American-Eurasian J. Agric. & Environ. Sci., 12 (8): 1042-1046, 2012
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2012.12.08.6551

Antibacterial Activitiesof Lemon Grass Methanol Extract and Essence on Pathogenic Bacteria

[1]Behboud Jafari, [2]Amirreza Ebadi, [3]Babak Mohammadi Aghdam and [2]Zarifeh Hassanzade

[1]Young Researchers Club, Ahar Branch, Islamic Azad University, Ahar, Iran
[2]Department of Microbiology, Ahar Branch, Islamic Azad University, Ahar, Iran
[3]Department of Chemistry, Ahar Branch, Islamic Azad University, Ahar, Iran

Abstract: According to presence of biological active compounds in lemon grass plant and use of this plant in traditional medicine, it seems that this plant possesses considerable antibacterial activity. The purpose of this study was investigatingthe antibacterial activity of lemon grass methanol extract and essential oil on the four reference strains of *Staphylococcus aureus* (*ATCC:25923*), *Bacillus cereus* (*ATCC:1247*), *Escherichia coli* (*ATCC:25922*) and *Pseudomonas aeruginosa* (*ATCC: 27853*). 20, 30, 50 and 400 mg/ml concentrations of plant methanol extract were prepared. Then their antimicrobial effects were assessed with agar well diffusion and dilution test methods. Results showed that methanol extract of lemon grass plant prevented bacterial growth of *Staphylococcus aureus, Bacillus cereus* and *Escherichia coli* which with increasing concentration, their antibacterial effect also increased. 1000 mg/ml concentration of this plant leaf essential oil indicated inhibitory effect on *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*. In this study no inhibitory effect of growth of*Pseudomonas aeruginosa*was observed. It can be concluded that despite the inhibitory effects of concentrations of methanol extracts and essential oil of this plant on the growth of pathogenic bacteria especially Gram-positive, to introduce it as an alternative to chemical antimicrobial drugs, wider investigation is required.

Key words: *Cymbopogon citratus* · Plant extract · Antibacterial activity

INTRODUCTION

Plants are still a potential source of medical compounds. In the world plants traditionally are used in oral health and to treat many disease especially infectious diseases including diarrhea, fever and cold [1] in addition, many recreational compounds used in traditional medicine have plant root [2]. According to World Health Organization (WHO) definition amedicinal plant, is a plant that can be usedfor therapeutic purposes and or its compounds be used as a pioneer in the synthesis of semi-synthetic chemical drugs [3].

Thereare 5 methods for choosing plants to investigate the pharmacological effects including: random approach that includes plants collection in the study area. Phytochemical targeting that includes all plant family members that their richness of biological active compounds has been proven. Ethno-directedsampling method is based on uses of traditional medicinal of one plant. Chemotaxonomic approach that is based on collection specific plant parts such as seeds [4].

With increasing number of bacterial strains resistant to various antibiotics, many attempts to use the antimicrobial potential of plants have been done. On the other hand emergence of resistant strains among Gram negative bacilli and Gram positive cocci such as *Pseudomonas, Klebsiella, Enterobacter, Staphylococcus* and *Enterococcus* has cased problems in treating infections caused by these bacteria [5]. Antimicrobial compounds obtained from plants with different mechanisms of actionagainst resistant microbial strains are of clinical importance [6]. Many studies on extracts prepared from collected plants randomly or by one of the above methods have been done. These studies further has focused on evaluation of the antimicrobial [7-10] antihelmintic [11], anti-viral [12], cytotoxic and mutagenicity effects [13] and also general pharmacological effects [14].

Corresponding Author: Behboud Jafari, Young Researchers Club, Ahar Branch, Islamic Azad University, Ahar, Iran.

Lemon grass plant with scientific name *Cymbopogon citratus* belongs to the Graminae (poaceae) family that contains about 660 genus and 9000 species, which are widely distributed in tropical and subtropical world. Lemon grass is native to India and Sri Lanka [15, 16].

