International Journal of Chemistry and Pharmaceutical Sciences

Journal Home Page: www.pharmaresearchlibrary.com/ijcps

Research Article **Open Access**

Chemical Composition and Antimicrobial activity of the Volatile oil of the leaves of *Cymbopogon citratus* (Lemongrass)

Trivikram Rawat[1*], I.P.Pandey[2], Suman Lata Chhimwal[2]

[1]*Member, American Association of Pharmaceutical Scientists, Unites States of America*
[2]*Department of Chemistry, D.A.V. (P.G.) College, Dehradun, 248001, India*

A B S T R A C T

The aim of the present study was to investigate the various chemical components present in the volatile oil of leaves of *Cymbopogon citratus* and to determine its antimicrobial activity. Nineteen compounds were identified in the *C.citratus* oil sample representing 99.8% of the detected compounds that included Citral, geranial, and myrcene as major component. The antimicrobial activity was assayed using the oil and a 2% cream formulation of the oil and a cup plate method by measuring the zone of inhibition of growth. The gram-positive organisms used were *Bascillus subtilis, Staphylococcus aureus, Bacillus megaterium and Bascillus coagulans*. The gram-negative organisms used were *Escherichia coli, Klebsiella pneumonia, Psedomonas aeruginosa and Salmonella typhii*. *In-vitro* antifungal studies were also carried out using, Candida albicans, *Aspergillus niger and Trichoderma lignorum.* The standard drug used was Ciprofloxacin, for gram-positive, gram-negative and also for fungi. Both the oil and the cream formulation displayed anti-microbial activity against fungi and bacteria.

Keywords: *Cymbopogon citratus*, volatile oil, Cream formulation, antimicrobial activity.

A R T I C L E I N F O

CONTENTS

Article History: Received 29 June 2015, **Accepted** 12 August 2015, **Available Online** 27 October 2015

***Corresponding Author**
Trivikram Rawat
Member, American Association of Pharmaceutical Scientists, Unites States of America
Manuscript ID: IJCPS2700

PAPER-QR CODE

Citation: Trivikram Rawat, *et al*. Chemical Composition and Antimicrobial activity of the Volatile oil of the leaves of *Cymbopogon citratus* (Lemongrass). *Int. J. Chem, Pharm, Sci.*, 2015, 3(10): 2030-2034.

1. Introduction

Lemongrass (*Cymbopogon citratus*) is a commercially important aromatic tropical grass of the family poaceae, commonly known as sweet grass family. Leaves of this plant contain high citral content in the essential oils with a typical strong lemon-like aroma. As a medicinal herb, lemongrass has been considered as carminative, insect repellent and widely used as herbal tea. Lemongrass oil is one of the most important essential oil produced in the world. India is a major producer of this oil**.** Chemical compounds present in varying concentration in lemongrass have a great demand due to their use in perfumery, flavor and pharmaceutical industry. The oil extracted from leaves of lemongrass is used for its anti-inflammatory, antipyretic, diuretic activity *(Negrelle and gomes, 2007)* and are also reported to have antimicrobial activity.

In recent year, multiple drug resistance, both in human and plant pathogenic microorganism have been developed due to indiscriminate use of commercial antimicrobial drugs used in infectious diseases *(service, 1995).* This prompts to the search for new, safe and effective antimicrobial agents from alternative natural resources like plant products. Antimicrobial agents from plant sources have much therapeutic potential; not only they are effective in the treatment of infectious diseases, but also lessen a large number of side effects that are often linked with existing antimicrobial agents *(Shakharkar PR et al., 1998; Jin L et al., 2009)*. One of the methods that reduce the resistance to antibiotics is by using antibiotic resistance inhibitor of plant origin *(Iinuma et al., 1994; Kim et al., 1995)*. The essential oil is watched today because of marked spread of antibiotic resistant bacteria. The essential oils have been reported to be active against. Methicillin resistant Staphylococcus aureus and other resistant bacteria *(Nelson, 1997)*. The present study analyze the chemical composition of the volatile oil from the leaves of *Cymbopogon citratus* and evaluates the antimicrobial activity of the oil and a cream formulation of the oil.

2. Materials and Methods

Plant material

Fresh leaves of *C.citratus (lemongrass) were* collected from the surroundings of Dehradun city located in Uttarakhand (India). The plant was properly identified and authenticated.

