

Effects of Antibacterial Activities Methanol Ofextract and Lemon Grass Essence on Pathogenic Bacteria

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Abstract: *Background:* Including common problems in the medical world, the issue is resistance of bacteria antibiotics, so finding new antimicrobial compounds with minimal side effects is necessary. According to presence of biological active compounds in plant lemon grass and use of this plant that was made in traditional medicine, it seems that this plant contain capacity considerable antibacterial. *Objective:* The purpose of this study is investigation antibacterial methanol extract and essential oil of lemon grass on the four reference strains of *Staphylococcus aureus* (ATCC:25923), *Bacillus cereus* (ATCC:1247), *Escherichia coli* (ATCC:25922), *Pseudomonas aeruginosa* (ATCC: 27853). *Methods:* This study was designed in vitro and antimicrobial effects of methanolic extract lemon grass after extraction were examined with soxhlet method on 4 bacteria, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*. 20 mg/ml, 30 mg/ml, 50mg/ml and 400 mg/ml concentrations was prepared of methanol extract by the solvent DMSO. Then their antimicrobial effects were assessed with Agar Well Diffusion and dilution test method. Analysis obtained data was done using ANOVA test and chi-square in $p < 0/01$ level. *Results:* Findings showed that methanol extract of lemon grass plant prevent of bacterial growth of *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* which with increasing concentration, their antibacterial effect also increases, addition 1000 mg/ml concentration leaves of this plant essential oil indicated inhibitory effect on *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*. In this study no inhibitory effect of growth on *Pseudomonas aeruginosa* bacteria were observed. *Conclusion:* Despite the effects of concentrations of methanol extracts and essential oil this plant on the growth of pathogenic bacteria especially kind of gram-positive, to introduce it as an alternative to chemical antimicrobial drugs, is required wider investigation.

Key words: Cymbopogon citratus • Plant extract • Antibacterial activity

INTRODUCTION

Plants is still as a potential source of medical compounds. In the world plants traditionally used to treat many disease especially infectious disease including diarrhea, fever, cold and also to control the birth rate is used in oral health [1] in addition, many recreational compounds used in traditional medicine have plant root [2] according to definition World Health Organization (WHO) medicinal plant, is plant that can use it order to therapeutic purposes and or its compounds be used as a pioneer in the synthesis semi-synthetic chemical drugs [3] there is 5 methods for choosing plants to investigate the pharmacological effects which include: random approach

that is included plants collection in the study area. Phytochemical targeting that is included all plant family members that their richness of biological active compounds have been proven that sampling method Ethno-directed is based on uses of traditional medicinal of one plant. Chemotaxonomic approach specific plant parts that is based on collection specific plant part such as seeds [4]. With increasing number of bacterial strains resistant to various antibiotics, many attempts to use the antimicrobial potential of plants has been done. On the other hand emergence of resistant strains among negative Bacillus and gram positive cocci such as genus *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Staphylococcus* and *Enterococcus* has caused problems in treating

infections caused by these bacteria [5]. Antimicrobial compounds obtained from plants with different mechanisms of antibiotics removed bacteria that this issue in treating infections caused by resistant microbial strains is of clinical importance [6]. According to again approach for the use drugs and herbal products study properties medicinal endemic plants to each area is important. Many studies on extracts prepared from collected plants randomly or one of the above methods has been done. These studies further has focused on evaluate the antimicrobial effects [7-10] antihelminthic [11], anti-viral [12], cytotoxic and mutagenicity effects [13] and also general pharmacological effects [14]. In this study the antibacterial properties of methanol extracts and essential oils of lemon grass plant has been studied on a number of plant pathogenic bacteria. Lemon grass plant with scientific name *Cymbopogon Citratus* is belongs to the Graminae (poaceae) family that contains about 660 genus and 9000 species, which is widely distributed in tropical and subtropical world. Lemon grass is native to India and Sri Lanka [15-16].

