Pharma-cognostic Standardization of *Cymbopogon citratus* (DC.) Stapf. Leaf

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Abstract

attira' ascribed to plant species *Cymbopogon citratus* (DC.) Stapf. belongs to family *Poaceae*. In Ayurveda, it is widely used for treatment of various diseases. It is an important ingredient of Ayurvedic Formulations viz. Sheetaprashamana mahakashaya, Ayurvadya taila, Mahapanchagavya ghrita, Ayurvedic chay etc. It is also used for flavouring soups and curries. An infusion of leaves is sometimes taken as a substitute for tea, a refreshing beverage. The essential oil from *C.citratus* is widely used in perfumery, cosmetic preparations and in aromatherapy. The diagonostic characters obtained from investigated parameters such as organoleptic, microscopical, powder characters, physico-chemical constants, TLC fingerprinting profile of leaf are given. The data obtained by this study lead to Pharmacognostic Standardization of *Cymbopogon citratus* (DC.) Stapf. leaf.

Key words: *Cymbopogon citratus* (DC.) Stapf, Pharmacognosy, Physico-chemical studies

Introduction

'Kattira' ascribed to plant species *Cymbopogon citratus* (DC.)Stapf. syn. *Andropogon citratus* DC. belongs to family *Poaceae*. It is popularly known as Lemon grass. The plant is aromatic, bitter, acrid, stimulant, thermogenic, anthelmentic, laxative, appetizer, alexipharmic, antispasmodic and anaphrodisiac and is useful in helminthiasis, flatulence, gastric irritations, anorexia, poisonous bites, bronchitis, epilepsy, leprosy, skin diseases, cholera, neuralgia, sprains, fever (Sharma *et al.,*2002). In Ayurveda, it is widely used for treatment of various diseases. It is an important ingredient of Ayurvedic Formulations viz. Sheetaprashamana mahakashaya, Ayurvadya taila, Mahapanchagavya ghrita, Ayurvedic chay etc.

Lemon grass is also used for flavouring soups and curries. (Anonymous,1950). An infusion of leaves is sometimes taken as a substitute for tea, a refreshing beverage (Kurup *et al.,*1979). The essential oil from *C.citratus* is widely used in perfumery, cosmetic preparations and in aromatherapy.

Some fragmentary information regarding pharmacognostic evaluation of leaves is available in literature. It is also reported that other species of *Cymbopogon* are used as its adulterants (Datta and Mukerji,1952). Therefore detailed studies in respect of pharmacocognostic standardization was carried out in

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view of need for identification and authentication of leaves of this particular species.

Material and Methods

Drug sample collected from Ghaziabad and identified with the help of standard flora (Bor,1982). It was thoroughly washed to get rid of any unwanted foreign organic or inorganic matter, adhered soil and other unwanted parts etc. After washing, it was finally cut to suitable sizes for further proceedings. Hand sections were cut, stained and mounted in Canada balsam for anatomical studies. For powder study Harold *et al*.(1981) was followed. To determine physico-chemical constants, Israili (1971), Indian Pharmacopoeia (2001) was consulted. Behaviour of the powdered drug towards specific reagent was recorded according to Kapoor *et al.*(1975). For fluorescence study schedules mentioned by Chase and Pratt (1949), Kokoski *et al*.(1958) and Trease and Evans(1978) were followed. The colouration was recorded according to Anonymous (1978). Standard prescribed procedures for histochemical studies (Johansen,1940; Datta and Mukerji, 1950; Youngken,1951; Cromwell,1955; Trease and Evans,1978; Henry,2001; Anonymous,2002), Organic group detection (Johansen,1940; Youngken,1951; Robinson,1963; Anonymous,1966; Saxena,1975; Rathore,1977;

Dan *et al*.,1978; Trease and Evans,1978; Gupta *et al.,1*980; Brahman and Saxena,1989), UV Spectroscopy (Willard *et al*. (1965) and Chromatography (Wagner *et al*, 1984) were adopted.

