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Evaluation of antibacterial activity of *Lemon verbena (Lippiacitriodora)* leaves

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ABSTRACT

Medicinal plants are the oldest known source for treatment of disease. Using pharmaceutical plants and plant extracts have been at great attention. In this research, the antibacterial activity of the ethanolic and aqueous extracts of Lemon verbena Leaves cultured in research farm Islamic Azad University , Azadshahr Branch were tested against nine pathogen bacteria strains. Crude extracts were obtained by using ethanol and hot sterile distilled water as the extraction solvent. Four concentrations(1000 mg/ml, 500 mg/ml, 250 mg/ml and 125 mg/ml) were used to check the antibacterial activity of this plant .The antibacterial activity of ethanolic and aqueous extracts was determined by Disk diffusion and agar well diffusion methods. Results of this research indicated that ethanolic Extract despite of aqueous extract was showed considerable antibacterial effects . The most susceptible bacteria were E.feacalis ,S.epidermidis, S.aureus and B. cereus while the most resistant bacteria were gram negative bacteria for example Pseudomonas aeroginosa, Salmonella typhimorium and Shigella dysentriae. The largest zone of inhibition was against E. feacalis , S. epidermidisand S. aureus in agar well diffusion method 24,22,20 (mm) respectively while in disk diffusion methodlarsest zone of inhibition was S. epidermidis and S. aureus 15,12(mm) respectively. Yersinia entrocolitica was the most susceptible among gram negative bacteria. Generally Effect of ethanolic extract of lemon verbena leaves was more than theire aqueous extract and gram positive bacteria were more sensitive than gram negative bacteria.future studies are recommended to determine the Antibacterial effect of ethanolic extract of lemon verbena leaves on animal models.

Key words: Antibacterial activity, *Lemon verbena*, ethanolic and aqueous extracts, Disk diffusion method, Agar well diffusion methods

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INTRODUCTION

Plant derived products have been used for medicinal purposes during centuries. Herbs and Spices are generally considered safe and proved to be effective against certain ailments [1, 2]. History of herbal medicines in treatment of diseases return to the first years of human life which has developed and evolved over the centuries. According to World Health Organization is now about 80% of the world population use herbal medicine to treat. The statistics is under in developed countries and higher in less developed countries [2].

They are also extensively used, particularly, in many Asian, African and other countries. In the recent years, because of their beneficial effects, using of Spices and herbs has gradually increased in developed countries, also [1].

The scientific name of lemon herb *Lippia citriodora* (*Verbena citriodoraCav, Aloysia citriodora* Ort, (Verbenaceae) is a shrub . The plant originally native to South America and in countries like Peru, Argentina and Chile have been reported. Other countries, including European countries and Iran entered the plant and are grown and are used for benefit properties. In popular culture, leaves of the brewing plant as a sedative, anticonvulsant, diuretic and a resolver is used palpitations and dizziness. Today, the beauty and fragrance is pleasant and in warm temperate areas, such as areas of Mediterranean and Europe are grown. In gardens of Iran northern provinces are planted , but is not native there[2].

The lemon in the treatment of indigestion, bloating, headaches, unilateral, neuralgia, dizziness and cold symptoms are used. As well as spices are used in domestic applications[2].

Escherichia coli , Salmonella typhimurium , Shigella dysenteriae , Staphylococcus aureus , Yersinia entrocolitica , Staphylococcus epidermidis , Bacillus cereus , Pseudomonas aeroginosa and *Entrococcus faecalis* are the most important human pathogens and causing of considerable mortality and morbidity in human population.

This study was done in order to assessment of antibacterial activity aqueous and ethanolic extracts of *Lippia citriodora* leaves against nine species bacteria by disk diffusion and agar well diffusion methods.

MATERIALS AND METHODS

Collection of Plant Samples

Lemon verbena(*Lippia citriodora*) leaves were collected from research farm of Islamic Azad University , Azadshahr branch in north of Iran in March 2013 and were classified reference vouchers and deposited at the Herbarium of the Faculty of botany, in Islamic azad university of Gorgan branch .

