African Journal of Microbiology Research Vol. 6(1), pp. 16-19, 9 January, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMRX11.016
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Antibacterial activity of *Lippa citriodora* herb essence against *MRSA Staphylococcus aureaus*

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Accepted 22 November, 2011

The search continues for safe and effective antimicrobial agents with which to treat, therapeutically and prophylactically, a wide variety of bacterial infections. The aim of this study was to determine the effect of *Lippia citriodora* against *MRSA*. *Lippa Citrofora* were tested aginst *Staphylococcus aureus* by minimal inhibitory concentration (MIC) and disk diffusion methods. It was found that the percentage of growth inhibition increasing with increasing concentration steadily up to 55 µl/ml and IC50 value of this assay was 0.245. MIC for each bacterial sample showed only *MRSA* inhibited in concentration of 15 µl/ml of oil. The use of plants to heal diseases, including infectious one, has been extensively applied by people. Our results reveal the great potential of plants for therapeutic treatment, in spite of the fact that they have not been completely investigated.

Key words: *Lippia citriodora*, *Staphylococcus aureus,* minimal inhibitory concentration (MIC).

INTRODUCTION

The search continues for safe and effective antimicrobial agents with which to treat, therapeutically and prophylactically, a wide variety of bacterial infections. This need has been heightened recently by the emergence of many antimicrobial-resistant organisms (U.S. Congress., 1995; Ayliffe, 1997) and by the potential use of many hard-to-treat, life threatening micro-organisms as weapons of terrorism. The best therapeutic antimicrobial agents cause virtually no adverse reactions, have a wide spectrum of activity, and are not likely to encounter resistance to their therapeutic effects. A number of natural products, specifically some essential oils and certain fats (monoglycerides), could possess some of these ideal characteristics.

Staphylococcus aureus is a major cause of hospital infections, its prevalence is also increasing. These bacteria cause a wide range of diseases, including endocarditic, osteomyelitis, toxic shock syndrome, pneumonia, boils, abscess. In many cases, employees are the source of infection and they are the carrier of the bacteria in their nose which, that estimated about 25 to 30% of people in various communities in the nose are carriers of *Staphylococcus aureus*. The increasing spread of antibiotic resistant strains of *Staphylococcus aureus* and the emergence of antibiotic-resistant strains of bacteria in the daily number of antibiotics available to treat this infection are decreased. So that now most of these bacterial strains were resistant to penicillin and cephalosporins, and shortly after the release of methicillin-resistant penicillinase to penicillin-resistant strains were created. In 1961 the first strain of methicillin-resistant *Staphylococcus aureus* (methicillin-resistance *Staphylococcus aureus* = MRSA) was identified in Europe. Since then and especially during the last two decades, the prevalence of these strains has increased in many parts of the world. It is important to understand the importance of infections caused by MRSA and can even cause death in patients. Therefore, antibiotic resistance is a concern and should always be controlled. Enzyme beta-lactamase production was independent of resistance to methicillin and its prevalence varies in different countries and time (Merlino et al., 2002). History of herbal medicines in treating diseases 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

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world population use herbal medicine to treat the statistics in underdeve-loped countries and higher in less developed countries. The scientific name of lemon herb Lippia citriodora (Verbena citriodora Cav, Aloysia citriodora Ort, (Verbena triphylla L'Herit Shah of family-friendly (Verbenaceae) is a shrub (Figure 2). 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 date properties. In popular culture, plant health and medicine to Iran, leaves The brewing plant as a sedative, anticonvulsant, diuretic and a resolver is used palpitations and dizziness. Today, the beauty and fragrance is the most pleasant and warm temperate areas, such areas are grown in Mediterranean Europe. Iran also brought in examples of it and have planted gardens in the northern provinces of Iran, but not native. Formed the essential oil of the lemon leaves to 0.5% form. This essential oil is lighter than water; its color is yellow-green and lemon-like odor (Hejab et al., 1991). 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. Topical use of lemon essential oil may cause skin sensitization. Leaves and lemon oil to be away from light, in a cool place and kept in a closed container (Ranjbarian, 2002). The effect of four plant extracts to, cinnamon, caraway and fennel on Helicobacter pylori by Seddigh et al (2004) in the disk diffusion method and flow cytometry showed that the extract has the effect of four antimicrobial effects on H. pylori and Most of the antibiotics amoxicillin and ciprofloxacin and tetracycline is comparable (Seddighi et al., 2004). The aim of this study was to determine the effect of *lippa citriofora* against MRSA.