Extraction of volatile oil

Freshly collected leaves were subjected to hydrodistillation (leaves: water ratio = 1:6) for the extraction of essential oil for 3 hrs using a Clevenger (Borosil, Mumbai) apparatus *(Clevenger, J.H., 1928).* The distilled volatile oil was dried over anhydrous sodium sulphate, filtered and stored at 4°C. The percentage yield of essential oil was 0.35 % (v/w).

GC-MS analysis of oil

GC-MS analysis was performed by using Perkin Elmer Clarus-500 equipped with a flame ionization detector. The GC column was (30 m × 0.25 µm) fused silica capillary column. The GC conditions were as follows: injector and detector temperature,250°C; oven temperature programmed to rise from 110°C to 230°C at 10°C per minute. Injection volume: 2µl. Helium, the carrier gas, was used at a flow rate of 1 mL/min. The oven temperature was programmed from 70°C to 260°C at 10 °C/min. the effluent of the GC column was introduced directly into the source of MS. Mass spectra were obtained in EI mode with 70 eV ionization energy. Ion source and transfer line temperature was kept at 300°C. The mass spectra were obtained by centeroid scan of the mass range from 40 to 500 amu for 2 sec. percentage of components of the essential oil was matching their recorded spectra with the data bank mass spectra of Wiley library provided by the instrument software.

Formulation of essential oil Cream

The distilled essential oil was formulated in to a cream by using a suitable base (oil in water type: O/W) listed in the Table 1. Aqueous cream was just as effective as the test agents because of its easy penetration into the skin *(Muller MJ. 2003).* Stearic acid, cetosteryl alcohol, liquid paraffin and petroleum jelly were melted on a steam bath at 75 °C. The remaining ingredients were dissolved in water and boiled at 75°C. The aqueous solution was than added to the above oily phase with agitation. Potassium hydroxide reacts with a portion of stearic acid to form potassium stearate which emulsifies the un-reacted stearic acid and becomes a dispersed phase. Excessive agitation was avoided so that air was not entrapped within the cream. 2 ml of volatile oil was mixed into the above prepared cream base with uniform stirring using a mechanical stirrer. Then glycerin (7 mL) was added and mixed in. The formulated cream was stored in a suitable plastic container.

Table 1: Composition of cream base

Ingredients	Composition w/w
Stearic acid	16.00 %
Potassium hydroxide	0.50 %
Cetosteryl alcohol	5.00 %
Liquid paraffin	3.50 %
Petroleum jelly	3.50 %
Methyl paraben sodium	0.16 %
Propyl paraben sodium	0.04 %
Glycerin	7.00 %
Purified water	64.30 %

Antimicrobial screening of the oil and its cream Formulation

The well agar assay *(Kavanagh, F., 1972)* of drug potency is based on the measurement of the diameter of zone of microbial growth inhibition surrounding the well (cups). The gram-positive organisms tested were, *Bacillus coagulans*, *Bacillus subtilis*, *Bacillus megaterium and Staphylococcus aureus*, and the gram-negative organisms used were, *Escherichia coli*, Pseudomonas *aeruginosa*, *K.pnemonia* and *Salmonella typhii*. The fungal strain used was *Aspergillus niger, Candida albicans and Tricoderma lignorum*. All the microorganisms were collected from Institute of microbial technology (IMT) Chandigarh (India).

Petri dishes were cleaned, dried and sterilized. Then they were filled with nutrient agar (Himedia, Mumbai) and Sabouraud's dextrose agar (SDA) (Himedia, Mumbai) medium for bacteria and fungi, respectively. After solidifying, the plates were inoculated with the organisms. Three and four holes were made in each plate with a sterile stainless steel borer with a height of 10 mm and internal diameter of 6-8 mm. The essential oil (0.1 mL), 200 mg of cream and cream base was added to each hole. Then the plates were refrigerated for two hrs to allow diffusion. Then the plates were incubated at 37 ± 1°C for 24 hrs and 28 ± 1°C for 48 hrs for bacteria and fungi, respectively. The standards used were Ciprofloxacin.

TLC Finger prints of *Cymbopogon citrates*

Sample application: Band wise application of volume 10µl of each extract.