*Cymbopogon citratus*is usually used in folk medicine for the treatment of neurological and gastrointestinal disorders and as an antispasmodic, analgesic, antibacterial, anti-pyretic, diuretic and sedative [17]. Citratus species cultures widely in some Asian countries and African due to the high rate sytral (70-80%) in its essence [18]. This plant is an important source that provides essential oils used in food and hygienic industry [19]. Lemon grass essential oil has the ability to control bacterial growth and fungal pollutants in food such as *Staphylococcus aureus* and *Escherichia coli*, also the antioxidant activity of plant essential oil has proven, so that lemon grass essential oil in comparison with alpha-tocopherol has stronger antioxidant activity and acts as BHT equivalent (Butylated Hydroxy Toluene) [20]. Hindumathy in a study in 2011 showed that lemon grass plant due to having alkaloids and phenols contains antibacterial properties. Research conducted on essential oils of*Cymbopogon Citratus*, its antimicrobial properties compounds such as citronel and, geraniol acetat was carried out [21, 22]. In this study the antibacterial properties of methanol extracts and essential oils of Lemon grass plant were tested against a number of pathogenic bacteria.

MATERIALS AND METHODS

Fresh leaves of Lemon grass plant collected from Jiroft, Dezful and Masjed Soleiman endemic areas of south and west of the Iranwere approved by the botanical botany part of Islamic Azad University of Ahar. Essential oils were obtained from hydrodistillation using and clevenger apparatus [24]. Three hundreds gr of powdered dried leaves with one liter distilled water was heated in clevenger apparatus. Essential oil collected after removing moisture was kept in opaque glass and sealed at 2°C away from light. To prepare methanol extract; 60gr of dried plant powder with methanol as a solventwere placed for 8 hours in a soxhlet extraction apparatus [25], the extract was concentrated by placing this solvent at 40°C and using rotary apparatus. Four concentrations of the methanolic extract by using 5% Dimethyl-sulfoxide with 20, 30, 50 and 400 mg/mlconcentrations were prepared. Tested Microorganisms includings *Bacillus cereus* (*ATCC: 1247*), *Staphylococcus aureus* (*ATCC: 25923*), *Pseudomonas aeruginosa* (*ATCC: 27853*) and *Escherichia coli* (*ATCC: 25922*) were obtained lyophilized from Tehran university of microbial collections. Microbial samples were reduced according to standard methods and the microbial suspensions wereprepared according to tube 0/5 McFarland standard (equivalent turbidity $1/5\times10^8$CFU/ml). In this study the antimicrobial effect of methanol extract was investigated with two methods agar well diffusion and dilution [25]. In well diffusion method; 500µL of $1/5\times10^8$CFU/ mlmicrobial suspension was inoculated onto Muller Hinton agar medium. Then wells with 6 mm diameter were created at agar surface, 100µL of 20, 30, 50 and 400 mg/ml concentrations of methanol extract was injected into the wells. The negative controlwas (5% DMSO) and the antibiotic chloramphenicol was used as positive control then plates were incubated at 37°C for 24 hours and inhibition zone was measured in millimeters. By using tube dilution method, the minimum growth inhibitory concentration and the minimum bactericidal concentration of methanol extract were determined. Active bacterial suspension ($1/5\times10^8$CFU/ml) was added to each of the prepared methanol extract concentrations 6.25, 12.5, 25, 50, 100 and 200 mg/ml. Finally tubeswere incubated at 37°C for 24 hours. After incubation time, tubes were examined for turbidity and the last dilution with no turbidity was considered as MIC. Then from all tubes in which no bacterial growthwas observed, samples were culturedon platesto determine the minimum lethal bacterial concentration (MBC). Then plates wereincubated at 37°C for 24 hours. Tube containing the lowest concentration extract with lack of bacterial growth was visible as MBC. The antimicrobial properties of essential oils of leaves were tested in agar dilution method [25]. A control that contains only DMSO and culture medium without essential oil was used. Inoculated culture media were placed at 37°C for 24 hours and then were studiedfor growth of bacteria. To reduce experimental error each of the above experiments were repeated 4 times. To determine significant differences at obtained results ANOVA and chi-square tests were used and differences between groups were determined at $p<0/001$ significant level.

