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Evaluation of Antimicrobial Effects of Three Medicinal Plants in South of Iran against the *Staphylococcus Aureus*, *Pseudomonas Aeruginosa* and *Escherichia Coli.*

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Research Article

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ABSTRACT

Nowadays, natural treatment of bacterial diseases using substances which are herbal is of utmost importance. In the present study effects of three antibacterial medicinal plants in south of Iran against the separated Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli from Clinical samples and standard strains was investigated. Hydro-alcoholic extracts of Ziziphora tenuior, Origanum Vulgare and Mentha longifolia were prepared after being dried at shade. In the following the antibacterial activity of the extracts against separated Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli from clinical samples of the Shiraz hospitals and standard samples using disk agar diffusion method was investigated and the drug resistance in the studied samples was conducted using the standard Kirby-Bauer method. The growth of the all studied Staphylococcus aureus and Escherichia coli was stopped by hydro-alcoholic extracts of Mentha longifolia, Ziziphora tenuior and Origanum Vulgare but Pseudomonas aeruginosa showed growth zone of inhibition only against Origanum Vulgare. From statistical viewpoint Origanum Vulgare had also the most antimicrobial effect against Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli in the all studied samples ($P<0.05$). Extracts of Mentha longifolia, Origanum Vulgare and Ziziphora tenuior have a potent antibacterial effect against the gram-positive and gram-negative. But on the nosocomial pathogen, Pseudomonas aeruginosa, only Origanum Vulgare has growth inhibitory effect.

INTRODUCTION

The belief that some plants have the treatment capability goes back many years. Some of these natural products contain Substances that we commonly know them as antimicrobial agents and use them [1, 2].On the other hand, strength of bacteria to antibiotics is increasing every day which it causes to think more about replacing effective antimicrobial agents with fewer side effects instead of antimicrobial substance with less and unexpected side effects.

Ziziphora tenuior plants belong to the Lamiaceae family. This plant is a bush with height of 20 to 50 cm and has small leaves, cross, more or less lanceolate form and is sessile. It also has small flowers and perfect which are white, pink and purple. Treatment of digestive disorders such as diarrhea and nausea can be named as its medicinal properties [5]. Additionally Ziziphora tenuior has antibacterial effects [6, 7], antioxidant [8, 9], disinfection of intestine [5, 6, and 10], expectorant and anti-cold [8].

Origanum Vulgare is a plant of the Labiatae family and series of Plantae [11]. This plant is seen in large parts of Europe, especially in South of this continent, North of Africa and large parts of Asia can be seen as well. Also in Iran has been scattered in the most North and North West parts and in the southern warm regions cannot be found. Origanum Vulgare is used in the traditional medicine as an antiseptic, antispasmodic, carminative, anti-worms and is used to eliminate liver and gall bladder pain [13, 14, 15].

Marjoram containing one percent essence, major part of it constitutes of phenols, monoterpene hydrocarbons and alcohols. Marjoram essence generally contains 25 compounds such as 26.9% thymol, 40.7% carvacrol, 7.3 percent gamma terpinene [13, 14, 16].

Mentha longifolia is one of Labiatae family species, which includes 20 species which are distributed around the world. This medicinal plant is one of the Mint species that typically is known as Pennyroyal. The natural habitat of this plant is in Europe, North of Africa and Asia Minor and the Middle East [17]. Aerial parts including Mentha longifolia flowers normally are used as effective substance in treating colds, sinusitis, cholera, food poisoning, inflammation of the bronchi, and tuberculosis as well [18]. This medicinal plant also contains properties such as carminative, expectorant and diuretic, antitussive and anti-menstruation [19]. Antimicrobial medicinal plant powder and oil of Mentha longifolia has been determined [20].

Methicillin-resistant Staphylococcus aureus (MRSA) is the most common bacteria that spread via infection. This bacterium is found in the skin and other body parts, like nose of people. MRSA bacterium enters the body through a wound or cut. MRSA bacterium resists many antibiotics therefore it is very difficult to treat infections caused by the bacteria. MRSA is one of the most common infections that a person will suffer from during hospitalization [21,22]. Pseudomonas aeruginosa is hospital pathogens. This organism is entered to hospital via fruits, vegetables, visitors and patients who are transferred from other wards. Development and dissemination of the disease to other patients via hospital staff hands and direct contact of the patient with infected sources such as contaminated drinking water and food occurs as well [23, 24].

Increasing expansion of antibiotic-resistant to Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia species is one of the problems that medicals and veterinaries are dealing with todays. And due to the rise of antibiotic-resistant strains of the bacteria, the number of antibiotics available for treatment of these infections is reduced daily. Pseudomonas aeruginosa and Escherichia cause Sepsis, Wound infections, Gastroenteritis and Neonatal Meningitis in hospitals as well, respectively. Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia are opportunistic nosocomial pathogens [25, 26, 27].

