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Analyzing and Determining the Activity of Antimicrobial, Functional Group and Phytochemicals of *Cymbopogon citratus* using Well Diffusion, FT-IR and HPLC

R. Uma Maheswari[1], V. EuginAmala[2]

[1]Department of Microbiology, Idhaya College For Women, Kumbakonam, Tamilnadu, India

[2]Research Scholar, Department of Biochemistry, Government Arts College (Autonomous), Kumbakonam, Tamilnadu, India

Abstract: ***Cymbopogon citratus belongs to the family Gramineae is herb worldwide known as lemongrass. The prefix 'lemon' owes to its typical lemon like odour, which is mainly due to the presence of citral, a cyclic monoterpene. Cymbopogon citratus fast growing, perennial aromatic grass native to South India and Sri Lanka, now widely cultivated in the tropical areas of America and Asia. Freshly collected and partially dried leaves are used medicinally and are the source of the essential oil. Cymbopogon citratus possesses various pharmacological activities such as anti-amoebic, anti-bacterial, anti-diarrheal, anti-filarial, anti-fungal and anti-inflammatory properties. Various other effects like anti-malarial, anti-mutagenicity, anti-mycobacterial, anti-oxidants, hypo-glycemic and neuro-behavioral have also been studied, plant is used extensively in ayurvedic medicine. Well diffusion method was used for antibacterial and antifungal susceptibility testing. Phytochemical screening was done to know the phytoconstituents present in the plant material. FT-IR and HPLC have been done to know the functional group and to analyze the compounds. Hence this results demands further research to unfold its therapeutic values.***

Keywords: *Cymbopogon*, FT-IR, HPLC, Gramineae, Pseudostem.

1. Introduction

Lemongrass is an aromatic plant belonging to the Gramineae family[1]. It is a tall, clumped perennial grass growing to a height of 1 meter. The leaf-blade is linear, tapered at both ends and can grow to a length of 50cm and width of 1.5cm[2]. The leaf-sheath is tubular in shape and acts as a pseudostem. This plant produces flowers at matured stages of growth[3]. According to World Health Organization (WHO) definition of a medicinal plant is a plant that can be used for therapeutic purposes and or its compounds be used as a pioneer in the synthesis of semi-synthetic chemical drugs[4]. Essential oils are natural products obtained from lemon grass plants. They were formed by varied and complex volatile mixtures of chemical compounds, with predominance of terpene associated to aldehyde, alcohols and ketone which were deposited in various structure of the plant[5]. Lemongrass contains citral[6] and 1 to 2% essential oil on a dry basis[7]**.** Plants are still a potential source of medicinal compounds. In the world this plants traditionally used in oral health and to treat many disease especially infectious diseases including diarrhea, fever and cold[8]additionally, many recreational compounds used in traditional medicine have plant root[9].Alkaloid may be useful against HIV infection[10], flavonoids have strong anticancer activity[11]and tannin have antimicrobial activity. Lemongrass is a folk remedy for coughs, elephantiasis, flu, gingivitis, headache, leprosy, malaria, ophthalmic, pneumonia and vascular disorders.

The lemon grass is a good cleanser that helps to detoxify the liver, pancreas, kidney, bladder and the digestive tract. It cuts down uric acid, cholesterol, excess fats and other toxins in the body while stimulating digestion, blood circulation and lactation; it also alleviates indigestion and gastroenteritis. It is said that lemon grass also helps improve the skin by reducing acne and pimples and acts as a muscle and tissue toner also it can reduce blood pressure. A recent study by the Food and Nutrition Research Institute of the Department of Science and Technology (DOES) showed lemon grass can help to prevent cancer[12].

2. Materials and Methods

Identification and Authentication:
Leaves of *Cymbopogon citratus* were collected from Idhaya College campus, Herbal garden situated in Kumbakonam, Tamilnadu, India. The plant was authenticated by Dr. John Britto, Rapinat Herbarium, St. Joseph's College, Trichy. The voucher specimen No: UM001 dated 09.02.2015. Herbarium has been deposited in department of Botany St. Joseph's College for future reference.