RESULTS

The results of the antimicrobial activity of lemon grass plant methanolic extract methanolic extract by well diffusion method were shown in Table 1. Both *Staphylococcus aureus* and *Bacillus cereus* had the highest microbial sensitivity and this inhibitory effect increased with increasing methanol extract concentration.

Table 1: Inhibition zone diameter (mm) of the methanolic extract of lemon grass plant against four bacterial strains

	Extractconcentrations (mg/ml)					
Bacteria strains	400	50	30	20	Negative control	Positive control
Staphylococcus aureus	18	10	9	7	--	20
Bacillus cereus	18	8	8	--	--	19
Escherichia Coli	12	--	--	--	--	26
Pseudomonas aeruginosa	--	--	--	--	--	22

Table 2: Minimum inhibitory and minimum bactericidalconcentrations of methanol extract of lemon grass plant on tested bacteria in term of (mg/ml)

	Extractconcentrations (mg/ml)	
Bacteria strains	MIC	MBC
Staphylococcus aureus	12/5	25
Bacillus cereus	6/25	12/5
Escherichia Coli	100	200
Pseudomonas aeruginosa	--	--

Also results obtained showed that growth inhibitory effect of methanol extract of lemon grass leaves on tested Gram negative bacteria was very low, so that no growth inhibition effect on the *Pseudomonas aeruginosa*was detected with slight inhibitory effect against *Escherichia coli* at the highest extract concentration used (400 mg/ml concentration). The results in Table 2 show that 25 mg/ml concentration of methanolic extract of lemon grass planthad a bactericidaleffect on *Staphylococcus aureus*. Lethal concentration of this extract against *Bacillus cereus*was obtained at 12.5 mg/ml. These results indicated that among tested bacteria in term of sensitivity to methanolic extract of lemon grass there was a significant difference (p<0/001). In the other words *Bacillus cereus*showed the highest sensitivity to methanolic extract lemon grass and the least sensitivity was displayed by *Pseudomonas aeruginosa*. Experiments related to the 1000µg/ml concentration effect of leaf essential oil against the tested pathogens showed that *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli* showed inhibitory effects and no inhibitory effect on *Pseudomonas aeruginosa*were observed.

DISCUSSION

In recent years much research has been conducted in the field of antimicrobial effects of different plants. In this study it was foundthat the methanol extract of lemon grass plant in concentrations around 30 mg/ml prevented the growth of tested Gram positive bacteria. While, inhibition of Gram negative bacteria needed higher concentrations. Its essential oil also has a significant inhibitory effect on *Staphylococcus aureus, Bacillus cereus* and *Escherichia coli*. Also the results showed that the methanol extract effect against Gram negative bacteria was much weaker than the Gram positiveones as. 400 mg/ml concentration of methanol leaf extract showed weak inhibitory effect against *Escherichia coli*. None of the concentrations tested had inhibitioneffect on *Pseudomonas aeruginosa* that its probable cause is presence of cell wall polysaccharides which probably prevent reaching the active compounds, essential oil and extracts to the cytoplasmic membrane of Gram negative bacteria [26]. In general, herbal products lead to granular cytoplasmand,cytoplasmic membrane rupture [27] and inactivation or inhibition of intracellular and extra-cellular enzymes activity and being disintegrated into cell wall [28].

Our results are consistent with findings of other researchers [21-23, 26-29] so that most plant extracts have inhibition effect on Gram positive bacteria and little effect on Gram negative bacteria. This inhibition effect can be related to its active compounds that include: steroids and terpenoids, alkaloids, citral, geraniol, flavonoids, eugenol, cytronolal, geranyl acetate, beta cariofiln, tannins, phenolic compounds, saponin and farnsul [15-21].