Cymbopogon Citratus 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 due to the high rate sytral (70-80%) in its essence in some Asian countries and African cultures widely [18]. This plant is an important source provideessential oils used in food industry and hygienic as flavor and aromatic and so in pharmaceutical industry is also considered [19]. Lemon grass essential oil to completey clear be controlled bacterial growth and fungi pollutants food such as *Staphulococcus aureus* and *Escherichia coli*, also antioxidant activity of plant essential oil proven, so that lemon grass essential oil in comparison with alpha-tocopherol has stronger antioxidant activity and acts BHT equivalent (ButylatedHydroxy Toluene), also with plant contains properties such as powerful antibacterial, appetizer, anti worm, carminative and its use topical reduce rheumatic pain and neurological [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 oil *CymbopogonCitratus*, its antimicrobial properties existence compounds such as citronel, geraniolacetatand is attributed its other compounds [21-22]. According to the presence of various phytochemical with potential antibacterial significant in lemon grass plant is needed experimental studies in order to determine the quality and range of material effect on pathogenic microorganisms is performed.

MATERIALS AND METHODS

Fresh leaves of plant collected from Jiroft, Dezful and MasjedSoleiman endemic area of south and west of the country and samples was approved of botanical botany part of Islamic Azad Unevirsiy of Ahar. To essential oil obtained from hydrodistillation using and clevenger apparatus was used. So that 300gr of powdered dried leaves with one liter distilled water was heated in clevenger apparatus. Essential oil collected after removing moisture kept by sodium sulfate in opaque glass and sealed at 2°C away from light. For prepare methanol extract 60gr of dried plant powder with methanol as a solvent for 8 hours in a soxhletextractant apparatus was placed, this solvent at 40°C temperature and with using rotary apparatus gently evaporation and it concentrated extract was obtained.of concentrated extract by 5% solvent Dimethyl-sulfoxide with 20, 30, 50 and 400 mg/ml concentrations and were prepared for use in determine test minimum inhibitory concentration and disc diffusion. Microorganisms tested included *Bacillus cereus* (ATCC:1247), *Staphylococcus aureus* (ATCC:25923), *Pseudomonas aeruginosa* (ATCC:27853), *Escherichia coli* (ATCC:25922) were prepared as Lyophilized from Tehran university of microbial collections. Microbial samples were reduced according to standard methodes, since the number of inoculated bacteria is one of the most important variables that affect the results of this study, inoculated microbial suspension density should be dtandard, thus for prepare microbial suspension of new culture bacteri 4-5 colony transferred to the Muller hinton broth medium until turbidity microbial suspensions prepared according to tube 0/5 McFarland standard (equivalent turbidity $1/5 \times 10^8$ bacteri in per ml) should be regulated. To achieve the $1/5 \times 10^6$ concentration, bacteria in per ml microbial suspension with a turbidity equal tube 0/5 McFarland to rate 0/01 was diluted, to determine the antimicrobial effects of methanol extracts of 4 concentration 20, 30, 50 and 400 mg/ml from methanolic extract herb was prepared in a solvent of 5% DMSO. In this study the antimicrobial effect of methanol extract were investigated with two methods agar well diffusion and dilution. In well diffusion method 500 μ L of $1/5 \times 10^6$ cfu/ml microbial suspension was transferred onto Muller hinton agar medium and in 3 directions were cultured by sterile swabs. Then wells to 6 mm diameter and to 2/5 cm distance was created from each other at agar surface, incontinous 100 μ L of 20, 30, 50 and 400 mg/ml concentrations of methanol extract was injected into the wells. Negative control using solution that for solving were used extracts (5% DMSO) were obtained and of the antibiotic chloramphenicol was used as positive

