrass leaves were analyzed and shown in Table 2. The moisture content of lemongrass leaves was 5.36 % dry weight. The ash content was 13.42 % dry weight. The crude protein and fat content were 14.64 and 1.23 % dry weight, respectively and the crude fibre in lemongrass leaves was 27.72 % dry weight. The low moisture content in the leaves indicate that they have capacity to prevent microbial attack and allows for high storage capacity. Our results were in agreement with Oloyede (2009) and Nambiar and Hema Matela (2012) who observed that the leaves of lemongrass have low moisture content. In the present investigation, the ash content was 13.42 % dry weight. The ash content is generally recognized as a measure of quality for the assessment of the functional properties of foods. Ash in food contributes the residue remaining after all the moisture has been removed as well as the organic material (fat, protein, carbohydrates, vitamins, organic acid, etc). Ash content is generally taken to be a measure of the mineral content of the original food.

The crude protein and fat content were 14.64 and 1.23 % dry weight, respectively. The moderate amount of protein present in the lemongrass leaves indicates that the plant can form a part of human diet. In lemongrass leaves fat was found to be very less, i.e 1.23 % dry weight. Crude fibre is made up largely of cellulose together with a little lignin and it aids absorption of glucose and fat, enhances digestibility. In the present investigation, the crude fibre in lemongrass leaves was 38.86 % dry weight as reported by Akande and Tindall, (1986).

**Table 2:** Proximate composition of lemongrass leaves

S.No.	Composition	% Dry weight
1	Moisture Content	5.36
2	Ash Content	13.42
3	Crude protein	14.64
4	Crude fiber	38.86
5	Fat content	1.23

Values are means of three independent analyses  $\pm$  SD (n = 3)

### 3.5 Extractive value

Different plant species would obviously have different chemical profile. Chemical present in the plant material could be dissolved in different solvent for the purpose of further analysis. Therefore, three solvents - water, alcohol and ether were selected to determine the soluble substance. Water-soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating. The water-soluble extractive values of lemongrass is 10%, alcohol soluble extractive values is 15% and ether soluble extractive values is 8% (Table 3). It was observed that alcohol soluble extractives value is higher than water soluble and ether soluble extractive value. Our results were in agreement with the results of Oloyede (2009) and Nambiar and Hema Matela (2012).

**Table 3 :** Extractive values of extracts of *Cymbopogon citratus*

Parameters	Values
Alcohol soluble extractive (%)	15.0
Water soluble extractive (%)	10.0
Ether soluble extractive (%)	8.0

### 3.6 Nutrient analysis

The quantitative estimation of vitamin contents present in the plant were shown in Table 4. The content of vitamin E was found high (216.67 mg/gm of extract) followed by Vitamin C content (155.07 mg/gm of extract). Vitamin C aids in wound healing and also help in resisting infection. Its deficiency can cause scurvy (characterized by bleeding gum, poor healing of wound and low resistance to infection). The recommended dietary allowance of vitamin C is 45 mg per day (WHO, 1991). There is an increasing body of evidence that natural antioxidants such as vitamin C and E protect the body against a number of degenerative diseases such as atherosclerosis, aging and certain types of cancer. The substantial level of these molecules in lemongrass leaves is indicative of the plant nutritional and medicinal significance.

**Table 4 :** Antioxidant vitamin content of lemongrass leaves

S.No.	Composition	Content mg/gm of extract
1	Vitamin C	155.07
2	Vitamin E	216.67

Values are means of three independent analyses  $\pm$  SD (n = 3)

The mineral analysis of lemongrass leaves reveals various major and trace elements like calcium, sodium, potassium, zinc, manganese and iron (Table 5). The calcium content was 39.4 mg / 100 gm dw, sodium content was 56.3 mg / 100 gm dw, potassium content was 60.2 mg/100 gm dw, zinc content was 1.25 mg/100 gm dw, manganese content was 0.951 mg/100 gm dw and iron content was 0.034 mg/100 gm dw. The results were in agreement with reports of (Berry, 1998; Latham, 1997). Since calcium content is 39.4 mg/100 gm dw, this result suggests that the leaves may be of greater physiological significance to cure diseases related to bone system. Sodium is the principle extracellular cation and is used for acid base balance and osmoregulation in intermodular fluid. Potassium is the principal cation in intracellular fluid and functions in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle, cell membrane function. The high potassium content could be utilized for the management of hypertension and other cardiovascular conditions. Iron functions as haemoglobin in the transport of oxygen and is an important constituent of succinate dehydrogenase as well as a part of the heme of haemoglobin (Hb), myoglobin and the cytochromes (Chandra, 1990). Zinc boost the immune system and act as antioxidant (Ferguson *et al.*, 1993). Since the level of metals such as manganese and zinc were found at low level in the leaves is a beneficial in the light of the toxicity associated with metal accumulation in the body.

**Table 5:** Mineral content of lemongrass leaves

S.No.	Composition	Content mg / 100 gm dw
1	Calcium	39.4
2	Sodium	56.3
3	Potassium	60.2
4	Zinc	1.25
5	Manganese	0.951
6	Iron	0.034

Values are means of three independent analyses  $\pm$  SD (n = 3)

#### 4. Conclusion

The present study investigates the pharmacognostic and physicochemical properties of lemongrass leaves. The present study deals with various standardization parameters like morphological characters, leaf anatomy, fluorescent characters and physicochemical properties of lemongrass leaves. The proximate analysis showed the moisture content of lemongrass leaves was 5.36 % dry weight and crude protein 14.64% dry weight. It was observed that alcohol soluble extractive value is higher than water soluble and ether soluble extractive value. The content of vitamin E was found high (216.67 mg/gm of extract) followed by vitamin C content (155.07 mg/gm of extract). The mineral analysis revealed various major and trace elements like calcium, sodium, potassium, zinc, manganese and iron present in lemongrass leaves in various levels. Such study on the physicochemical parameters, organoleptic studies and behavioural pattern of lemongrass leaves provides important information which may be helpful in authentication and adulteration for quality control of this plant in future.

#### Conflict of interest

We declare that we have no conflict of interest.

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