Several investigators studied the antimicrobial activity of essential oil of lemon grass plant against pathogenic bacterial strains and found that *Enterococcus fecalis* was the most sensitive microorganism, while *Pseudomonas aeruginosa* was most resistant [29-31]. A study by Jeongand his colleagues [32] conducted on lemon grass plant has concluded that essential oil prepared from *Cymbopogon citrates* maybe a safe alternative environment inhibition of antimicrobial agentsfor various uses.It seems that generally antimicrobial properties of methanol extracts can be attributed to the presence of secondary metabolitesespecially flavonoids in first degree, in the second degree terpenes and in the third degree saponins [33]. Results of this study showed that most antibacterial effect of lemon grass and essence was against Gram positive bacteria. According to the considerable antibacterial effect of methanol extract of lemon grass leaves on pathogenic bacteria especially Gram positive bacteriathat are involved in creating variety of nosocomial and malicious infections this extract can be considered as a natural antibacterial herbal product.

ACKNOWLEDGMENT

The authors would like to acknowledge the people who assisted in this. The authors would liketo also acknowledge funding support fromYoung Researchers Club, Ahar Branch, Islamic Azad University, Ahar, Iran for research with title:Effects of Antibacterial Activities of Methanol extract and cymbopogon Essence on pathogenic bacteria.

REFERENCES

1. Mitscher, L.A., S. Drake, S.R. Goliapudi and S.K. Okwute, 1981. A modern look at folkloric use of anti- infective agents. Journal of Natural Products, 50: 1025-1040.
2. Deans, S.G. and K.P. Suboda, 1990. Biotecnology and Bioactivity of culinary and medicinal plants. Ag Biotech News and Information, 2: 211-216.
3. WHO (World Health organization), 1979. The selection of essential drugs. Second report of the WHO Expert Committee. WHO Technical Report Series, 641: 1-44.
4. Cotton, C.M., 1996. Ethnobotay principles and application. Wiley, Chi Chester, UK, pp: 119-115.
5. Oussalah, M., S. Caillet, L. Saucier and M. Lacroix, 2007. Inhibitory effects of selected plant Essential oils on the growth of four pathogenic bacteria: *E. coli* O_{157}: H_7 salmonella typhimurium, staphylococcus aureus and Listeria monocytogenes. Food Control, 18: 414-420.
6. Eloff, J.N., 1999. It is possible to use herbarium sepecimens to screen for anti bacterial components in some plants. Journal of Ethno- Pharmacology, 67(3): 355-60.
7. Khafagi, I.K. and A. Deweder, 2000. The efficitncy of random rersus ethno- directed research in the evalution of Sinai medicinal plants for bioactive compounds. Journal of Ethno-Pharmacology, 71: 365-376.
8. Khatibi, A., A.H. Shah, M.S. Ahmad, M.A. Yahya and M. Tariq, 1989. Saudi Folk Medicine Phytochemical and Antimicrobial Sciences, 2: 29-34.
9. Navarro, V., M.L. Villarreal, G. Rojas and X. Lozoya, 1996. Antimicrobial evalution of some plants used of infectionalas disease. Journal of Ethnopharmacology, 53: 143-147.
10. Rao, K.S., 1996. Antibaterial activity of some medicinal plants of Papua New Guinea. Internation of Journal of Pharmacognosy, 34: 223-225.
11. Naqvi, S.A.H., M.S.Y. Khan and S.B. Vohora, 1991. Antibacterial antifungal and anthelminitic investigations on Indian Medicinal Plants. Fitoterapia, 62: 221-228.
12. Vlietinck, A.J., L. Van- Hoof, J. Totte, A. Lasure, D.V. Berghe, P.C. Rwangabo and J. Mvukiyumwami, 1995. Screening of hundred Rwandese medicinal plants for Antimicrobial and Antiviral properties. Journal of Ethnopharmacology, 46: 31-42.
13. Alkofahi, A., R. Batshoun, W. Owais and N. Najib, 1997. Biological activity of some Jordanian medicinal plant extracts. Part II. Fitoterapia, 68: 163-168.
14. Nick, A., T. Rli and O. Sticher, 1995. Biological screening of traditional Medicinal plants from Papua New Guinea. Journal of Ethnopharmacology, 53: 143-147.
15. Clayton, W.D., 1968. Gramineae. In: Flora of West Africa: Tropical Africa, 3: 349-512.
16. Esmort, H.C., R.H. David and I.F. Dudley, 1998. Volatile constituents of the essential oil cymbopogon citratus stapf grown in zambia. Flavour and Fragrance, 13: 29-30.
17. Santin, M.R., A.O. Santos, C.V. Nakamura, I.V. Ferrira and T. Ueda- Nakamura, 2009. *In vitro* activity of the essential oil of cymbopogon citratus and its major component (citral) on Leishmania Amazonensis. Parasitol, 5: 1489-1496.
18. Robbing, S.R.J., 1983. Selected Markets for the Exxential oils of lemon grass, citronella and Eucalyptus. Tropical Products Report, pp: 13.
19. Adeleke, A., O. Kasali Adebola and O. Oyedeji, 2001. Volatile leaf oil constituents of cymbopogon citratus stapf. Flavour and Fragance, 16: 377-8.
20. Tizianna Baratta, M. and R. Giuseppe, 1998. Antimicrobial and antioxidant properties of some commercial essential oils. Flavour and Fragrance, 13: 235-44.
21. Hindumathy, C.K., 2011. *Invitro* study of Antibacterial Activity of cymbopogon citratus. World Academy of science, Engineering and Technology, 14: 193-197.
22. Ojo, O. and I. Anibijuwon, 2010. Studies on extracts of three Medicinal plants of south-western Nigeria: Hoslundia opposite, lantana Camara and cymbopogon citratus. Advances in Natural and Applied Sciences, 4: 93-98.
23. Mothana, R.A., A.J. AI-Rehaily and W. Schultze, 2010. Chemical analysis and biological activity of the essential oils of two endemic soqotri Commiphora species. Molecules, 15: 689-698.