Development chamber*: Camag Twin Trough Chamber 10 x 10cm*

Mobile Phase: Toluene: Ethyl acetate = 93: 07

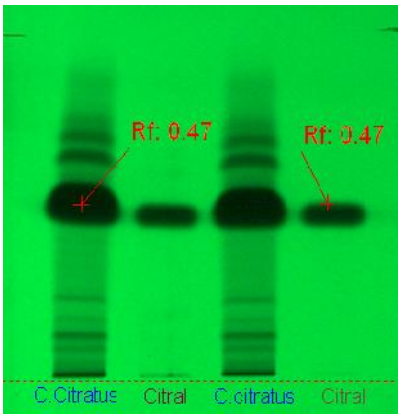

Figure 1: TLC Finger prints of *Cymbopogon citrates*

3. Results and Discussion

The total of 19 compounds was identified with 99.8% by GC-MS analysis. The oil is mainly composed of monoterpenes and sesquiterpenes. The major compound identified was citral which accounted for 74.09% of the total oil. Composition of volatile oil constituents of lemongrass has been reported *(Chisowa, E.H , 1998).* The essential oil of lemongrass is characterized by a high content of citral and its quality is generally determined by the amount of citral present in the oil. Citral is a mixture of two stereo-isomeric monoterpene aldehydes; geranial (trans-citral, called citral-a) and neral (cis-citral, called citral-b). Essential oil of C.citratus is mainly composed of citral with general predominance of geranial. Significant antimicrobial activity was observed against both gram-positive bacteria and gram negative bacteria (Table -2). However, the antimicrobial activity was more potent on the gram-positive than the gram negative species tested, particularly on *S.aureus* and B. subtilis. The oil was as potent on B. subtilis as the standard, Ciprofloxacin. Both the oil and 2% cream formulation displayed dramatic antimicrobial activity on all of the fungal strains tested, but were particularly notable on *T. lignorium* and *C. albicans (Table-3).* The essential oil has been reported to contain mono-, sesqui- and di-terpenes. The present investigation also indicated that the same mono- sesqui- and di-terpenes are present in the volatile oil. Although the volatile oil contain the same group of compounds but the percentages of individual components differ *(Koba Koffi, et al., 2009).* The essential oil obtained from this plant and its cream formulations exhibited antibacterial and antifungal activities. These activities may be attributed to the presences of the above noted compounds of mono-, sesqui- and di-terpenes *(Magiatis P,et. Al., 1999; Tzakou O et al., 2001; .Oumzil H et al., 2002).*

These chemical components probably exert their toxic effects against microorganisms through the disruption of bacteria or fungal membrane integrity since they are known to increase membrane permeability in yeast cells and in isolated mitochondria *(Uribe S et al., 1998).* This is strongly supported by the study on the effects of different essential oil components on outer membrane permeability in gram-negative bacteria *(Knoblock K et al., 1998).* The significant antimicrobial effect of lemongrass essential oil and the cream prepared by the oil against all the pathogens confirmed that the compounds present in the oil are responsible for the effective antimicrobial activity. Thin layer chromatography studies indicated the presence of citral and all different compounds confirms that the activity may be due to citral and other compounds (Fig. 1), confirming the synergistic action. In this study, it was found that both oil and 2% cream formulation yielded good activity against fungi and bacteria. This may be due to the presence of the high percentage of the citral (74.09%).

Table 2: GC-MS analysis of Essential oil

Retention time	Chemical constituents	Composition (%)
2.218	β-Phellandrene	0.7%
3.743	β -Cymene	0.2%
4.734	α –pinene	0.13%
6.140	Myrcene	12.25%
8.259	Dipentene	0.23%
10.721	Citral-b	32.27%
12.059	Citral-a	41.82%
14.125	β –Pinene	0.16%
15.572	Delta-3-catrene	0.16%
16.316	Geranyl acetate	3.00%

18.344	β -elemene	1.33%
21.782	Germacrene-D	0.75
23.578	δ- Cadinene	0.75
26.173	Geraniol	1.65%
31.600	α-Terpineol	0.40
33.518	β -Caryophylene	0.18%
34.953	Terpenolene	0.05%
40.559	Methyl heptanene	2.62%
42.497	Elemol	1.2%

Table 3: Antibacterial Activity of Essential oil and Cream Formulation of Lemongrass

S. No	Test Organism	Diameter of zones of inhibition		
		Essential oil (0.1ml)	**2% Cream formulation** (200 mg)	**Standard** (Ciprofloxacin)
1	Bacillus coagulans	13mm	10mm	18mm
2	Bacillus subtilis	18mm	15mm	19mm
3	*Bacillus megaterium*	16mm	13mm	20mm
4	Staphylococcus aureus	26mm	20mm	32mm
5	*E.Coli*	12mm	9mm	25mm
6	*Salmonella typhii*	11mm	9mm	24mm
7	*Pseudomonas aeruginosa*	10mm	9mm	20mm
8	*K.pnemonia*	14mm	10mm	28mm