Observations and Results

I. Organoleptic Characteristics:

A. The drug comprises of leaves which are linear, tapering upwards to a long setaceous point, approximately 90-115 cm in length and 1.4-2.0 cm in width. The leaves are eau-de-nil to sea green in colour. The leaves are glaucous, rough along margins, also with slightly rough surface because of protruding veins. The leaves possess parallel venation. Midrib is somewhat stout below and whitish on upper side. The leaves are chartaceous and possess a very strong lemon aroma and bitter taste (Figure 1).

B. The powdered drug is light olive green in colour with a powerful lemon aroma and bitter taste.

Fig. 1: Cymbopogon citratus (DC.) Stapf. Habitat of Plant and Macroscopical Features of Drug . A, Plant in Natural Habitat;
B, Fresh Leaf; C, Dried Leaf.

II. Micro-morphological Characteristics:

The transverse section of leaf shows an upper epidermis and lower epidermis covered with thick cuticle. Some of the cells of upper epidermis are comparatively larger (bulliform cells). In surface view, epidermis shows polygonal to tubular cells. Some of the marginal cells of leaf are provided with hair which are rhomboidal in shape and possess pointed end. Stomata are distributed on both surfaces and are multistomatic. It also shows abundance of hair bases or hair with elongated base and hemispherical to tapering apex, long cells more or less rectangular with slightly sinuous walls, short cells over the vein and silica bodies which are near to cross shape (Figure 2). Beneath

each epidermis, there are sclerenchymatous patches at close intervals. The vascular system contains a number of large and small vascular bundles. The bundle sheath of each vascular bundle possesses a tier of parenchymatous cells followed by another tier of palisade-like cells. Large vascular bundles have prominent sclerenchymatous patches on both and upper lower ends extending between the bundles and epidermal layers. The large bundles have distinct phloem towards lower epidermis and xylem towards the upper epidermis. Phloem consists of sieve tubes and companion cells. The xylem has two pitted, oval metaxylem vessels, tracheids in between metaxylem vessels, scanty xylem parenchyma and protoxylem vessel. Protoxylem vessel is located towards upper epidermis and is represented by a lysigenous cavity. The small vascular bundles are also surrounded by bundle sheaths and contain distinct but less developed xylem and phloem. The bundles are conjoint, collateral and closed. The transverse section of midrib shows similar structure as in case of a vein (Figure 3 and 4).