MATERIALS AND METHODS

Microbial culture growth conditions

S. aureus were derived from the stock cultures of the “research center microbiology laboratory” of the Ilam University of Medical Sciences.

Cultures and media

S. aureus were cultured in nutrient broth (heart-brain medium) at 37°C for 24 h. Broth cultures were diluted in sterile nutrient broth to a concentration of approximately 108 CFU ml^{-1}. Subsequent dilutions were made from the aforementioned suspension. Both spectrophotometer absorbance reading at 580 nm and plate counts using nutrient broth dilution blank and nutrient agar, were used to confirm the viable cell concentration. In subsequent trials, only spectrophotometer absorbance readings were used to estimate cell concentration. Diluted cultures were then used in the tests.

Selection of dilution solvent

Several solvents were tested for their antibacterial activity using the disk diffusion method on Mueller-Hinton medium. The solvent dimethylsulfoxide (DMSO) showing no antimicrobial activity was selected as a diluting medium for the oil. This solvent also served as control. Dilutions (1/2, 1/4, 1/8, and 1/16) of oils were made with DMSO. These dilutions were used in antibacterial analysis (7).

Antibacterial analysis

The fresh oil was tested for their antibacterial activities. The disk diffusion method was used for antibacterial screening as follows. Sterile Mueller-Hinton agar medium (Merck) was prepared and distributed into Petri plates of 90 mm diameter. This medium was used for antibiogram assays. The disk size used was 6 mm (Whatman No. 1) paper. Different dilutions of the oils were made with the DMSO. Microbial suspension was streaked over the surface of the Mueller-Hinton agar using a sterile cotton swab in order to get a uniform microbial growth on both control and test plates. Under aseptic conditions, the disks were placed on the agar plates and then 5 µl from each of the oil dilutions was put on the disks. A 5 µl dilution solvent (DMSO) was added to the disks on the control plates. The plates were refrigerated 2 h at 4°C and then incubated at 37°C for 24 h in order to get reliable microbial growth. Diameters of microbial inhibition zones were measured in mm.

Determination of the minimal inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) was determined by a broth dilution method in test tubes according to modified Gachkar et al. (2007). procedure (18). Tween 80 (0.5%) was added to the heart-brain nutriment broth and MIC was calculated as follows: 40 µl of each of various dilutions of the oils was added to 4.5 ml of nutrient broth containing 108 CFU ml-1 of live bacteria cell (0.5 ml of the bacterial suspension). After homogenization by vortexing, the tubes were incubated on an incubator shaker as to evenly disperse the oil throughout the broth in the tubes. The highest dilution (lowest concentration), showing no visible growth, was regarded as MIC (Jalali et al., 2006).

Toxicity assay: The cells were plated in 96- well flat bottom plates at 5000–1000 cells/well and were allowed to adhere to the wells overnight. Then the cells were treated with different concentrations of the oil (1-100 µl) with a maximal final ethanol concentration of 1%. After 24 h, MTT assay was used to monitor cell growth (Hansen et al., 1989). The absorbance of converted dye was measured at a wavelength of 570 nm with a background subtraction at 630 nm (Celike and Kavas, 2008).

RESULTS AND DISCUSSION

The findings of disk diffusion showed that the zone diameter around 5, 10, 15, 20 and 40 were 25, 30, 31, 44 and 44 mm, respectively.

The results showed the best effect of antimicrobial activity of oil against MRSA. It showed that the percentage of viability *Vero* cells line is 70 to 72% which are most suitable to perform cytotoxicity studies. The cytotoxicity study was carried out for essential oil .These oil was screened for its cytotoxicity against *Vero* cells line at different concentrations to determine the IC50 (50% growth inhibition) by MTT assay (Figure 1 and 2). It was found that the percentage of growth inhibition increasing