Due to the irregular use of antibiotics in the region, the study was performed in order to investigate Antimicrobial effects of three medical plants in south of Iran against the Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia.

METHOD AND MATERIALS

Origanum Vulgare, Mentha longifolia and Ziziphora extraction:

Origanum Vulgare, Mentha longifolia and Ziziphora tenuior plants has been gathered nearby mountains of Shiraz and Kerman in the spring and dried shade outdoors. After drying and cleaning the plant, it has been powdered by electric mill, then per each 100 grams of powdered Origanum Vulgare 1000 CC hydro-alcoholic 50% was added and about 72 hours has been kept in percolator in the lab temperature. After 72 hours the faucet of percolator has been opened and droplets of extract has been gathered and by separator funnel droplets of hydro-alcoholic was added simultaneously until the obtained extract does not have the same color of plant. The obtained extract has been condensed and the dense extract for drying has been kept in Desiccator for 24 hours which per each 100 grams plants, 21 grams dried crystal extract obtained [24].

Method for determining the antimicrobial effect

In this study, 60 microbial diseases samples were taken from at Shiraz's hospitals. After diagnosis and separation Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia, determination of their drug resistance was performed by standard Kirby – Bauer method. Simultaneously standard strains

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Pseudomonas aeroginosa (PTCC 1430), Staphylococcus aureus (PTCC 1298) and E.coli (ATCC25922) were used.

In order to determine the antimicrobial effects of Origanum Vulgare, Mentha longifolia and Ziziphora tenuior plants, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia have been grew within 10-15 ml of medium Mueller Hinton Broth (MHB) in sterile condition and have been kept in Incubator at 35-37 centigrade degree for 24 hours till turbidity proportion to 0.5 Mcfarland pipe were obtained.

In the next step the obtained extracts have been injected into 6 mm sterile paper discs (Schleicher No 2668Germany) using 20 micrometer volume sampler under sterile condition [30]. By microbial suspension linear growth on the Mueller Hinton agar medium was provided and discs including medical plant extracts have been placed on the medium by sterilized pence and then prepared plates have been kept under 35-37 centigrade degree for 24 hours. After finishing the Incubation time, plates of plant extract discs and also antibiotic discs were brought out from incubator and the diameter of growth inhibition zone around the discs using a ruler were written down.

Statistical analysis of data

The obtained data using SPSS software and variation analysis were analyzed and to investigate the difference among groups, Tukey test was used with signification level $p<0.05$. The all results were presented by standard deviation.

RESULTS

The mean of the obtained results of Antibacterial effects investigation of Origanum Vulgare, Mentha longifolia and Ziziphora tenuior against separated bacterium from Clinical samples using Agar diffusion method by disc are in tables 1, 2 and 3.

Table 1: mean of growth inhibition zone diameter of separated Staphylococcus aureus samples from patients.

Antibiotics and studied plants	growth inhibition zone diameter	growth inhibition zone diameter (standard)
Penicillin	15	29
Ampicillin	20	29
Gentamicin	14	15
Erythromycin	18	23
Tetracycline	17	19
Chloramphenicol	16	19
Origanum Vulgare (10 micro l/disk)	21	-
Ziziphora tenuior (10 micro l/disk)	16	-
Mentha longifolia (10 micro l/disk)	14	-

Table 2: mean of growth inhibition zone diameter of separated Pseudomonas aeruginosa samples from patients

Antibiotics and studied plants	growth inhibition zone diameter	growth inhibition zone diameter (standard)
Penicillin	0	0
Ampicillin	0	0
Gentamicin	10	15
Erythromycin	0	0
Tetracycline	14	19
Chloramphenicol	15	18
Origanum Vulgare (10 micro l/disk)	10	-
Ziziphora tenuior (10 micro l/disk)	0	-
Mentha longifolia (10 micro l/disk)	0	-

Separated Staphylococcus aureus from clinical samples had antibiotic resistance to penicillin and ampicillin and Origanum Vulgare extract had the most effect ($p<0.05$). But separated Escherichia coli in addition to penicillin, ampicillin and Erythromycin, was fairly resistant to Ziziphora tenuior. For

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Pseudomonas aeruginosa also clinical samples had antibiotic resistance to penicillin, ampicillin, Erythromycin, Mentha longifolia and Ziziphora tenuior extracts and Origanum Vulgare extract had the most growth inhibition effect ($p<0.05$). The analyzed results of antimicrobial effects investigation of Origanum Vulgare, Mentha longifolia and Ziziphora tenuior plants against standard strains using Agar diffusion method by disc are in tables 4, 5 and 6.

