IJBPR

Int. J. Biopharma Research
ISSN: 2287-6898
Research Article

Chemical Composition and Its Antibacterial Activity of Essential Oil from *Cymbopoton Jwarancusa*

Sarath Chandra Bose N[1*], Ammani K[2] and S Ratakumari[3],
[1]Department of Biochemistry and [2]Department of Microbiology,
Acharya Nagarjuna University, Guntur, Andhra Pradesh, India
[3]Crop physiology, Regional research Centre, LAM, Guntur, Acharya N.G Ranga Agricultural University

Received for publication: November 18th 2012; Revised: December 29th 2012; Accepted: Jan 30th 2013

Abstract: The present study was elucidating the chemical composition of essential oil from *Cymbopogon jwarancusa* and its antibacterial activity. Chemcial composition of the essential oils was determined by capillary gas chromatography (GC) and mass spectrometry. Major constituents of the oil were Piperitone (33.05%), Geraniol (20.30%), Δ4–Caren.e (16.9%), γ–terpinen (6.5%), β–Piniene (3.5%). and in *C. jwarancusa* 95.8% was identified. The antimicrobial assay using the agar well diffusion method showed that the essential oil markedly suppressed the growth of several species of *Citrobacter, Klebsiella pneumonia, Proteus mirabilis, Salmonella enterica* ser.typhi and *Shigella flexneri* at the dose of 105 CFU/ml. The most active compounds among the 19 examined 6 constituents shows antibacterial activity. Among the 6 constituents, geraniol completely inhibited the growth of the bacteria than fungi. The β – Pinene, Linalool,and α – terpeniol showed an inhibitory activity against some bacteria and fungi, whereas the other compounds lacked this property.

Keywords: *Cymbopogon jwarancusa*, Geraniol, B-Pinene, Linalool, Essential Oils, Hydro Distillation, Antimicrobial Activity.

Introduction

Cymbopogon is native to warm temperate and tropical regions. The word cymbopogon was introduced by Sprengel in 1815. At that time the genus consisted of a few species. They are then moved to the genus Andropogon and it is the sub type of Graminaeae. Use of essential oils extracted from aromatic plants in medicine was since 16th century[1]. The flourishing trade of Indian essential oils in ancient and medieval times has comparatively receded because of lack of scientific and technological progress. The chemical composition of the essential oils of Cymbopogon may vary from one species to another species[2]. Based on the composition of oil, the genus Cymbopogon has been partitioned into three series i.e., Schoenanthi, Citrati and Rusae[3].

The essential oils that are derived from Cymbopogon are of medicinal importance as some the extracts are having antimicrobial and antibacterial properties[4]. The quality of lemon grass oil is generally determined by the content of citral. Citral is a monoterpene that gives lemon aroma in lemongrass[5]. In general, the yield of essential oil is highly corelated with the yield of biomass. The essential oils of the grasses of species of Cymbopogon have an industrial profile. They are used in beverages, food stuffs, fragrances, house hold products, personal care products and in tobacco[6]. Essential oil and citral contents were influenced by factors such as temperature light intensity, soil moisture, fertilizer and maturity stage[7]. The oils of some Cymbopogon species have powerful germicidal and antibacterial properties[8]. The essential oil of palmarosa seeds at three different geographical locations in India (Bangalore, Hyderabad and Pantnagar) and found significant genetic variation which led to the crop improvement[9]. The essential oils are used in perfumes, flavoring, cosmetic and pharmaceutical preparations. The oils from some of the species are used in treatment of various human ailments such as cough, fever, gout, leprosy, stomach disorders and have sedative properties[10]. The aim of the present study was to extract the essential oils from *Cymbopogon jwarancusa* and its chemical constituents and to evaluate the antibacterial and antifungal activity of the chemical constituents.

Materials and Methods

Collection and Identification of Samples:

The leaves of *Cymbopogon jwarancusa* was collected from Acharya Nagarjuna University located NH-5 between two cities Vijayawada and Guntur, Andhra Pradesh.

Extraction of Essential Oil from the Leaves:

The extraction of essential oil may sound only to be of technical interest. It is one of the points which determine the quality of the oil. The essential oils are present in the oil glands, oil salts and glandular hairs of the plants. The essential oil from aromatic plants of Cymbopogon can be obtained either hydro distillation or steam distillation. Hydro distillation is cheaper than steam distillation. Therefore hydro distillation method was commonly

***Corresponding Author:**
Dr. Sarath Chandra Bose N,
Department of Biochemistry,
Acharya Nagarjuna University,
Guntur, Andhra Pradesh, India.
Email: sarathchandrabose@gmail.com

used for the extraction of essential oil. Clevenger apparatus is used for distillation process. The sample was submitted for 3 to 4 hours to hydro distillation using a Clevenger apparatus. The distilled oil should be left to stand for few hours. Anhydrous sodium sulphate (approximately 3%) is added to remove moisture. The oil was stored at -4oC until tested and analyzed.