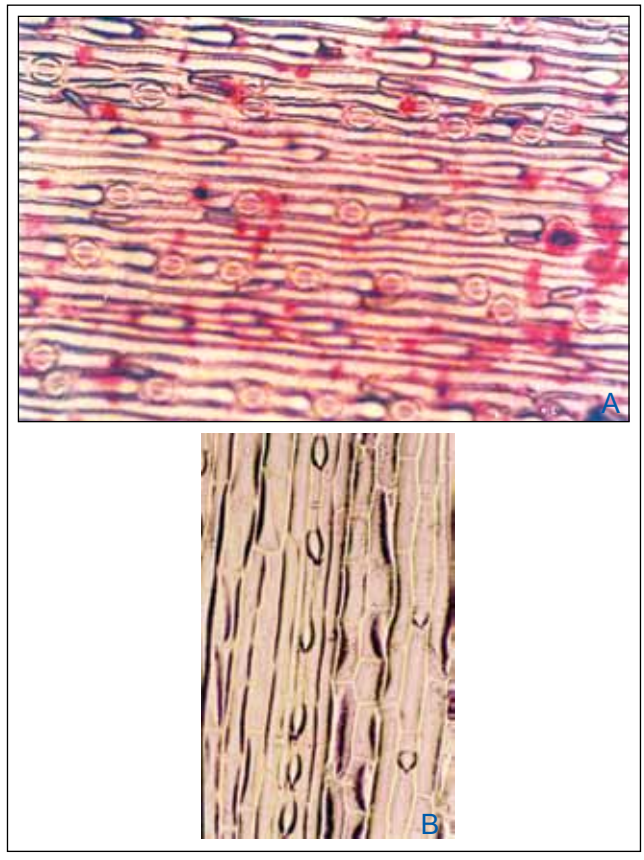

Fig. 2: Surface view of *Cymbopogon citratus* (DC.) Stapf leaf

A. Surface view of lower epidermis with stomata (St) and hair (H) 100X
B. Surface view of upper epidermis showing hair (H), long cell (Lc), short cell (Sc) and Silica bodies (Sibd) 200X

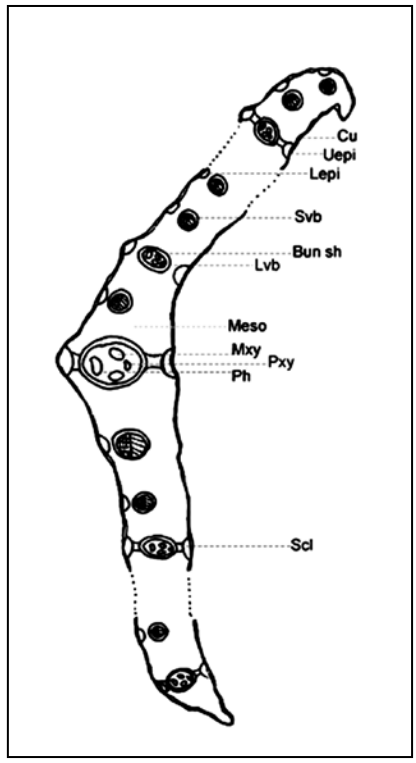

Fig. 3: Line diagram of Cymbopogon citratus (DC.) Stapf. in transverse view (60X)

Abbreviations: Bun sh-bundle sheath; Cu-Cuticle; Lepi-lower epidermis; Lvb-large vascular bundle; Meso-mesophyll cells; Mxy-metaxylem; Ph-phloem cells; Pxy-protoxylem; Scl-patches of sclerenchyma; Svb-small vascular bundle; Uepi-upper epidermis

The microscopic dimensions of individual cell of different tissues of drug and cell contents are enumerated in Table 1.

Table-1: Dimensions of cellular elements and cell contents in transverse section.

Cellular Elements/Cell Contents	Measurements(m)
	Length × Width
Cuticle	1.50-3.00-3.75 (T)
Upper epidermis cells	7.50-18.75-30.00 (D)
Bulliform cells	30.00-60.00-90.00 (D)
Lower epidermis cells	15.00-22.50-26.25 (D)
Stomata	30.00-30.00-45.00 × 9.40-11.25-11.25
Sclerenchyma cells	11.25-18.75-30.00 (D)

Cellular Elements/Cell Contents	Measurements(m)
	Length × Width
Spongy parenchyma cells	18.75-22.50-30.00 (D)
Metaxylem vessels	15.00-45.00-60.00 (D)
Protoxylem vessels	7.50-26.25-37.50 (D)
Phloem cells	7.50-15.00-22.50 (D)
Outer bundle sheath cells	26.25-30.00-37.50 × 15.00-18.75-22.50
Inner bundle sheath cells	15.00-22.50-30.00 (D)
Hair	15.00-22.50-30.00 (L)
Starch granules	3-5-6 (D)

Abbreviation: 'D' refers to diameter, 'T' refers to thickness and 'L' refers to length.

Quantitative Microscopic Characters:

The observations of stomatal indices of upper and lower surfaces and the palisade ratio of palisade-like cells on upper and lower surfaces are cited in the Table 2.

Table 2: Quantitative Microscopy.