Preparation of Microorganism
The bacterial strains used in this study were gram positive bacteria - *Staphylococcus aureus* gram negative bacteria- *Psuedomonas aeruginosa* and, *Klebsiella pneumonia and Escherichia coli* procured from Vaishnavi Lab, Kumbakonam, Tamilnadu, India. All chemicals and media components and impregnated discs were used in this study from Hi-Media, Mumbai, India.

Preparation of the plant extracts:
Fresh leaves of Lemon grass were washed and air dried in the shade for two weeks. They were ground with mortar and pestle sieved with a mesh of size 0.5mm. The powdered samples obtained were stored in clean air tight containers at ambient temperature until when needed for use[10, 11]. The

powdered leaf samples were subjected to successive extraction with acetone using Soxhlet Extractor. 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, the extract thus obtained was preserved for the usage of various analysis[12].

Antibacterial Screening

Nutrient broth had been used for *Staphylococcus aureus* and *Psuedomonas aeruginosa*, Lactose broth medium for *Klebsiella pneumonia.* These microbes were sub-cultured and used for the antibacterial activity. Acetone and aqueous extract was investigated with agar well-diffusion method**.** An in-vitro antimicrobial screening was carried out using Muller Hinton Agar (MHA) by Agar well -diffusion method according to Perez et al., 1990[13]. The petriplates were allowed to stand for one hour for pre-diffusion of the extract into the medium[14].

Antifungal screening

The agar diffusion method[15] was employed to test the organism. *Candida albicans was* inoculated into test tubes containing Rose Bengal broth and *Aspergillus niger* was in potato dextrose broth incubated at room temperature for 72 hours. The organisms were sub-cultured into Rose Bengal agar and potato dextrose agar by the pour plate method. A sterile cork borer (6mm) was used to bore holes in the culture media and the bases of the wells were sealed with a drop of molten agar to prevent unwanted spreading of the extracts. In a drop-wise manner, 1ml of the extract was added into each of the well and the cultures were allowed to stand for 30 minutes before incubation at room temperature for 48 hours. Thereafter active growth zone of inhibition were measured with the aid of a Kirby Bauer Meter rule considering the diameter of the cork borer, control plate was also prepared for each test organism.

FT-IR analysis

Fourier Transform Infrared Spectrometer (Make Perkin Elmer, Mode Spectrum RXI 2012) study was carried out in Archbishop Casmir Instrumentation Centre (ACIC), St.Joseph's College, Trichy, Tamilnadu, India to identify the functional groups present in *Cymbopogon citratus* with the adsorbents of 4000-400 cm^{-1} range. The adsorption capacity of adsorbent depends upon the porosity as well as chemical reactivity of functional groups at the adsorbent surface[16].

High Performance Liquid Chromatography

Chromatographic separation was performed by set up of High Performance Liquid Chromatography (HPLC) system, equipped with Pump-Lc-8A, Column - C 18, variable wavelength detector- SPD 20A at a flow rate of 2.0 ml/min & detector wavelength of 254 nm. The injection volumn was 1.0 µL and the run time was 14 min for each injection[17].

3. Results

Results of the antimicrobial activity of *Cymbopogon citratus* portrayed the growth inhibition effect of acetone and aqueous extract of Lemon grass leaves tested on gram positive bacteria - *Staphylococcus aureus* gram negative bacteria- *Psuedomonas aeruginosa*, *Klebsiella pneumonia and Escherichia coli* were detected. *Staphylococcus aureus* has 14mm,11mm, *Klebsiella pneumonia* has 12mm, 11mm, *Escherichia coli* has 13mm,10mm and *Psuedomonas aeruginosa* did not show any inhibitory effect, among this *Staphylococcus aureus* has high inhibitory to compare with other organisms.While experimenting with fungal species such as *Candida albicans* has 8mm, 7mm,*Aspergillus niger* has very low inhibitory effect described in Table 2. Identified the phytochemicals such as alkaloids, flavonoid, tannin, carbohydrate, saponin, glycoside, protein, amino acid and phenol respectively, among this tannin and phenol is richly present. Functional groups were illustrated as -C-H Alkanes (stretch), -O-H Alcohol and Phenols (stretch), -O-H Carboxylic acid (stretch), -C=C- Aromatic (stretch), -C≡C-H, Alkynes (stretch), C-N Amines (stretch), -C-H Alkanes (stretch), C-H Aromatic (Out of plane bending), C-O Ether group (stretch) shown in Table.1 and Figure.1.