Preparation of ethanolic extracts

Samples were dried at room temperature for 48 h. Collected plants were washed thoroughly and chopped into small pieces shade dried and grinded into powdered form. Clean and dry separating funnel was taken. Then 75 gm of ground material and 250 ml of 95% dehydrated ethanol was filled in the separating funnel and filtered using of standard filter paper for 48 hours. The filtrate was dried using of rotatory vacuum evaporator. Then, extract was heated at 72 °Cin a water bath shaker and the volume of the crude extract was reduced to 90% of its volume.[3,4,5] .All the extracts were kept in refrigerator prior to using. This extract was considered as the pure (1000mg/ml) concentration of the extract and Different dilutions of the extracts were made with appropriate volumes of dimethyl sulphoxide (DMSO).

Preparation of aqueous extracts

One hundred milliliter of hot sterile distilled water ,70-80ºC was added to the 30g powder plants which were allowed to soak for 24h in water bath at 45-50ºC .The extracts were sieved through a fine mesh cloth.[3] .This extracts was considered as the pure(1000mg/ml) concentration of the extract and Different dilutions of the extracts were made with appropriate volumes of sterile distilled water .

After preparation of extracts is evaluated antibacterial effect using by two Disk diffusion and agar well diffusion method.

Test Microorganisms and Preparation of standard culture inoculums of test organism

Nine species of bacteria used in the present study were obtained from Persian Type Culture Collection (PTCC).The species of bacteria were *Escherichia coli* (PTCC 1399) , *Salmonella typhimurium* (PTCC 1596) , *Shigella dysenteriae* (PTCC 1188) , *Bacillus cereus* (PTCC) , *Staphylococcus aureus* (PTCC 1436) , *Staphylococcus epidermidis* (PTCC 1435) , *Yersinia entrocolitica* (PTCC) , *Pseudomonas aeroginosa* (PTCC 1430) and *Entrococcus faecalis* (VanR 181).

A loopful of 24h surface growth on a MH agar slope of each species of bacteria was transferred individually to Nutrient broth after incubation at 37º C untile turbidity was adjusted to match that of a 0.5 McFarland standard (1.5 $\times 10^8$ CFU/ml).

Antibacterial activity assay using disk diffusion method

The antibacterial effects were tested by the disk diffusion method , briefly, Muller Hinton Agar plates were culture with a standardized inoculums (1.5 $\times 10^8$ CFU/ml equal to 0.5 McFarland) of each bacterial strains. Then the disks contain specific amount of extracts were carefully placed at the labeled seeded plate. The plates were incubated aerobically at 37º C for 24 hours. The diameter of inhibition zones were measured in mm and the results were recorded. Inhibition zone ≥ 12mm were considered as good inhibitory effect of extract[6,7]. Each experiment was done 3 times.

Antibacterial activity assay using Well method

Muller Hinton Agar plates were culture with a standardized inoculums (1.5 $\times 10^8$ CFU/ml equal to 0.5 McFarland) of each bacterial strains using a sterile cotton swab. Agar wells were prepared with the help of sterilized cork borer with 7mm diameter.Using a micropipette100 microliters of different concentration of spices extracts were added to different wells in the plate. The plates were incubated in an upright position at 37º C for 24 hours. The diameter of inhibition zones were measured in mm and the results were recorded. The inhibition zones with diameter ≥ 12mm were considered as having Antibacterial activity[4,8,9].

RESULTS AND DISCUSSION

The antibacterial activity of ethanolic and aqueous extracts *Lemon verbena(Lippia citriodora)* leaves were assayed against nine gram positive and negative bacteria by disk diffusion and agar well diffusion methods and the results of inhibition zones have shown in Table1 .

Results of this research indicated that ethanolic extract of this plant had inhibitory effect more than aqueous extract.

The most susceptible bacteria in agar well diffusion method were *E.faecalis* , *S.epidermidis*, *S.aureus* and *B.cereus* with diameter of inhibition zones 24,24,18,18 mm respectively, while the most resistant bacteria were *E.coli* , *Shigella dysenteriae,Pseudomonas aeroginosa* and *Salmonella thyphimorium*. The largest zone of inhibition obtained against *Entrococcus faecalis (24mm)* .The most susceptible gram negative bacteria was *Yersinia entrocolitica*.