Table 3: mean of growth inhibition zone diameter of separated Escherichia coli samples from patients

Antibiotics and studied plants	growth inhibition zone diameter	growth inhibition zone diameter (standard)
Penicillin	5	15
Ampicillin	3	17
Gentamicin	14	15
Erythromycin	9	23
Tetracycline	20	18
Chloramphenicol	21	19
Origanum Vulgare (10 micro l/disk)	20	-
Ziziphora tenuior (10 micro l/disk)	14	-
Mentha longifolia (10 micro l/disk)	3	-

Table 4: mean of growth inhibition zone diameter of Staphylococcus aureus samples standard strain

Antibiotics and studied plants	growth inhibition zone diameter	growth inhibition zone diameter (standard)
Penicillin	29	29
Ampicillin	29	29
Gentamicin	16	15
Erythromycin	21	23
Tetracycline	20	19
Chloramphenicol	20	19
Origanum Vulgare (10 micro l/disk)	32	-
Ziziphora tenuior (10 micro l/disk)	28	-
Mentha longifolia (10 micro l/disk)	27	-

Table 5: mean of growth inhibition zone diameter of Pseudomonas aeruginosa samples standard strain

Antibiotics and studied plants	growth inhibition zone diameter	growth inhibition zone diameter (standard)
Penicillin	0	0
Ampicillin	0	0
Gentamicin	15	15
Erythromycin	0	0
Tetracycline	20	19
Chloramphenicol	19	18
Origanum Vulgare (10 micro l/disk)	16	-
Ziziphora tenuior (10 micro l/disk)	0	-
Mentha longifolia (10 micro l/disk)	0	-

Table 6: mean of growth inhibition zone diameter of Escherichia coli samples standard strain

Antibiotics and studied plants	growth inhibition zone diameter	growth inhibition zone diameter (standard)
Penicillin	14	15
Ampicillin	17	17
Gentamicin	16	15
Erythromycin	22	23
Tetracycline	20	19
Chloramphenicol	18	18
Origanum Vulgare (10 micro l/disk)	25	-
Ziziphora tenuior (10 micro l/disk)	18	-
Mentha longifolia (10 micro l/disk)	15	-

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DISCUSSION

Variety of geographic and climatic conditions has resulted in existence of a diverse and powerful source of plant species in our country. Some of these plants have medical properties such as antibacterial activity [2]. In this study antimicrobial effect of three plants (Origanum Vulgare, Mentha longifolia, Ziziphora tenuior) was investigated in south of Iran. The results indicated that the investigated plants had the most growth inhibition effect on studied separated Staphylococcus aureus and Escherichia coli from clinical samples and standard strains respectively. But just Origanum Vulgare had appropriate growth inhibition properties on Pseudomonas aeruginosa. Quance's study in turkey showed the native Ziziphora tenuior of that region has effect on Gram-negative and Gram-positive bacteria but did not has effect on Pseudomonas aeruginosa [33]. The result of Salehi and et.al shows that Ziziphora tenuior is able to prevent from Gram-positive bacteria growth which is in agreement with our results. Mentha longifolia is a medical and eatable plant as well. In the previous studies it is indicated that Mentha longifolia has a strong antibacterial effect [35 and 36]. The methanol extract of Mentha longifolia has shown antibacterial and Anti-Fungal effects on a wide range of Gram-negative and Gram-positive bacteria and fungus as well [37]. There are typically Peritoneum (60-80%), Beta caryophyllene (5-15%), 1 and 8 Cineol (2-7%) and Hesperidin and quercetin Flavonoids in hydro-alcoholic Mentha longifolia extract [38].

In our study Mentha longifolia extract had antibacterial activity on Staphylococcus aureus and Escherichia coli but it did not have effect on Pseudomonas aeruginosa. According to the study of Taba tabaii nejad and et.al in 2002 and the study of Farahani and et.al on the extract and essence of Origanum Vulgare, it did not have significant antibacterial effect on Pseudomonas aeruginosa, is different [39,40]. The obtained results indicates that Origanum Vulgare had 10 mm growth inhibition zone diameter in Pseudomonas aeruginosa clinical samples and 16 mm growth inhibition zone diameter in standard Pseudomonas aeruginosa which this difference could depend on the place and the time of gathering the plant, soil of the region and the site of taking bacterial sample. Even though our results show that Origanum Vulgare has the most antibacterial effect on three studied bacteria (Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli). So it is proposed to be done more study on Origanum Vulgare, its antimicrobial effect combination, growing place and condition.

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