Gas-Chromatography-Mass Spectrometry:

GC-MS analysis was recorded on a Agilent 6890 GC with 5973 Network Mass Selective Detector. Column: HP-5MS (30m x 0.25mm id x 0.25µm). Over temperature kept initially at 50oC for 2 minutes and then increased at 10oC/minute to 280oC and maintained for 5 minutes. The carrier gas used was helium at 1.2 ml/minute flow rate. Injector and detector were 250oC and 280oC, respectively. The area percentage data were obtained on a chemstation integrator.

The constituents were identified by comparing their GC retention times and their identity confirmed by a computer matching of their mass spectral pattern with that of known compounds in computer library software coupled with GC-MS. Fragmentation pattern studies with those were reported in literature were also made[11,12].

Test Organisms:

The test organisms were procured from microbial type culture collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, Punjab. Gram negative bacterial strains such as *Citrobacter* (MTCC6805), *Klebsiella pneumoniae* (MTCC9544), *Proteus mirabilis* (MTCC9493), *Salmonella enterica* ser. typhi (MTCC8767) and *Shigella flexneri* (MTCC1457) were used as test organisms for antibacterial activity experiments. Fungal strains such as *Candida albicans* (MTCC7533), *Trichophyton rubrum* (MTCC3018), *Aspergillus niger* (MTCC1344), *Aspergillus fumigatus* (MTCC2483) were used as test organisms for antifungal activity experiments.

Preparation of Innoculum:

Stock cultures of bacterial and fungal strains were maintained at 4°C on slopes of nutrient agar and Sabouraud dextrose agar, respectively. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton broth for bacteria and Sabouraud dextrose broth for fungi that were incubated without agitation for 24 hours at 37°C and for 48 hours at 25°C, respectively. Activated cultures of bacterial and fungal strains in Mueller-Hinton broth for bacteria and Sabouraud dextrose broth, respectively were adjusted to 108 CFU/ml.

Determination of Antimicrobial Activity:

The antimicrobial tests were carried out by Agar well diffusion method

Antibacterial Activity:

This assay was performed by Agar well diffusion method. The agar well diffusion assay was used to screen the antibacterial activity of the essential oils of different plant species. NAM was prepared as per the composition given below. The pH was adjusted to 7.2 using 5 M sodium hydroxide, and then sterilised in an autoclave maintained at 121oC (15 1bs/sq.in) for 20 minutes

Antifungal Activity:

Peeled potatoes (20 grams) were cut into small pieces and boiled with 100 ml of water for 30 minutes. The pieces were crushed during boiling and the pulp was removed after cooling by filtration through muslin cloth. PDA was prepared as per the composition given below.

Results and Discussion

In the crude oil of the third plant 19 components were identified (table-1). The major components are Piperitone (33.05%), Geraniol (20.30%), Δ4–Carene (16.9%), γ–terpinen (6.5%), β–Piniene (3.5%).Identification of the essential oil constituents was accomplished by comparing the retention times of the chromatography peaks and was confirmed by a computer matching with that of known compound in computer software. the chemical composition of essential oil from *Cymbopogon nardus* (citronella oil) through GCMS and reported that the major constituents of the oil were geraniol (35.7%) of total volatiles, citral (14.2%) geranyl acetate (9.7%) and citronellol (4.6%). The chemical composition of *Cymbopogon winterianus* through GC-MS and reported that the major components were Geraniol (23.6%), Citronellol (10.8%), Linalool (6.2%), Nerol (9.6%) and B. Caryophyllene (5.3%).13 The 25 different components were extracted from the essential oil of *Cymbopogon citratus*. He also stated that the oil was dominated by monoterpene hydrocarbons which accounted for 94.25% of the total oil and characterised by a high percentage of geranial (39.53%), neral (33.3%) myrecene (18.4%) whereas the remaining components were present in minor quantities[14].

The antimicrobial activity of all the chemical components were tested against Gram negative bacterial strains *Citrobacter , Klebsiella pneumoniae, Proteus mirabilis, Salmonella enterica* ser. typhi and *Shigella flexneri* and fungal strains *Candida albicans, Trichophyton rubrum, Aspergillus niger* and *Aspergillus fumigatus* (table-2) and found few of the chemical components are very much effective against these pathogens[15]. Tetracycline is used as positive control for bacteria and Nystatin is used as

a positive control for fungi. The susceptibility of 1,114 strains belonging to 24 genera and 105 species against lemon grass oil, of which that 38.2% of the tested strains were sensitive. Among the tested strains Bacillus species and Streptococci are predominantly sensitive, whereas the species Enterococci and Staphylococci are predominantly resistant[16].