HPLC analyzed the quantity of compounds present in *Cymbopogon citratus* and reviewed the peak area percentage of Resorcinol (75%) and Ellagic acid (25%) in the retention time of 3.117 and 4.569 depicted in Table 3 and Figure2.

Table 1: Systematic Organic analysis for Functional Group Identification –FT-IR

S.No	*Frequency*	*Wave Length*	*Functional Groups*	*Bonds*
1	2800-3000	2820.77 2922.63	Alkane (Stretch)	-C-H
2	3200-3500	3259.85 3403.56 3468.87	Alcohol Phenol (Stretch)	-O-H
3	2500-3300	2737.39 2820.77 2922.63 3959.85	Carboxylic Acid (Stretch)	-O-H
4	1500-2600	1595.12	Aromatic (Stretch)	-C=C-
5	2100-2270	2154.49	Alkynes (Stretch)	-C≡C-H
6	1385-1370	1383.23	Alkanes (Stretch)	-C-H
7	1260-1000	1107.32	Ether (Strech)	C-O
8	500-900	668.78 768.03	Aromatic (Out of Plane Bending)	C-H

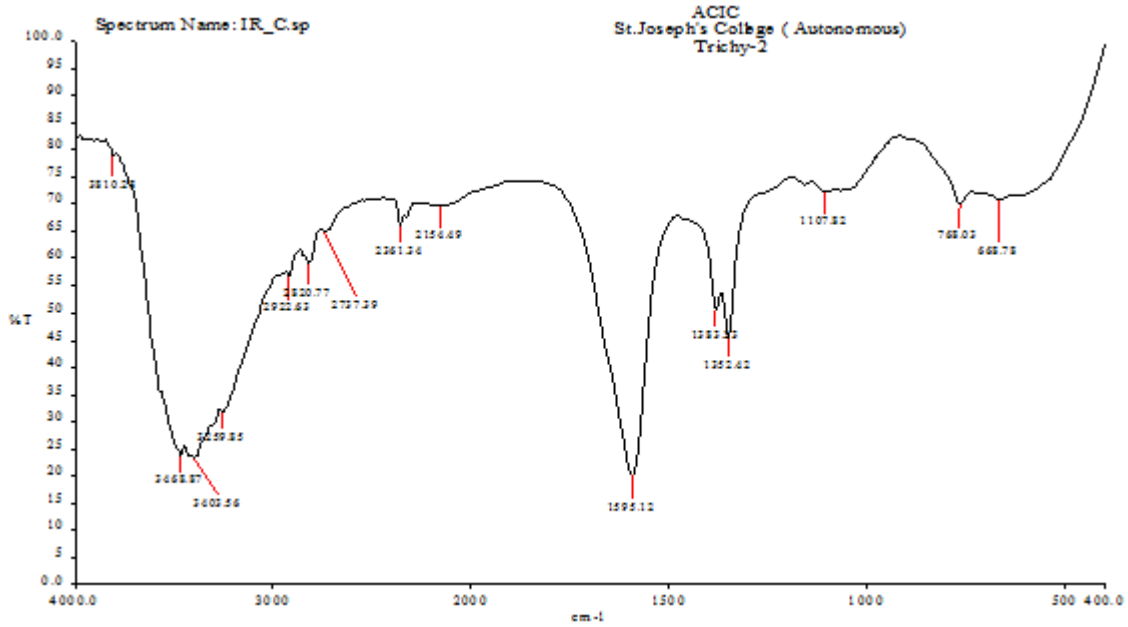

Figure 1: FT-IR Spectrum *Cymbopogon citrates*

Table 2: Antibacterial and Antifungal Screening of *Cymbopogon citratus* extracts against Micro Organisms --U @C:\HPLC\DATAS\--U.lcd

