Research Article

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Antibacterial activity profile and quality standards of *Cymbopogon citratus* Stapf- an aromatic grass used in Indian system of medicine

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ABSTRACT

Background: *Cymbopogon citratus* stapf. an aromatic grass found cultivated commonly in India, used in ancient texts of Ayurveda to cure infectious diseases related to respiratory system. The aromatic, long leaves are major used part of this drug. **Materials and methods:** Leaves collected form mature plant, cleaned properly, macro-microscopic characters recorded as per standard methodology. Physico-chemical constituents and major secondary metabolites found in the leaves are marked. HPTLC fingerprints of methanolic extract of test drug drawn out. *In vitro* antibacterial activity of aqueous and methanolic extract of test drug carried out as per disc diffusion method, zone of inhibition measured. **Results:** Macro-microscopic characters, physicochemical standards are a measure of its quality. Secondary metabolites like triterpenoids, tannins, flavanoids etc revealed its chemical nature. HPTLC fingerprint further prove its chemical nature. Both methanolic and aqueous extract of the drug has shown sensitivity against all four bacterial strains. **Conclusion:** Methanoilc extract of test drug has shown better zone of inhibition against *S. pyogen*, *S. aureus*, *P. auregenosa*, thus proved a promising source of antimicrobial drug.

Keywords: *Cymbopogon citratus*, Macro-microscopic, HPTLC, Disc diffusion method.

INTRODUCTION

Cymbopogon citratus stapf. a tufted perennial aromatic grass popularly known as Citronella or lemon grass belongs to family *Graminae* [1]. It grows wild in South India, Srilanka, Moluccas and also cultivated in gardens [2]. In Indian system of medicine it forms an important source for *Kuttrana/Gandhatrina* and said to be *krimighna(*antimicrobial*)* and used as a best remedy in respiratory system related diseases [3]. *Sheeta prashamana mahakashaya, agurvaadi taila, mahapanchagavya ghrita* are some important formulations prepared out of this drug [4]. Lemon grass is even taken as a tea for digestive problem. It relaxes muscles of the stomach and gut, relieves spasm and flatulence [5]. The essential oil from lemon grass contains a volatile oil, with citral (70%), citronellal, geraniol and myrcene as its main constituents [6]. It showed vibriocidal activity against vibrio cholera [5]. Increased incidences of infectious diseases and multidrug drug resistant strains of pathogens; resulted in a search of safe, natural and effective source of drug to face the situation. In this paper an antibacterial activity profile of *C. citratus* stapf. along with its macro-microscopic features and phytochemical investigation data are recorded.

MATERIAL AND METHODS

Plant material

Naturally grown, fresh matured aerial parts of *Cymbopogon citratus* stapf were collected from Dakshina Kannada district of Karnataka, India. It was identified and authenticated referring floras and databases. Plant samples were cleaned under running water to remove adherent extraneous matter and sample deposited at SDM centre for research in Ayurveda and Allied sciences, Udupi (Voucher specimen number 750/16041101). The sample collected was left in FAA for more than 48 hours to record cellular structures. Rest of the plant sample was shade dried, coarse powder was prepared (60 mesh) and preserved in airtight container.

Macroscopic evaluation

Detailed morphological features are noted and documented. Macroscopic characters like size, shape, texture, colour were noted in detail [7].

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Microscopic evaluation

Free hand sections were taken, stained with saffranine, followed by HCL and iodine respectively to test the lignifications of cell wall and to check the starch grains. The surface preparation was done by scrapping method. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the scale-bars [8].

Analytical parameters

Physico-chemical constants of test drug, like moisture content, total ash, water soluble ash and acid insoluble ash were calculated following the procedures recommended by World Health Organization [9]. Note on aqueous and alcohol soluble extractive were done.

Qualitative parameters:

Aqueous and methanolic extract of plant powder was tested for the presence of secondary metabolites as per standard guidelines [10].

HPTLC

Methanolic extract of the sample was taken for this study [11]. The solvent system used for HPTLC was Toluene: Ethylacetate: Diethyl amine: Methanol: Chloroform:: 10:6:2:2:1.

Antibacterial activity

In vitro antibacterial activity of aqueous and methanolic extracts of test drug carried out as per disc diffusion method against four main bacterial pathogens [12].

Preparation of extracts

*Aqueous extraction*cloth once in every 2hr and centrifuged at 5000rpm for 15 minutes. From this supernatant was collected. This particular method was repeated two times and after 6hr supernatant concentrated to get final volume one-fifth of the original volume. Extract was then autoclaved at 121° C and 15 Ibs pressure and stored at 4° C.