S. No.	Parameter	Surface of leaf	Mean±SD
1.	Stomatal indices	Adaxial	4.53 ± 1.40
		Abaxial	15.78 ± 0.85
2.	Palisade ratio	Adaxial	18.40 ± 1.14
		Abaxial	17.95 ± 0.57

The leaves of ***C.citratus*** have parallel venation and there, vein-islet number of the same could not be determined as they possess no definite vein-islets.

Powder Study:

The powdered drug is characterized by fragments of lower epidermis in surface view showing stotata and hair, fragments of marginal cells with hair which are rhomboidal in shape and possess pointed end, vessels with annular and reticulate thickenings and simple pits, mesophyll cells with oil content, starch granules, simple, round to oval, sclereids with pits, fragments showing fibres and associated sclereids and parenchyma cells in longitudinal view, broken fibres with tapering ends, fragments showing sclerenchyma and hair (Figure 5).

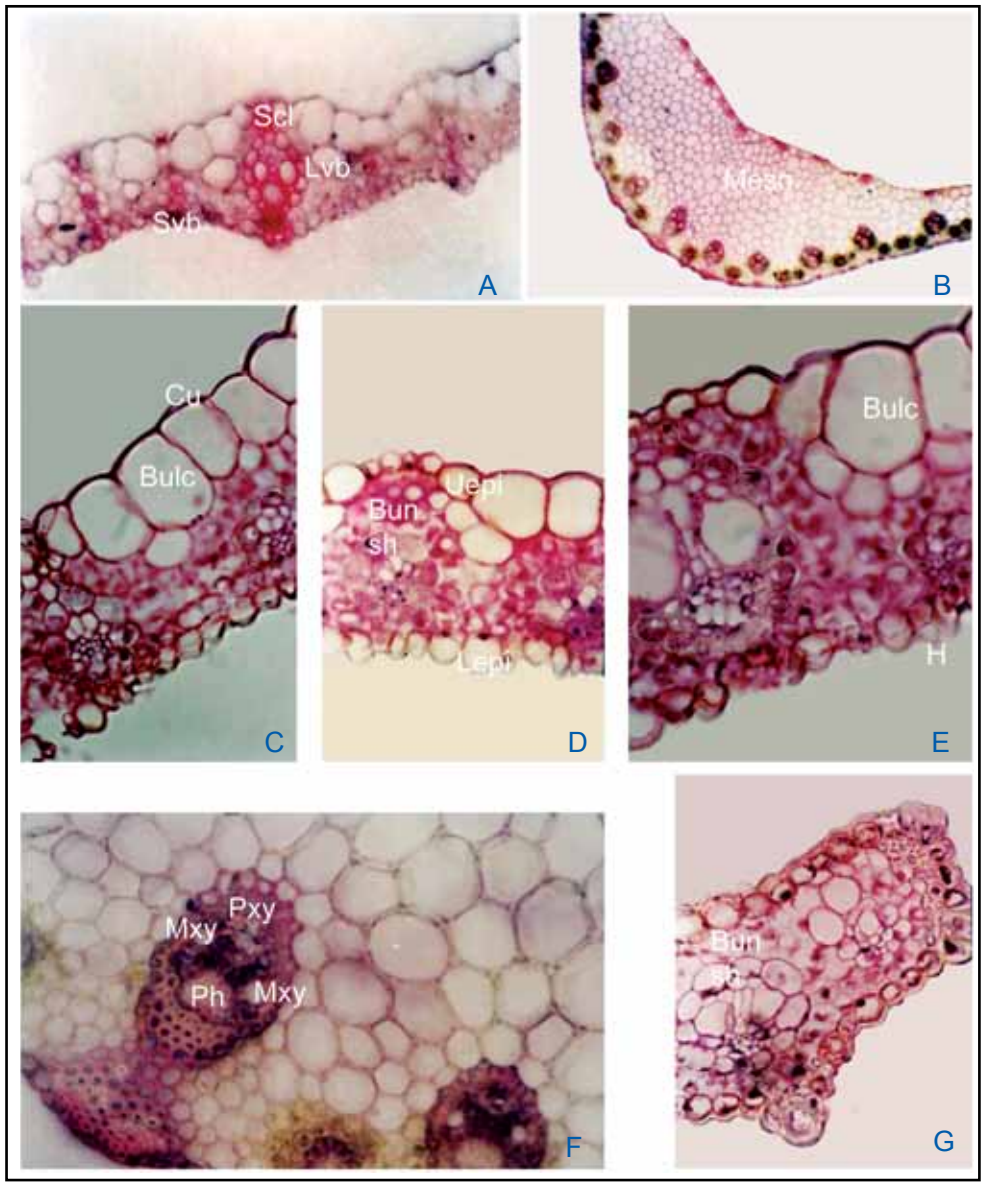

Fig. 4. Photomicrographs of *Cymbopogon citratus* (DC) Stapf. in transverse view

A	Lamina portion showing large and small vascular bundle and patches of sclerenchyma	200X
B.	TS through Midrib	40X
C.	Cuticle and bulliform cells	400X
D.	Upper and lower epidermis and bundle sheath	400X
E.	Bulliform cells and hair	400X
F.	Phloem cells, protoxylem and metaxylem	200X
G.	Bundle sheath	400X

Abbreviations: Bulc-bulliform cells; Bun sh-bundle sheath; Cu-Cuticle; H-hair; Lepi-lower epidermis; Lvb-large vascular bundle; Meso-mesophyll; Mxy-metaxylem; Ph-phloem cells; Pxy-protoxylem; Scl-patches of sclerenchyma; Svb-small vascular bundle; Uepi-upper epidermis