Test organisms	Antibacterial activity		Antifungal activity	
	Acetone extract (mm)	Aqueous extract (mm)	Acetone extract (mm)	Aqueous extract (mm)
Staphylococcus aureus	14	11	-	-
Psuedomonas aeruginosa	Nil	Nil	-	-
Klebsiella pneumonia	12	11	-	-
Escherichia coli	13	10	-	-
Candida albicans	-	-	8	7
Aspergillus niger	-	-	6	Nil

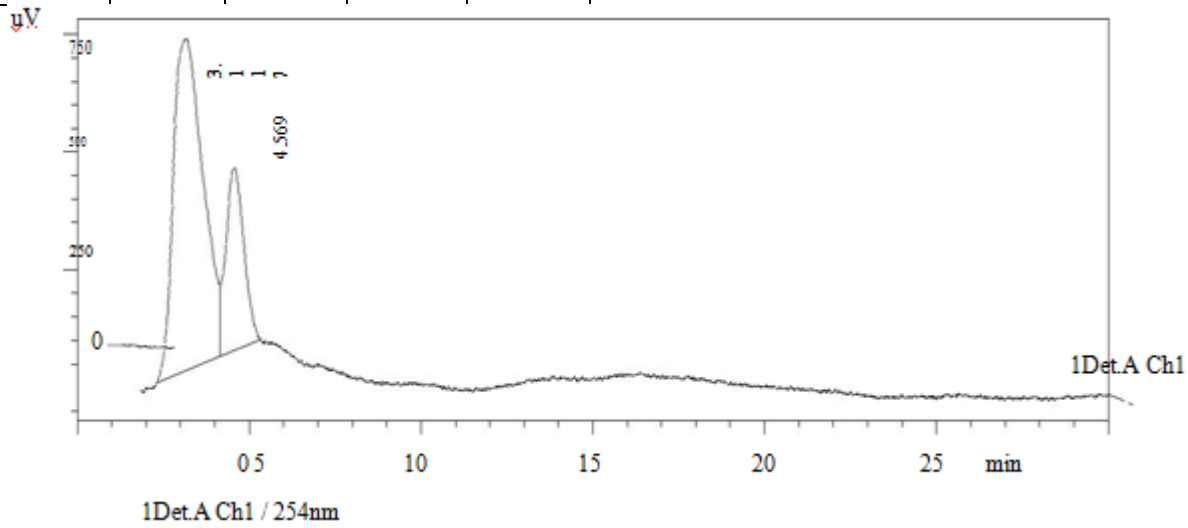

Figure 2: HPLC Chromatogram of *Cymbopogon citrates*

Table 3: Compounds of *Cymbopogon citratus* – HPLC

Peak	Compound name	Ret. Time	Area	Height	Area%	Height %
1	Resorcinol	3.117	42634	705	75.089	64.530
2	Ellagic acid	4.569	14144	388	24.911	35.470
			56778	1093	100.000	100.000

4. Discussion

Medicinal plants are very important to human beings in preserving our health. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine. All the extracts were acidic in nature (pH values ranging between 3-5). The acidity combined with bioactive components might enhance the antimicrobial activity of the extracts especially against the bacteria. Qualitative phytochemical investigation revealed that the extracts contained some phytoconstituents such as saponins, tannins, alkaloids and flavonoids are present in the acetone extracts; alkaloids and flavonoids in aqueous extracts. These bioactive components, beside other water soluble components which are naturally occurring in most plant materials, are known to be bactericidal or bacteriostatic, fungicidal or funfistatic in nature thus conferring the anti-microbial property to plants[18, 19, 20]. Lemongrass has great interest due to its commercially

valuable essential oils and widely used in food technology as well as in traditional medicine. Owing to the new attraction for natural products obtained from lemon grass a proper photochemical and pharmacological study is required, which shall opens new pharmacological avenues for this magnificent plant which are helpful for clinical experimentation and also in the development of novel drugs.

5. Conclusion

Ultimately, this study states about various activity of *Cymbopogon citratus* such as determining the phytocompounds, identifying the functional groups, evaluating the antimicrobial activity and analyzing the compounds of *Cymbopogon citratus* depicted more medicinal, pharmaceutical, antibacterial and antifungal properties. Therefore it has hall mark and boulevard to the growing society.

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