Methanolic extraction

10g of dried test drug powder was extracted with 100ml of methanol for 24hr. Thereafter filtered and centrifuged at 5000gm for 15 minutes. Next to this supernatant was collected, evaporated to get the final volume one-fifth of original one and stored at 4° C in airtight bottles.

Methodology

Antimicrobial susceptibility was tested on Muller-Hinton agar for *Staphylococcus aureus, Escherichia colli* and *Pseudomonos auriginosa*, while *Streptococcus pyogens* was tested on sheep blood agar. The broth suspension of the organism was prepared. These were inoculated in their respective media by lawn culture or carpet culture. A sterile borer was used to punch wells in the inoculation media (per plate 5 wells). In each well 100 microlitre of the plant extract was placed. Then standard control discs were put in the centre of the plate, to test the efficacy of the procedure. (for *Staphylococcus aureous* and *Streptococcus pyogens* Ampicillin disc was used, while Gentamycin was used as a standard antibiotic for *Escherichia coli* and *Pseudomonos auriginosa*).The plates were further incubated overnight at 37°C. The inhibition zone was recorded (in mm) after overnight incubation using a transparent scale. Minimum inhibitory concentration of each test solution against microorganisms *(S. aureus, S. Pyogen*, *P.auriginosa, E.coli)* were recorded and results analyzed.

RESULTS

Macroscopic features

Leaf blade linear; long thin towards the base and tapering to a long setaceous point up to over 80 cm by 16-20 cm wide. Leaves firm, glaucous green, glabrous smooth, rough along margin. Prominent midrib, stout below. Strong lemon smell and rough, dry to touch. (Figure no. 1)

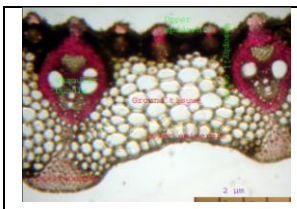
C. Citratus stapf. at its natural habitat

Macroscopic view of leaf and flower *C. citratus* stapf.

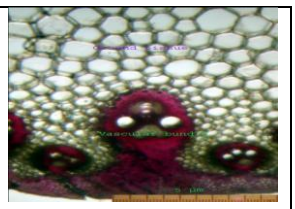
Figure 1: Macroscopic view *C. citratus* stapf.

Microscopic records

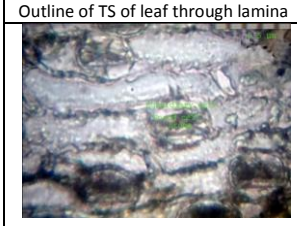
TS of leaf show both upper and lower epidermis, beneath which a layer of sclerenchymatous fibres placed at closed intervals. Stomata found distributed on lower surface of leaf abundantly than on upper surface. Ground tissue made of parenchymatous cell, between which are placed vascular bundles surrounded by a layer of mesophyll cells. These mesophyll cells are filled with oil content. Marginal cells of leaf provided with trichomes of pointed end (Figure no.2).



Outline of TS of leaf through lamina



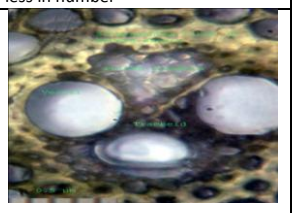
Outline of TS of leaf through idrib



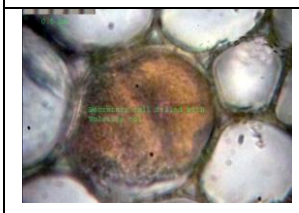
Stomata on lower surface of a leaf more in number

Stomata on upper surface of a leaf less in number

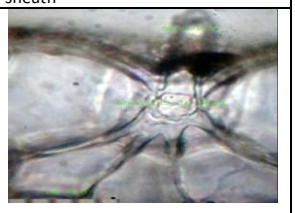
TS of outline of base of a leaf



Vascular bundle enclosed in undle sheath



Secretory cell among ground tissue



Trichome

Figure 2: Microscopic view Cymbopogon citratus stapf.

Physicochemical standards, HPTLC

Physicochemical standards of the leaf are displayed at Table no. 2. Methanolic extract of the drug investigated for the presence of different secondary metabolites and results shown at Table no. 3. HPTLC photodocumentaion of test drug drawn at Figure 3 and Rf values at Table no. 1.

Table 1: R_f values of Methanol extract of *Cymbopogon citratus* staff.

At 254 nm	At 366 nm	White light
0.17(Light Green)	0.15(pale blue)	0.28(Yellow)
0.68(Green)	0.22(Pink)	0.77(Pale green)
0.93(Dark Green)	0.44(Pink)	0.92(Yellow)
-	0.91(Pink)	-

Table 2: Physico-chemical standards of *Cymbopogon citratus* stapf.