24. Vadlapudi, V. and K. Chandrasekhar Naidu, 2009. *In vitro* bioefficiency of marine mangrove plant activityof Rhizophora conjugate. International Journal of PharmTech Research, 1: 1598-1600.
25. Boyanova, L., G. Gergova, R. Nikolov, S. Derejian, E. Lazarova, N. Katsarov, I. Mitov and Z. Krastev, 2005. Activity of Bulgarian propolis against 94 Helicobacter pylori strains *in vitro* by agar-well diffusion, agar dilution and disc diffusion methods. Journal of Medical Biology, 5: 481-483.
26. Duraipandiyan, V., M. Ayyanar and S. Igancimuthu, 2006. Antimicrobial activity of some Ethno,edicinal plants used by paliyar Tribe from Tamil Nadu, India. BMC Complement Altern Med., 6: 35.
27. Caccioni, D.L.R., D.M. Guzzardi, A. Renda and G. Roberto, 2000. Relationships between volatile components of citrus Fruit Essential oil and Antimicrobial action on pencillium Digitatum and penicillium Italicum. Inter Journal of Food Microbial, 88: 770-75.
28. Kraft, K. and C. Hobbs, 2004. Poket Guide to Herbal Medicine. New York: Thieme Stuttgart, pp: 61-62.
29. Bassole, I.H.N., A. Lamien - Meda, B. Bayala and L.C. Obame, 0000. Antimicrobial activityof cymbopogon citratus and cymbopogon giganteus essential oils alone and in combination. Phytomedicine, 18: 1070-1074.
30. Yazdani, D., S.H. Rezazadeh and N. Shahabi, 2003. Identify and introduce the components of the volatile oil of lemon grass plants grown in northern Iran. Journal of Medicinal Herbs, 9: 69-80.
31. Olivero-Verbal, J., L.S. Nerio and E.E. Stashrnko, 2010. Bioactivity against tribolium castaneum herbst (coleopteran: Tenebrionidae) of cymbopogon citratus and Eucalyptus citrodora essential oils grown in colombia. Pest Managa, 66: 664-668.
32. Jeong, M.R., P.B. Park, D.H. Kim, H.S. Jeong and S.H. Choi, 2009. Essential oil prepares from cymbopogon citratus exerted an Antimicrobial activity against plant pathogenic and Medical Microorgenisms. Mycobiology, 37: 48-52.
33. Eleyinmi, A.F., 2007. Chemical composition and Antibacterial and Antibacterial of Gongronema Latifolium. J. Zhejiany Univ. Sci. B., 8: 352-358.