Pharmacognostic Standardization of Cymbopogon citratus (dc.) stapf leaves

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ABSTRACT

Context: To ensure reproducible quality of herbal products, proper control of starting material is important. The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. Objective: The present work has been designed to study the Pharmacognostic parameters of the leaves of Cymbopogon citratus. Materials and methods: Various standardization parameters like morphological characters, microscopic evaluation, physicochemical evaluations (loss on drying, ash values, extractive values), preliminary phytochemical screening and TLC chromatographic profile of the extract were carried out and the quantitative microscopy were reported. Results: The standardization parameters provide referential information for correct identification of the plant material and will also be useful in preparation of monographs on these plants.

Key words: Cymbopogon citratus, Pharmacognostic parameters, Preliminary Phytochemical Screening.

INTRODUCTION

Cymbopogon citratus, family Poaceae also called as lemongrass is a widely used herb in tropical countries, especially in Southeast Asia. The essential oil of the plant is used in flavour, fragrancing and aromatherapy, medicinal tea, culinary herb[1] and treatment for skin diseases.[2] It is known as a source of ethno medicines.[3] C. citratus is used in different parts of the world in the treatment of digestive disorders, fevers, menstrual disorder, rheumatism and other joint pains.[4] The chemical composition of the essential oil of C. citratus varies according to the geographical origin, the compounds as hydrocarbon terpenes, alcohols, ketones, esters and mainly aldehydes have constantly been registered.[5] The essential oil (0.2 to 0.5%, West Indian lemon grass oil) consists of mainly citral.[6] Citral is a mixture of two stereoisomeric monoterpenic aldehydes, the Trans isomer (40 to 62%) dominates over the cis isomer neral (25 to 38%).[7] Flavonoids are also reported to be the phytoconstituents of Cymbopogon citratus. It consists of luteolin and and its 6-C and 7-O- glucosides,[9] isoorientin 2’-O-rhamnoside[10] and isolation of the flavonoids quercetin, kaempferol and apigenin[11] from aerial parts. The phenolic compounds elimicin, catecol, chlorogenic acid, caffeic acid and hydroquinone isolated from plant.[12]

Various pharmacological activities of Cymbopogon citratus, have been reported such as Anti amoebic,[13] Antibacterial,[14-17] Antidiarrhoeal,[18] Antifungal,[19] Antimalarial,[20] Antiinflammatory[21] and Anti-anxiety.[22] Cymbopogon citrates (DC) Stapf (Graminae) (C. citratus) are an herb worldwide known as lemongrass. The tea made from its leaves is popularly used as antispasmodic, analgesic, anti-inflammatory, antipyretic, diuretic and sedative.[23] In lemongrass, antioxidant[24], and antinociceptive[25] activities have been studied. A few ethno botanical reports on treatment of fever and headache were investigated.[25]

MATERIALS AND METHODS

Plant material
Cymbopogon citratus leaves were collected in the month of February 2007, from Punjab University, Chandigarh, India. The taxonomic identity of the plant was confirmed by Dr. H.B. Singh, Head, Raw Materials Herbarium & Museum, National Institute of Science Communication and Information Resources (CSIR), New Delhi 110067.
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**Preparation of leaf extracts**
Leaves of *Cymbopogon citratus* (DC.) stapf were dried in shade and powdered. One hundred grams of powdered leaves were subjected to successive Soxhlet extraction by solvents in increasing order of polarity viz. petroleum ether (60-80°C), chloroform and methanol. Before each extraction the powdered material was dried in hot air-oven below 50°C. Finally, marc was digested with distilled water for 24 hours to obtain the aqueous extract. Each extract was concentrated by distilling off the solvent and then evaporating to dryness on the water-bath. Extracts were weighed and percentage was calculated in terms of the air-dried weight of the plant material.

**Pharmacognostic Evaluation**

**Organoleptic Evaluation**
Organoleptic features of the plant were evaluated by observing color, odour, taste, size, shape of morphology and special features like texture. A part of quantitative microscopy, stomatal number, stomatal index, was determined by using fresh leaves of plant.