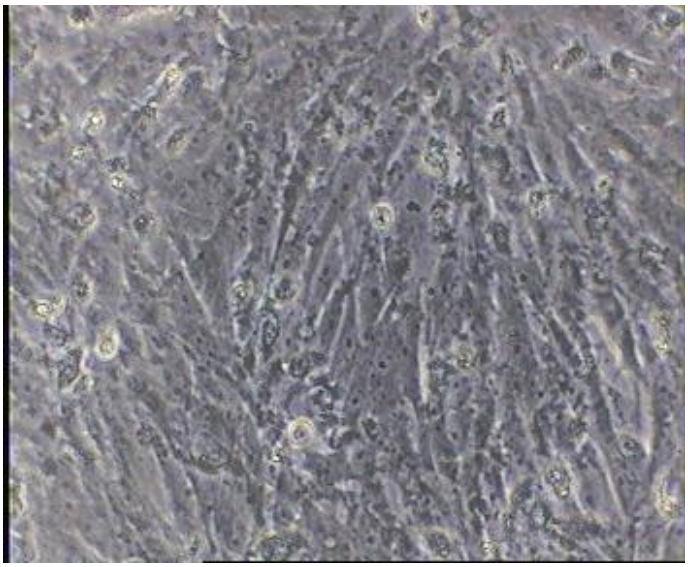

Figure 1. Confluent vero cell.

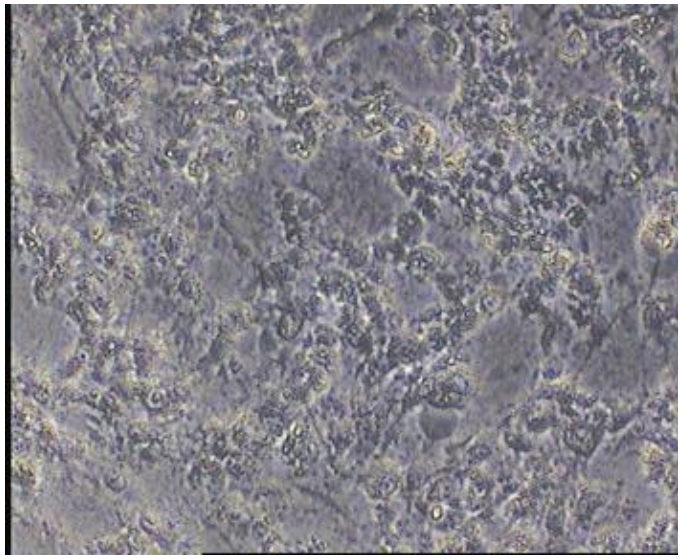

Figure 2. Cytotoxicity of *Lippia citriodora*.

with increasing concentration steadily up to 55 µl/ml and IC50 value of this assay was 0.245. Minimal inhibitory concentration (MIC) for each bacterial sample showed only MRSA inhibited in concentration of 15 µl/ml of oil. The use of plants to heal diseases, including infectious one, has been extensively applied by people. Our results reveal the great potential of plants for therapeutic treatment, in spite of the fact that they have not been completely investigated. Therefore, more studies need to be conducted to search for new compounds. Bioassays have demonstrated the toxicity of extracts from different plants. Therefore, our results revealed the importance of plant extracts when associated with antibiotics, to control resistant bacteria, which are becoming a threat to human health. The survey was conducted in accordance with the toxicity of the extract is highly toxic to humans and it has no side effects (Norajit, 2007). So with regard to Iran, mostly based on the use of traditional medicine and medicinal plants is based record high capacities in prevention and treatment of diseases and conditions Due to the increasing resistance of *Staphylococcus aureus* to antibiotics, especially the very good results and low dilutions of MRSA strain can be important. We conclude that the lemon essential oil has antimicrobial properties against bacteria tested in laboratory conditions and also has very low toxicity on cells, but to realize the antibacterial in its natural condition and the body needs to more studies. Thionines have toxic effects on different Gram-positive and Gram-negative pathogenic bacteria. Fabatins, which are recently, identified peptide molecules from fava beans appeared to inhibit the growth of *E. coli* and *P. aeruginosa* (Zhang and Lewis, 1997). The tea and petroleum extract of St John's wort (*Hypericum perforatum*) were proved to have antibacterial effect against methicillin resistant *Staphylococcus aureus* strains, which are among the most common and most problematic resistant strains to eradicate (Reichling et al., 2001). Abietane diterpenoids extracted from *Salvia sclarea* were shown to be bacteriostatic and bactericid for the cultures of *S. aureus* and *S. epidermidis* strains, regardless to their antibiotic susceptibility profile (Kuzma et al., 2007). The results of this study also showed different concentration of the Lippa Citrofora essential oil has been affected against MRSA.

Conclusion

In conclusion, the findings released the *Lippa Citrofora* essential oil is effectiveness against MRSA and more study needed to give the more comment for substitution of essential oil instead of current antibiotics.

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