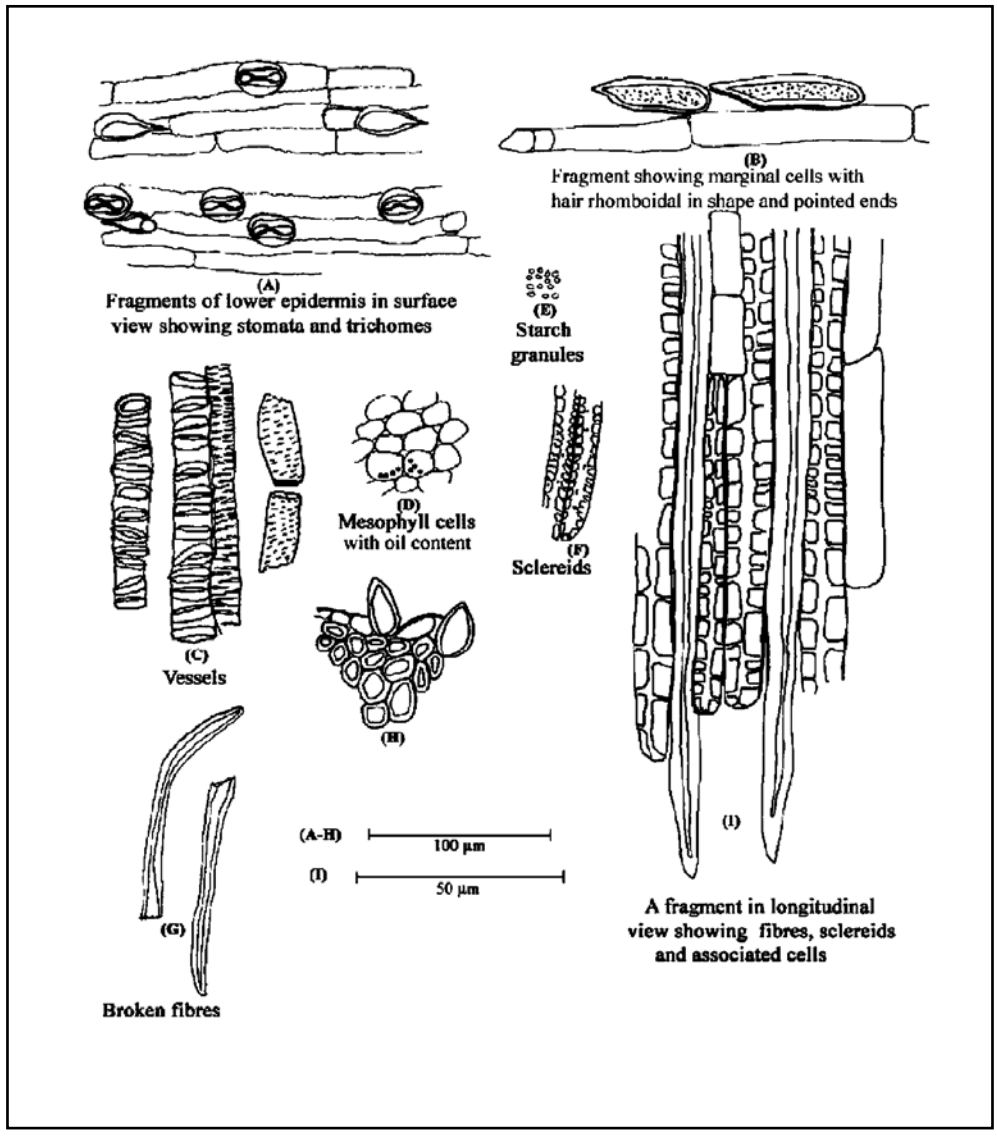

Fig. 5. Powder Characteristics

III. Histochemistry

A. *Micro-Chemical Tests and Behaviour of Specific Reagents Towards Plant/ Drug Tissues:* Observations and results pertaining to the observations of Micro-chemical Tests and behaviour of specific reagents towards Plant/ Drug Tissues Table-3.

Table-3: Micro-chemical Tests and behaviour of specific reagents towards plant tissues and cell contents.

Sl. No.	Reagents	Test	Infer-ence	Histological zone/Cell contents responded
1.	Dragendorff's reagent	Alkaloid	+	Cells of epidermis, vascular bundle and sclerenchyma
2.	Wagner's reagent	Alkaloid	+	Same as above
3.	Acetic acid	Calcium oxalate	−	Not responded
4.	Hydrochloric acid + Potassium hydroxide solution (5% w/v)	Calcium oxalate	−	Same as above
5.	Iodine sol. followed by sulphuric acid	Cellulose	+	Cells of parenchyma and bundle sheath
6.	Sudan III	Fixed oils and fats	+	Oil globules in mesophyll and sclerenchyma cells
7.	Phloroglucinol-HCl	Lignin	+	Cells of sclerenchymatous patch, xylem and bundle sheath
8.	Lugol's solution	Protein	+	Cells of epidermis, vascular bundle and sclerenchyma
9.	Saturated aqueous solution of copper acetate	Resin	+	Cells of bundle sheath of large vascular bundle
10.	Iodine solution weak	Starch	+	Starch grains in cells of bundle sheath and parenchyma
11.	Potassium hydroxide solution (5% w/v)	Starch	+	Same as above
12.	Sudan III	Suberin	+	Cells of sclerenchyma and epidermis
13.	Fehling's solution	Sugar	+	Cells of epidermis and phloem
14.	Aqueous ferric chloride	Tannin	+	Cells of sclerenchyma

B. *Behaviour of Powdered Drug towards Specific Reagents:* The powdered drug was treated with specific reagents and the resultant behaviour is represented in Table-4.

Table-4: Behaviour of Powdered Drug on Treatment with Different Chemical Reagents.