**Microscopic and Histological Techniques**

**Study of Transverse Sections**
The leaves of *Cymbopogon citratus* were boiled with water until soft. Free hand sections of the leaves were cut transferred on slides cleared by warming with chloral hydrate and mounted in glycerin. The lignified and cellulosic tissues were distinguished using differential staining techniques.

**Photomicrography**
Microscopic evaluations of tissues were supplemented with micrographs. Photographs of different magnifications were taken with Nikon Labpot 2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grain and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars.

**Powder microscopy**
A few drops of chloral hydrate solution were added to a sample of powdered plant material on a slide, was covered with a glass slip and heat gently over a microbunsen. Vigorous boiling was avoided. The slide was examined under the microscope. When the clearing process is completed a drop of glycerol solution was added which will prevent crystallization of the mountain on cooling.

**Physicochemical analysis**
Physicochemical analysis i.e., alcohol (90% ethanol) and water soluble extractive values, total ash, acid-insoluble ash, and loss on drying of the powdered drug were determined.

**Phytochemical Screening**
The various extracts of *Cymbopogon citratus* were subjected to qualitative chemical examination.

**Thin Layer Chromatography Profile**
TLC glass plates (5×15 cm), 0.25 mm thick were prepared using Silica gel G. The plates were activated at 110°C for 30 minutes. The TLC profiles of the extracts were studied using different solvent systems. TLC plates were developed in TLC chamber. Thin layer chromatogram was visualized under 254/366 nm UV light and after keeping in Iodine Chamber.

**RESULTS**

**Organoleptic features of the leaves (Figure 1)**

- **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- to 10 mm wide
- **Shape**: Leaf blades linear & tapered to both ends, sheath terete
- **Margin**: Entire
- **Surface**: Flat, very coarse
- **Venation**: Parallel

**Microscopic Evaluation**
Leaf is dorsiventral with prominent midrib, smooth adaxial surface and rehivate abaxial surface. The lamina is 80-90 μm thick. It consists of prominent adaxial epidermis which has radical oblong thin walled cells. Some of the adaxial epidermal cells are dilated into large circular or radially wedge shaped cells called bulliform cells or motor cells (Figure 2 and 3). The abaxial epidermis is thin and the cells are thick walled, the outer tangential walls form semicircular papillate projection (Figure 3).

The mesophyll tissue is not differentiated into palisade cells and spongy parenchyma. The vascular bundles...
of the lateral veins are equal in size and occur at uniform intervals in a horizontal row. The vascular bundles are surrounded by radiating cylindrical chlorenchyma cells, which represent the palisade mesophyll (Figure 3), in between the vascular bundles, these are vertical band cells; the cells beneath adaxial epidermis is dilated and hyaline, other cells after vertical band are chlorenchymatous.

The vascular bundles are collateral with adaxial group of a few xylem elements and phloem elements. The vascular bundle of the smaller vein is surrounded by a ring of circular dilated cells. Each bundle is capped by small group of fibres both on the upper and lower ends.

The larger vein has two wide, circular metaxylem elements and one or two protoxylem elements. A prominent circular mass phloem is seen in between the metaxylem elements, the entire bundle is unsheathed by thick sclerenchyma cells (Figure 2).

**Midrib (Figure 4 and 5).**
The midrib is concavo convex in transactional view. The adaxial side is broadly concave and the abaxial side is convex.
The midrib is 380 μm in vertical plane and 650 μm in horizontal plane and thus the midrib is cradle shaped in sections. The upper surface of the midrib is smooth while the lower surface has small conical ridges, opposite to the smaller vascular bundle (Plate 5). The epidermal layer in the region of adaxial midrib is thin and less prominent. The abaxial epidermis is thin, but distinct with spindle shaped cells and thick cuticle.

The ground tissue is parenchymatous, fairly thick walled angular and compact. The vascular system of the midrib consists of a central median larger vascular bundle placed towards the basal part; these are several smaller vascular bundles placed all along to basal abaxial part (Figure 4). The larger median bundle has two wider metaxylem elements and three protoxylem elements, phloem occurs in wide mass in between metaxylem elements. The vascular bundle is surrounded by thick bundle-sheath fibres (Figure 5). The smaller bundles are circular with a small group of xylem and bundle sheath parenchyma cells. A prominent mass of fibres can be seen beneath each smaller vascular bundle.