Table.1: Essential oil composition of *Cymbopogon jwarancusa*

S. No	Component	Retention time in min	Percentage
1	β – Pinene	5.98	3.5
2	Camphene	6.09	0.9
3	Myrcene	6.80	0.8
4	Δ^4 – Carene	7.45	16.9
5	Linalool	8.60	1.0
6	Allo – Ocimene	8.90	0.8
7	Trans – Crysanthemal	9.00	0.7
8	Citronellal	9.43	1.2
9	Citronellol	9.58	0.9
10	γ – Ter pinen	9.89	6.5
11	α – Terpeniol	10.00	0.7
12	Geraniol	10.96	20.36
13	Geranic Acid	12.50	2.4
14	Geranyl Acetate	12.70	1.6
15	Geranyl Propionate	13.50	1.2
16	Elemene	16.90	0.9
17	Eudesmol	21.90	0.6
18	Elemol	24.15	0.8
19	Piperitone	28.50	33.5
	Total		**95.26**

Table.2: Antimicrobial activity of essential oil of *Cymbopogon jwarancusa*

Volatile compounds (95 mg/L)	Growth inhibition								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
β – Pinene	++		++		++		+		+
Camphene									
Myrcene									
Δ^4 – Carene	-	-	-	-	-	-	+	-	+
Linalool	++	++	+	-	++	+	-	+	++
Allo – Ocimene									
Trans – Crysanthemal	-	-	-	-	-	-	-	-	-
Citronellal	-	-	-	-	-	-	-	-	-
Citronellol							+		+
γ – Ter pinen									
α – Terpeniol	++			+			++	+	+
Geraniol	++ (22)	++ (19)	++ (19)	++ (21)	++ (20)	++ (17)	++ (18)	++ (15)	++ (17)
Geranic Acid									
Geranyl Acetate	-	-	-	-	-	-	-	-	-
Geranyl Propionate									
Elemene									
Eudesmol									
Elemol	-	-	-	-	-	-	-	-	-
Piperitone									

Conclusion

The present study confirms that the gram negative bacteria that are human pathogens are very much sensitive to geranial. The dangerous fungal pathogens and Candida albicans are also sensitive to geraniol. Geraniol is used for the treatment of different diseases like skin allergies, gout cardio vascular diseases. The antimicrobial activity of geraniol was evaluated for the first time.

Acknowledgements

One of the authors, N. Sarath Chandra Bose, expresses his gratitude to K.P. Sharma, Principal scientist, CIMAP, Hyderabad for his guidance and to the Department of Biochemistry, Acharya Nagarjuna University for providing the necessary facilities to carry out the project.

References

1) Cobley, L.S. 1956. In: Introduction to the Botany, of Tropical Plant, Longman, London, pp. 357.

2) Gildemeister, E., Hoffman, F. 1956. Die Arthrisehen Ole, Vol. IV. Akademie Verlag, Berlin, 307-419.

3) Stapf, O. 1960. The oil grasses of India and Ceylon, Kew Bull. 1906: 297-363.

4) Anonymous, 1958. Essential oils and Aromatic chemicals, Symposium at Dehradun, Kolkatte, C.K. and Varma, K.C. 1971. Indian Jour. Agri. Sci. 41: 382-385.

5) Miyazaki, Y. 1965. Grass and oil yields from lemon-grass and the quality of oil. Japanese. J. Trop. Agric. 37-41.

6) Bor, N.L. 1960. The grasses of Burma, Ceylon, India and Pakistan, pp. 767, Pergamon Press, London.

7) Kazuhiko Nakahara, Najeeb S.Alzoreky, Tadasi Yoshihashi, Huong T.T. Nguyen and Gassinee Trakoontivakorn. 2003. Chemical composition and Antifungal activity of essential oil from Cymbopogon nardus (Citronella Grass). JARQ 37(4): 249-252.

8) Sharma, J.K. and Ram, R.S. 2000. Genetics and genotype improvement of Cymbopogon species. The Aromatic Grass, Monograph, Pub CIMAP, Lucknow, 5: 85.

9) Sugumaran M, Joseph s, Lee K L W, Wong K W.2005. Hass of Malaysia. Shah alam. Federal publication.

10) Adamas, R.P. 1989. Identification of essential oil by ion trap mass spectrometry, Academic press, Sandiego, USA.

11) Silverstein, R. M and Webster, F. X. 1998. Spectrometric identification of organic compounds, VI Edn., John Wiley and Inc, Newyork, 45-70.

12) Khan, M.S.A and Ahmad. I. 2011. In vitro antifungal, anti-elastase and anti-Keratiase activity of essential oil of Cinnamomum – syzygium and Cymbopogon species against as per gillus fumigates. Phyto medicine 19(1): 48 – 55.

13) Kazuhiko Nakahara, Najeeb S. Alzoreky, Tadasi Yoshihashi, Huong T. T. Nguyen and Gassinee Trakoontivakorn. 2003. Chemical composition and Antifungal activity of essential oil from Cymbopogon nardus (Citronella Grass). JARQ 37(4): 249-252.

14) Dorman, H.J.D and Deans, S.G. 2000. Antimicrobial agency from plants. Antibacterial activity of plane volatile oils. J. Applied Microbiol, 83: 308-316.

15) Rios, J.R and Recio, M.C. 2005. Medicianal plants and antimicrobial activity. Journal of Ethnopharmacology, 100: 1 – 2, 80 – 84.

Source of support: Nil
Conflict of interest: None Declared