Sl. No.	Reagents	Behaviour of the powdered drug
1.	Acetic acid	Powdered drug settles at bottom giving a primrose solution.
2.	Dragendorff's reagent.	Most of the particles float on surface and rest remain suspended in solution giving a deep orange solution.
3.	5% aqueous ferric chloride solution	Most of the particles float on surface and rest settle down giving a golden brown solution.
4.	Hydrochloric acid	All the particles float on surface giving a light jasmine solution.
5.	Iodine solution	Most of the particles settle down and few particles float on surface giving a light purple brown solution.
6.	Lactic acid	Powdered drug floats on surface giving a royal ivory solution.
7.	Nitric acid	All the particles come to upper side giving a traffic yellow solution.
8.	Saturated picric acid	Some of the particles float on surface and rest of the particles settle down giving a lemon solution.
9.	5% potassium hydroxide	Entire powdered drug settles down giving a middle stone colour.
10.	Concentrated sulphuric acid	All the particles come to upper side giving a middle brown solution.
11.	Water	Particles float on surface giving a middle buff solution.

IV. Identity, Purity and Strength

Physico-Chemical Characteristics: The analytical values in respect of physico-chemical characteristics of drug were observed and their statistical data is quoted in the Table-5.

Table-5: Statistical Data of Physico-Chemical Constants of Drug

Sl. No.	Physico-chemical constants	Mean±sd
1.	Moisture content, %w/w	12.55 ± 0.18
2.	pH	7.17 ± 0.03
3.	Total ash, %w/w	9.54 ± 0.30
4.	Acid insoluble ash, %w/w	5.81 ± 0.07
5.	Water soluble extractives, %w/w	12.39 ± 0.04
6.	Alcohol soluble extractives, %w/w	5.25 ±0.03

V. Fluorescence and Spectroscopy

A. *Fluorescent Characteristics of Powdered Drug on Screening under Ultra-Violet Light:* Powdered drug was screened for fluorescent characteristics with or without chemical treatment. The observations of fluorescence analysis of powdered drug pertaining to their colour under normal day light and UV light are presented in the Table-6.

Table-6: Fluorescent Characteristics of Powdered Drug under Ultra-Violet Light.

Sl. No.	Treatment	Colour in normal light	Colour in UV light
	Powder		
1.	mounted in 1N sodium hydroxide in methanol.	Light olive	Sage green
2.	mounted in 1N hydrochloric acid.	Light jasmine yellow	Sea green
3.	mounted in 1N sodium hydroxide in water.	Light brown	Sea green
4.	treated with concentrated nitric acid diluted with an equal amount of water.	Primrose	Sea green
5.	treated with concentrated sulphuric acid diluted with an equal amount of water.	Orange brown	Light bronze green
6.	in water.	Middle buff	Sea green
7.	with ethyl acetate.	Light olive	Eau-de-Nil
8.	with petroleum ether.	Frost green	Cool breeze
9.	with acetone.	Canary yellow	Sea green

Sl. No.	Treatment	Colour in normal light	Colour in UV light
	Powder		
10.	with benzene.	Mid cream	Eau-de-Nil
11.	with chloroform.	Light olive	Sea green
12.	with carbon tetrachloride.	Light olive	Sea green
13.	with methanol.	Light olive	Eau-de-Nil
14.	with ethanol.	Nut brown	Light bronze green
15.	as such.	Light stone	Light olive

B. *Ultra-Violet Spectroscopy*: The data pertaining to UV spectrophotometric characteristics is computed in the Table-7 (Figure 6).

Table-7: Ultra-Violet Spectrophotometric Characteristics of Drug.