Powder microscopy

The leaf pieces were cleared and made transparent by treating then with chloral hydrate. These leaf fragments exhibit several thin and thick, straight parallel lateral veins. In the witercostal regions are seen epidermal cells and stomata. The adaxial epidermis is apostomatic (without stomata). The epidermal cells are vertically rectangular and thin walled (Figure 6).

The abaxial epidermis is stomataferous. The epidermal cells are narrow, rectangular and the anticlinal walls are thick and undiluted (Figure 7 and 8). The stomata occur in regular, parallel longitudinal rows. The stomata are paracytic type with two bracket shaped subsidiary cells lying parallel to the guard cells.

The epidermal tissues were stained with Toluidine blue in order to show the lignification of the cells. The vascular strands of the veins and guard cells stained blue, showing that they have lignified walls. The stomatal number is 700-800/mm².

![Figure 5: Transverse section of leaf through midrib. [AdE: Adaxial epidermis MX: Metaxylem, Sc: Sclerenchyma, GT: Ground tissue, Ph: Phloem, VB: Vascular bundle. AbE: Abaxial epidermis.]](image)

![Figure 6: Powder Microscopy of leaf shows the adaxial epidermis is apostomatic (without stomata). [AdE: Adaxial epidermis V: Vessel.]](image)
Determination of Leaf constants
The surface parameters of leaves of *Cymbopogon citratus* were measured (Table 1).

Physical Evaluation
The physical parameters of powdered leaves of *Cymbopogon citratus* were evaluated (Table 2).

Preliminary Phytochemical Screening
The preliminary phytochemical investigation of the petroleum ether (60-80°C), chloroform and methanol and water extracts of *Cymbopogon citratus* leaves shows the presence of carbohydrates, proteins, saponins steroids, flavonoids, phenolic compounds and tannins (Table 3).

Thin layer chromatography
TLC of the methanolic extract on silica gel G using n-butanol: acetic acid: water (4:1:5) under UV (366 nm) shows one fluorescent zone at Rf value of 0.91 (violet). On exposure to iodine vapour, three spots appeared at Rf values of 0.76, 0.47, 0.21 (all yellow). On spraying with 5% methanolic-sulphuric acid reagent and heating the plate at 105°C for ten minutes a single spot appears at Rf 0.91 (grey).

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<tr>
<th>Table 1: Surface data of leaves of <em>Cymbopogon citratus</em>.</th>
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<td>Stomatal No</td>
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<td>Stomatal Index</td>
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<th>Table 2: Physical parameters of leaves of <em>Cymbopogon citratus</em>.</th>
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<td>Physical Parameter</td>
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<tr>
<td>Loss On Drying</td>
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<td>Total Ash</td>
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<td>Acid Insoluble Ash</td>
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<td>Ethanol Soluble Extractives</td>
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<td>Water Soluble Extractives</td>
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DISCUSSION

As a part of standardization, the macroscopical examination of leaves of *Cymbopogon citratus* was studied. Macroscopical evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of drugs. The macroscopical characters of the fruits of plant can serve as diagnostic parameters. The microscopic examination of fruits of *Elaeocarpus sphaericus* and extractive values, ash values and loss on drying of the powdered drug and phytochemical screening of the extract have been carried out which would be of considerable use in the identification of this drug. Percentages of the extractive values, ash value and loss on drying were calculated with reference to the air-dried drug. The percent extractives in different solvents indicate the quantity and nature of constituents in the extracts. The extractive values are also helpful in estimation of specific constituents soluble in particular solvent. Thin layer chromatography (TLC) was examined in short UV (254 nm) and long UV (366 nm) which is particularly valuable for the preliminary separation and determination of plant constituents. This finding is useful to supplement the existing information with regard to identification and standardization of *Cymbopogon citratus* even in the powdered form of the plant drug to distinguish it from drug and adulterant. These studies also suggest that the observed pharmacognostic and physicochemical parameters are of great value in the quality control and formulation development.

CONCLUSION

The present study may be useful to supplement the information with regard to its standardization and identification and in carrying out further research and its use in traditional system of medicine.

**REFERENCES**


