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Antimicrobial and cytotoxic evaluation of some herbal essential oils in comparison with common antibiotics in bioassay condition

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ABSTRACT

Background: Since ancient times, various infectious diseases have been treated using herbal drugs. Today, efforts regarding the discovery of the effectual components of plants possessing antimicrobial properties are advanced. Herbal essential oils are widely used for treatment of various diseases, and they play an important role in health care considerations.
Methods: The antibacterial activity of *Artemisia kermanensis*, *Lavandula officinalis*, and *Zataria multiflora* Boiss essential oils against *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (PTCC 1310), and *Klebsiella pneumonia* (PTCC 1053) was evaluated using the disk diffusion method as well as determination of the minimal inhibitory concentration and minimal bactericidal concentration. The composition of the three essential oils was determined with gas chromatography-mass spectrometry. Variable amounts of different components (such as oxygenated monoterpenes, thymol, carvacrol, and 1,8-cineol) were found in all three oils. Among the tested bacteria, *S. aureus* was the most sensitive to the three essential oils.
Results: The obtained results showed that each of the three essential oils has an inhibitory effect on pathogenic strains. Of these three oils, *Z. multiflora* Boiss essential oil showed the highest inhibitory effect on microbial strains. Furthermore, comparison of the antibacterial effects of these three essential oils with ampicillin and tetracycline revealed that these antibiotics have a better effect in controlling pathogenic strains.
Conclusion: The essential oils used in the present study with different components showed antibacterial activity (especially *Z. multiflora* Boiss essential oil), and therefore they can be used as a new antibacterial substance.

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1. Introduction

In recent decades, increasingly drug-resistant bacteria have been a major concern. Drug resistance is common among pathogenic staphylococci. *Staphylococcus aureus* is a facultative anaerobic Gram-positive coccobacillus naturally found in parts of the skin and nasal cavity.[1] The inherent virulence of *S. aureus* and its ability to create a diverse array to life-threatening infections and capacity to adapt to different environmental conditions are the main concerns about this pathogen.[2] *Pseudomonas aeruginosa* is Gram-negative aerobic motile basil.[3,4] This bacterium is commonly found in most environments in hospitals. *P. aeruginosa* often exist in small numbers in the normal intestinal flora and on human skin. It becomes pathogenic only when introduced into areas without normal defenses such as the skin and the mucus layer.[3] This bacterium can express a variety of efficient efflux pump and antibiotic inactivating enzymes, so it can be resistant to antibiotics. *Klebsiella pneumonia* (which belongs to the Enterobacteriaceae family) is a nonmobile and encapsulated Gram-negative, facultative anaerobic bacillus, and is found in the normal flora of intestines.[3] This bacterium causes infections in hospitals and communities.[5] The majority of hospital infections caused by *K. pneumonia* are nosocomial pneumonia, urinary tract infections, diarrhea, and intra-abdominal infections.[6] *K. pneumonia* may be attributable to multidrug efflux systems.[7] The mounting concern about drug resistance has led researchers to focus more attention on natural products, including plants, with antimicrobial properties as the future source of antimicrobial agents.[8,9] For thousands of years, humans have been using natural products derived from plants for therapeutic purposes.[9] The World Health Organization reported that in 2008, more than 80% of the world population depended on traditional medicine for their primary health care needs.[10] *Artemisia* is a genus belonging to the Asteraceae family. Many members of this genus are important medicinal plants. *Artemisia kermanensis* is an endemic plant in Iran and important medicinal plant in the south of Kerman Province.[11,12] *Lavandula officinalis* (*L. angustifolia*) is an important species of the family Laminaceae, and is broadly distributed in the Mediterranean region.[13] In Iranian flora, lavender is chiefly distributed in the northern parts of the country. Lavender oil is known for its excellent aroma and is widely used in flavor, perfume, and cosmetic industries; it is also recommended for its anti-inflammatory and anti-infectious effects.[14] In Europe, lavender is used as an antispasmodic, carminative, and mild tranquilizer for digestive and mild nervous disorders. Lavender oil's antifungal and antibacterial activities oil have been reported.[14,15] Moreover, it has been found that lavender oil is active against many species of bacteria and fungi. For example, *L. angustifolia* oil was indicated to have *in vitro* antibacterial activity against methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococcus faecalis* at a concentration of < 1%.[16] *Zataria multiflora* Boiss, a member of the Labiatae family, is a native plant of Iran, Pakistan, and Afghanistan. It is traditionally used for anesthetic, antiseptic, and antispasmodic purposes. *Z. multiflora* has also been shown to have anti-inflammatory analgesic effects.[17,18] This plant is also used as a condiment and has many therapeutic applications in traditional folk medicine (Iranian Herbal Pharmacopoeia). In this study, we examined the antibacterial activity of *A. kermanensis*, *L. officinalis*, and *Z. multiflora* Boiss against three pathogenic bacteria (*P. aeruginosa*, *S. aureus*, and *K. pneumonia*).

2. Materials and methods

2.1. Origin and isolation of essential oils

Fresh *Z. multiflora*, *L. officinalis*, and *A. kermanensis* plants were gathered from Lorestan and Chaharmahal provinces in Iran (2012). Their scientific names were searched through the Herbarium part of Institution of Traditional Medicine in Iran (nos. 2359, 2360, and 2361, respectively). At first, the aerial parts of the herbs were kept at room temperature for 3 days, and after complete dryness was attained, the parts were powdered by mill. Making of essential oil was done with water using the essential making machine, Clevenger apparatus (model BP, Ashke Shisheh Co., Tehran, Iran & mantle model H610, Fater Electronic, Tehran, Iran) based on boiling point. For each batch, 100 g of the powder was placed in a 1-L balloon of Clevenger, and then water was added. After 5 hours of distillation, the essence—which was a yellow to green liquid with a good smell—was gathered.[19] The oils were dried over anhydrous Na_2SO_4 and stored at 4 °C in sealed amber vials until use.[20]

2.2. Gas chromatography-mass spectrometry

Analysis was carried out using a GC-mass chromatograph with an HP-5MS column (30 m × 0.25 mm, film thickness 0.25 m). Helium was used as the carrier gas at a flow rate of 0.8 mL/minute. The column temperature was kept at 50 °C for 2 minutes, and then it was programmed to 200 °C at a rate of 3 °C/minute and kept constant at 200 °C for 10 minutes. The injection was performed in split mode with ratio of 50:1 at 250 °C. The compounds were identified by comparison of the relative retention indices with those reported in the literature and also by comparison of their mass spectra with published mass spectra.[20,21] The retention indices for all the components were determined according to the Van Den Dool method[22] using *n*-alkanes as standards.

2.3. Antimicrobial activities assays

2.3.1. Preparation of bacterial cells

The bacterial species consisted of *S. aureus* (ATCC 25923), *P. aeruginosa* (PTCC 1310), and *K. pneumonia* (PTCC 1053), which were prepared at the Traditional Medicine Institute of Isfahan (Isfahan, Iran). First, the Muller-Hinton agar (MHA) medium was prepared and transferred in sterilized Petri dishes (5 cm thick). Under aseptic conditions, the samples of bacteria were taken from basal culture using an applicator and then inoculated in the medium.

2.3.2. Antibacterial assay

In order to evaluate the antimicrobial effect, the disk diffusion method (which is known as Kirby–Bauer and