λ max, nm	Maximum absorption peaks at dilution 0.5/25 ml/ml
667.0	0.050
266.0	0.803
235.1	3.221
231.9	1.940
226.0	2.523

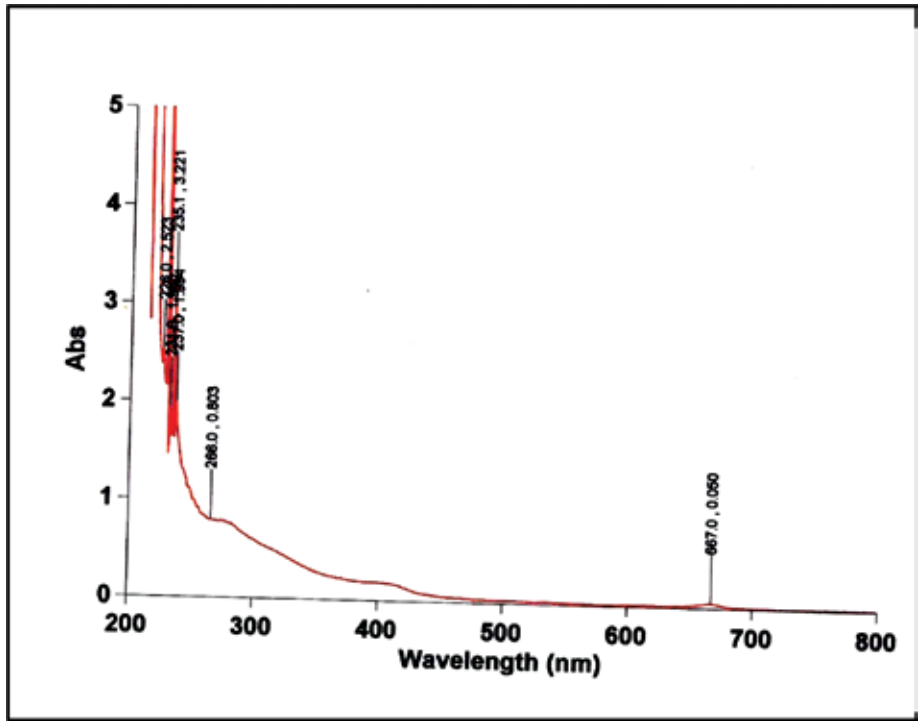

Fig. 6. Ultra-Violet Spectrophotometric Characteristics of Cymbopogon citratus (DC.) Stapf

VI: Chromatography

Thin-Layer Chromatography: The TLC fingerprinting data is presented in Table-8.

TLC of Hexane extract on aluminium plate precoated with silica gel 'G' 60 F254 of 0.2 mm thickness using Toluene: Ethyl acetate (9:1) as solvent system and when seen under UV 366 nm shows bands at Rf 0.25 (Red), 0.34 (sky blue) and 0.40 (Red). On dipping in *Vanillin Sulphuric Acid reagent* and on heating at 105^0 for 5 minutes bands appear at Rf 0.10, 0.26, 0.38 (All grey).

Table-8: TLC Fingerprinting Data

Sl. No.	Technical detail	Specification
1.	Stationary phase	Hexane extract on aluminium plate precoated with silica gel 'G' 60 F254 of 0.2 mm thickness
2.	Solvent system	Toluene: Ethyl acetate (9:1)
3.	Derivatizing reagent	Vanillin Sulphuric Acid reagent
4.	Extract	Hexane extract
5.	Volume of test solution applied	7ml
6.	Distance traveled by solvent system	8 cm
7.	Spots in visible light, Rf	-
8.	Spots under UV(366 nm), Rf	3 spots, 0.25 (Red), 0.34 (Sky blue) and 0.40 (Red)
9.	Spots after derivatization, Rf	3 spots, 0.10, 0.26, 0.38 (All grey)

Discussion

The pharmacognostic study of *Cymbopogon citratus* (DC.)Stapf..was attempted by a few workers. Datta and Mukerji (1952) described macro- and microscopic characters of the drug which are in accordance with the findings of present study. However Chauhan and Pillai (2007) reported presence of prismatic crystals of calcium oxalate in powder characteristics of drug which are not found in this investigation. It is an important medicament of cosmetics, certain classical and patent and proprietary preparations. These pharmacognostic and other related quality parameters can be used to identify and authenticate commercial available lemon grass.

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