Tests	Result n = 3 (% w/w)
LOD	5.6
Total ash	9.6
Acid insoluble ash	3.8
Water soluble extractive	5.2
Alcohol soluble extractive	6.8

Table 3: Phytochemical moieties of *Cymbopogon citratus* stapf.

Alkaloid	-
Protien	+
Flavanoid	+
Carbohydrate / glycoside	-
Phenol	+
Tannins	+
Triterpenoid	+
Saponins	-

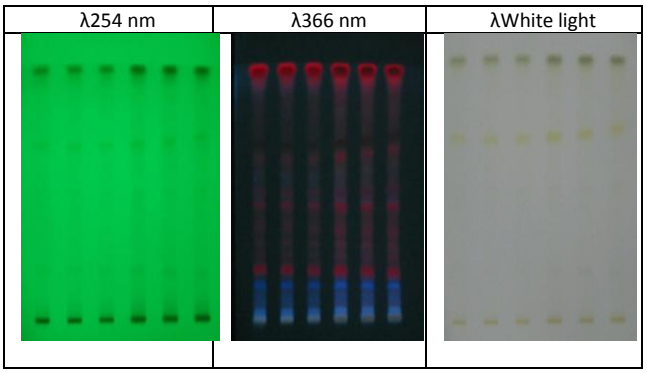


Figure 3: HPTLC photodocumentation methanolic extract of *Cymbopogon citratus* stapf

Antibacterial activity

Aqueous and methanolic extract of leaf tested for the sensitivity against four bacterial pathogens as per disc diffusion method. It is an in-vitro study and zone of inhibition measured in comparison to standard drug displayed at Table no. 5 & 6.

Table 4: Antibacterial activity (Methanolic extract) of *C. citratus* stapf.

S. aureus			*S. Pyogen*			*E. coli*			*P. auregenosa*		
C	M	T	C	M	T	C	M	T	C	M	T
41	19	13	31	20	14	18	14	12	28	18	13

C- Control group, M- Methanol group, T- Test group, Zone of Inhibition in (mm)

Table 5: Antibacterial activity (Aqueous extract) of, *C. citratus* stapf.

S. aureus		*S. pyogen*		*E. coli*		*P. auregenosa*	
C	T	C	T	C	T	C	T
41	11	30	13	18	10	29	11

C- Control group, T- Test group, Zone of Inhibition in (mm)

DISCUSSION

Since ancient times leaves of *Cymbopogon citratus* stapf. are used in various therapeutic conditions in Indian system of medicine. Leaves of this tufted grass having scented smell are said to be best *Krimighna*(antimicrobial) along with other therapeutic properties. Increased interest in medicinal plant products in recent time emphasized its quality and efficacy based scientifically published data [13]. Pharmacognostic profile on leaf along with antibacterial activity records delineated here would be adequate to ensure their authenticity, quality and efficacy.

Loss on drying reveals about the moisture content of the drug. Here LOD 5.6 indicates less moisture presence in leaves. 9.6(w/w%) total ash indicative of its carbonaceous matter, whereas 3.8(w/w%) acid insoluble ash value shows its less contamination with silicaceous matter. Extractive values of test drug ie, 5.2 (w/w%) and 6.8 (w/w%) shows their solubility with water and alchohol media.

Methanolic extract of drug has shown the presence of protein, flavanoid, phenol, tannins and triterpenoids as phytochemical component. Alkaloids and saponins are not detected out of this extract. HPTLC records on methanolic extract of test drug outlined three peaks at 254nm and four peaks at 366nm.

Historically phytomedicines have shown great promise to combat many infectious diseases [14]. Secondary metabolites of plants like terpenoids, alkaloids, flavanoids etc. proved as a source of antimicrobial [15]. In this study antibacterial effect of *C. citratus* leaf extract (aqueous and methanolic) was recorded using disc diffusion method. Results showed different sensitivity levels for the tested strains of bacterial pathogens. The results were compared with standard antibiotic drug. In this screening work no extract of *C. citratus* were found inactive against any organism. Methanolic extract of test drug has shown better zone of inhibition against *S. pyogen* [14], *S. aureus* [13], *P. auregenosa* [13], whereas it was less with *E. coli* [12]. Sensitivity of aqueous extract of test drug observed found to be less compared to methanolic group, however plant extract has shown ZOI against all four bacteria. Flavanoids, triterpenoids, phenols and tannins present in methanolic extract of *C. citratus* stapf. resulted in good antimicrobial activity.

CONCLUSION

Natural products are used since ages to combat infectious disease. Antibacterial activity profile of *Cymbopogon citratus* stapf. proved it as a promising source of future antimicrobial drug. Macro-microscopic and chemical data make known about its quality and purity.

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