control then plates were incubated at 37°C for 24 hours and after a certain period of microbial cultures of formation or lack of inhibition zone was measured in milimeters. By using tube dilution method, the minimum growth inhibitory concentration and the minimum bacterial concentration of methanol extract was determined. In this method to determine the MIC of prepared methanol extract 6/25, 12/5, 25, 50, 100 and 200 mg/ml dilution serials and was obtained in Muller hinton broth medium. Then, to each of dilutions was added active bacterial suspension $1/5 \times 10^6$ cfu/ml. besides tubes was used of positive control (medium containing bacteri free extracts) and negative control (culture medium without bacterai). Finally tubes were incubated at 37°C temperature for 24 hours. After incubation time, tubes evaluted of turbidity due to inoculated bacterial growth and last dilution which no turbidity was observed (lack growth) was considered as MIC. Then from all tubes in which was observed no bacterial growth, was sampled and by culture in plate were determined the minimum lethal bacterial concentration (MBC). Then plates were incubated at 37°C temperature for 24 hours. Tube containing the lowest concentration extract that in plate related to that lack of bacterial growth was visible as MBC that material was considered, to proven antimicrobial properties essential oils leaves were used in agar dilution method, so that 1000 µg/ml concentration essential oil in DMSO at Muller hinton agar medium was prepared. Plates were incubated at 30 minutes at 25°C temperature; microbial suspension was prepared concentration and were inoculated at its special place. Beside each of plates above from a plate as a control that only contains DMSO and culture medium without essential oil was used. Inoculated culture medium were placed at 37°C temperature for 24 hours and then were studied growth or lack of growth bacteria. To reduce experimental error each of the above experiments were repeated 4 times. To determine significant differences at obtained results was used ANOVA test and chi-square and differences between groups were determined at $p < 0/001$ significant level.

RESULTS

The results of the concentration effect methanolic extract lemon grass with well diffusion method is shown at table 1. Comparison between 20, 30, 50 and 400 mg/ml of methanol extract with well diffusion method on 4 bacteria strains of *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* showed that both *Staphylococcus aureus* and

Table 1: Diameter of inhibition zone in terms mm 4 bacteri strains of methanol extract in different concentrations

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

Table 2: The minimum inhibitory concentration and minimum bacterial concentrations of methanol extract lemon grass plant on tested bacteria in term of (mg/ml)

Bacteria strains	Extract concentrations (mg/ml)	
	MIC	MBC
<i>Staphylococcus aureus</i>	12/5	25
<i>Bacillus cereus</i>	6/25	12/5
<i>Escherichia Coli</i>	100	200
<i>Pseudomonasaeruginosa</i>	--	--

Bacillus cereus bacteria at the methanol extract had the highest microbial sensitivity and this inhibitory effect with increasing methanol extract concentration increased on these two bacteri that was observed as inhibition zone increase.

Also results obtained of lack of growth inhibition zone showed that growth inhibitory effect of methanol extract of lemon grass leaves on tested gram negative bacteria was very low, so that have not any growth inhibition effect on the *Pseudomonas aeruginosa*. 400 mg/ml concentration of leaves methanol extract showed slight inhibitory effect on *Escherichia coli*. Amounts related to the minimum inhibitory concentration (MIC) and bactericidal concentration (MBC) lemon grass leaf methanol extract against 4 tested strains are marked at table 2. The results show that 25 mg/ml concentration of methanolic extract of lemon grass plant, has a bacterial effect on *Staphylococcus aureus*. Lethal concentrations this extract against *Bacillus cereus* obtained 12/5 mg/ml. these results indicate that among tested bacteria in term of sensitivity methanolic extract of lemon grass there is a significant difference ($p < 0/001$). In the other words there is the highest sensitivity to methanolic extract lemon grass in *Bacillus cereus* and the least sensitivity is about *Pseudomonasaeruginosa*. Experiments related to the 1000 µg/ml concentration effect of leaf essential oil against the *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli* showed inhibitory effects and no inhibitory effect on the *Pseudomonas aeruginosa* bacteria and reduce growth were observed.