Exceptionally *Yersinia entrocolitica* that showed zone of inhibition against extract of *lemon verbena* ,other gram negative bacteria i.e. *Pseudomonas aeroginosa*, *Escherichia coli, Shigella dysenteriae* and *Salmonella thyphimorium* were found to be resistant to this extract.

Gram-positive bacteria were more susceptible than gram-negative bacteria to this plant extract.

The greater susceptibility of Gram-positive bacteria has been previously reported for South American [10], African[11,12] and Australian [13]plant extracts.Susceptibility differences between gram-positive and gram-negative bacteria may be due to cell wall structural differences between these classes of bacteria. The Gram-negative bacterial cell wall outer membrane appears to act as abarrier to many substances including antibiotics [14].

Table1. Antibacterial activity of ethanolic extract of *Lippia citriodora* against nine species bacteria using disk diffusion and agar well diffusion method

		Well method				Disk method			
S.N.	ORGANISMS	1000mg/ml	500mg/ml	250mg/ml	125mg/ml	1000mg/ml	500mg/ml	250mg/ml	125mg/ml
1	*E.coli*	9*	0	0	0	0	0	0	0
2	*S.aureus*	18	16	14	13	11	8	0	0
3	*P.aeroginosa*	0	0	0	0	0	0	0	0
4	*E.feacalis*	24	22	21	20	0	0	0	0
5	*S.epidermidis*	22	21	19	17	15	12	10	8
6	*S.dysentry*	0	0	0	0	0	0	0	0
7	*S.thyphimorium*	0	0	0	0	0	0	0	0
8	*Y.entrocolitica*	14	12	0	0	0	0	0	0
9	*B.cereus*	18	15	13	11	10	8	0	0

**Zone Of Inhibition (mm)*

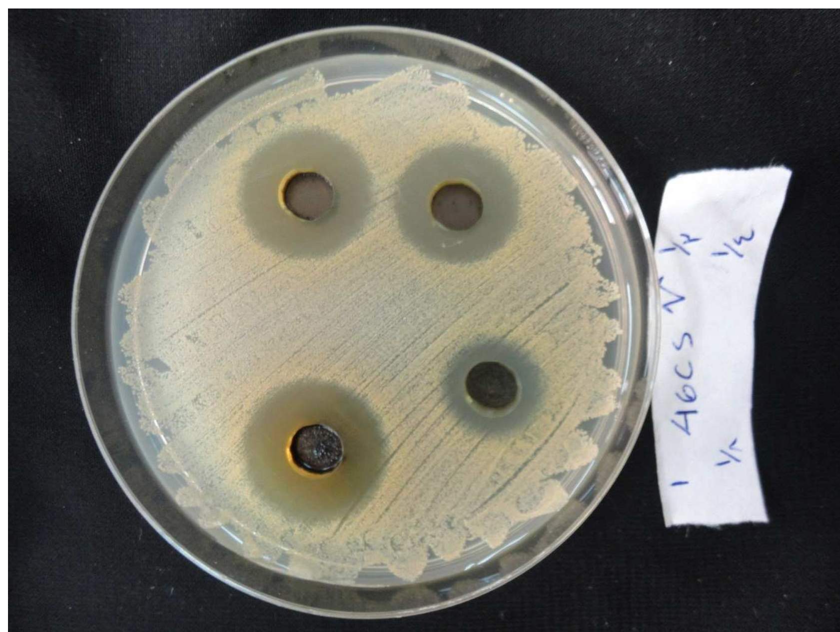

Fig1.Diameter of inhibition zones of *S.aureus* in presence of ethanolic extract *Lippia citriodora* in agar well diffusion method

CONCLUSION

The results of screening this plant extract for antibacterial activity was summarized in Table 1. in this study ethanolic extract of *Lemon verbena(Lippia citriodora)* leaves is more effective against gram positive bacteria than gram negative.Result showed that the ethanolic Extract of this plant was stronger than aqueous extract.

The results of this work suggest that ethanolic extract of *Lemon verbena(Lippia citriodora)* leaves have a broad spectrum of antibacterial activity , which can be used as an alternative for antibiotics. Therefore, pharmacological test is necessary to isolate and characterize its active compounds. Moreover, this plant ethanolic extract should be investigated *in vivo* to better understand its safety, efficacy and properties.

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