Table 4: Antifungal Activity of Essential oil and Cream Formulation of Lemongrass

S. No.	Test Organism (Fungal strain)	Diameter of zones of inhibition		
		Standard (Ciprofloxacin)	**Essential oil (0.1ml)**	**2% Cream formulation (200mg)**
1	*Aspergillus niger*	18mm	15mm	11mm
2	*Candida albicans*	19mm	18mm	15mm
3	*Tricoderma lignorum*	17mm	16mm	14mm

4. Conclusion

From this study it was observed that the product developed in the form of a cream formulation (2%) from the essential oil of *C. citratus* possibly can be used as an antimicrobial cream to cure the skin diseases. Further clinical studies on human volunteers will be conducted to confirm these activities.

5. Acknowledgement

The authors are thankful to Dr. S. Farooq, President 'The Himalaya Drug Company' for valuable assistance and providing us the lab facility to complete our research work.

6. References

1. Chisowa, E.H., Hall DR and Farman DI. Volatile constituents of the essential oil of Cymbopogon citratus Stapf grown in Zambia. Flavour Fragr J. **1998**, 13: 29–30.
2. Clevenger, J.H. Apparatus for the determination of Volatile oil. J.Am.Pharmaceut. Assoc., **1928**, 17, 346.
3. Iinuma M, Buchiya H, Flavonones with potent antibacterial activity against methicillin resistant Staphylococcus aureus. J.Pharm. Pharmacol. **1994**, 46: 892-895.
4. Jin L, Song S H, Li Q, Chen Y, Wang Q, Hou S H.Identification and characterisation of a novel antimicrobial polypeptide from the skin secretion of a Chinese frog (*Rana chensinensis*). *International J Antimicrobial Agents*. **2009**, 33: 538-42.
5. Kavanagh, F. (**1972**). Analytical Microbiology. F. Kavanagh(ED), VOL II, Academic press, New York and London, P: 11.
6. Kim H, Park SW. Screening and isolation of antibiotic resistant inhibitors from herbs materials

I-Resistant inhibition of 21 Korean Plants. Nat. Prod. Sci. **1995**, 1: 50-54.

7. Knoblock K, Pauli A, Iberi B, Weis N, Weigand H. Antibacterial activity and antifungal properties of essential oil componenets. J Essent Oil Res. **1998**, 1: 119-128.
8. Koba Koffi, Sanda Komla, Guyon Catherine, Raynaud Christine, Chaumont Jean-Pierre and Nicod Laurence. *In -vitro* cytotoxic activity of Cymbopogon citratus L. and Cymbopogon nardus L. essential oils from Togo. Bangladesh J Pharmacol. **2009**, 4: 29-34.
9. Magiatis P, Melliou E, Skaltsounis AL, Chinou IB, Mitaku S. Chemical composition and antimicrobial activity of the essential oils of Pisacia lentiscus var. chia. Planta Med. **1999**, 65: 749-752.
10. Muller MJ, Hallyoak MA, Moaveni Z, Tim LH, Herndon DN, Heggers JP. Retardation of wound healing by silver sulfadiazine is reversed by Aloe vera and nystatin. Burns. **2003**, 29: 834-836.
11. Negrelle RRB, and Gomes EC. Cymbopogon citratus (D.C) Stapf: chemical composition and biological activities. Rev Bras Pl Med. **2007**, 9: 80–92.
12. Nelson RRS. *In-vitro* activities of five plant essential oils against methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus faecium. J. Antimicrob. Chemoth. **1997**, 40: 305-306.
13. Oumzil H, Ghoulami S, Rhajaoui M, Ilidrissi A, Fkih- Tetouani S, Faid M. Antibacterial and antifungal activity of essential oil of *Mentha suaveolens*. Phytother Res. **2002**, 16: 727-731.
14. Sakharkar P R and Patil A T. Antimicrobial activity of *Cassia alata*. *Indian J Pharmaceutical Sci*. **1998**, 60: 311-2.
15. Tzakou O, Pitarokili D, Chinou IB, Harvala C. Composition and antimicrobial activity of essential oil of *Salvia ringens*. Planta Med. **2001**, 67: 81-83.
16. Uribe S, Ramirez T. Effects of β-pinene on yeast membrane functions. J Bacteriol. **1998**, 161: 195-200.