DISCUSSION

In recent years much research has been conducted in the field antimicrobial effects of different plants. In this study indicated that some plants have effects such as chemical drugs or more than them [23]. Lemon grass is also a traditional medicinal plant that has many uses in traditional medicine and is applied to treat stomach bloating, nerve and dislocation pains, fever, appetizer, laxative, anti-worm.... In this study determined that methanol extract of lemon grass plant in concentrations around 30 mg/ml prevents of the growth tested gram positive bacteria. While for effect on gram negative bacteria to higher concentrations it is necessary. Its essential oil also have a significant inhibitory effect on *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*. The results obtained of this study showed antibacterial properties methanol extract of lemon grass plant is against gram positive and gram negative bacteria. Growth inhibition effect this extract on gram positive bacteria begins concentration about 30 mg/ml, with increasing extract concentration until 400 mg/ml lack of growth area dramatically increase. Also the results show that the methanol extract effect against gram negative bacteria is much weaker than the gram positive. Such that in 400 mg/ml concentration of methanol extract leaves shows weak inhibitory effect against *Escherichia coli*. In none of the concentrations tested had no inhibition effect on *Pseudomonas aeruginosa* that its probable cause lipoprotein presence of cell wall polysaccharids probably prevented from reaching the active compounds essential oil and extracts to the cytoplasmic membrane of gram negative bacteria [24]. In general, herbal products leading to a granular cytoplasm, cytoplasmic membrane rupture [25]. Inactivation or inhibition of intracellular and extracellular enzymes activity and being disintegrated into cell wall [26] that these results are consistent with findings other researchers [21-27] so that most plant extracts had inhibition effect on gram positive bacteria and had little effect on gram negative bacteria that of this among *Pseudomonas aeruginosa* bacteria is the most resistant bacteria than most plant extracts. Hindumathy research also showed methanol extract lemon grass plant has inhibition effect on gram positive bacteria but has no effect on the *Pseudomonas aeruginosa* bacteria and this inhibition effect lemon grass plant knows related to its compound that include: steroids and terpenoids, alkaloids, citral, geraniol, flavonoids, eugenol, citronellal, geranyl acetate, beta cariofilin, tannins, phenolic compounds, saponin and farnesol [15-21] also Rezazade and his colleagues studies that done identifying and introducing compounds the

volatile oil of lemon grass plants grown in northern Iran confirmed these compounds [28] in studies conducted by Bassole and colleagues observed that lemon grass plant has a different chemical composition that granules (1/48%), neral (6/34%), myrcene (11%) are its major constituents and showed that this plant essential oil contain variable degrees of antimicrobial activity against various strains. *Enterococcus fecalis* was the most sensitive microorganism to its necessary oil, while *Pseudomonas aeruginosa* was most resistant [27-29]. Its Leaves are used to make tea which can relieve stomach and gut problems. In many countries it is used to treat feverish conditions and as a relaxant and sleeping aid. It helps with emotional states and it is an antidepressant agent. Cymbopogon citrates contains active ingredients like myrcene, an antibacterial and pain reliever, citronellal, citronellol and geraniol. A study by Sang hoonchoi and colleagues conducted on lemon grass plant have concluded that essential oil obtained from this plant contain antifungal effect and cause complete growth inhibition *Aspergillus* Nayjer and *Colletotrichum gloeosporioides* and showed that the essential oil prepared from Cymbopogon citrates maybe a safe alternative environment inhibition of antimicrobial agents for various uses [30]. According to reports said this plant is full of flavonoids, sesquiterpene and citral [21-27] also the best solvent for extraction flavonoids is methanol [24]. However, methanol extracts addition to flavonoids extracted alkaloids, saponins, tannins, anthraquinones and terpenes [27-31] it seems that generally antimicrobial properties of methanol extracts can be attributed the presence of secondary metabolites especially flavonoids in first degree, in the second degree terpenes and in the third degree saponins [32]. Mothana and his colleagues research is confirming flavonoid and terpenes compound extraction by methanol solvent and finally their antibacterial properties [31].

CONCLUSION

Results of this study showed that most antibacterial effect of lemon grass and essence is against gram positive bacteria, so that the active compounds in this extract on *Pseudomonas aeruginosa* that is qualify outer membrane with purines with very small pore have no growth inhibitory effect. According to considerable antibacterial effect of methanol extract of lemon grass leaves on pathogenic bacteria especially gram positive samples that are involved in creating variety of nosocomial and malicious infections this extract can be considered as a natural herbal products.

ACKNOWLEDGMENT

The authors would like to acknowledge the people who assisted in this. The authors would like to also acknowledge funding support from Young 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.

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