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Short Communication

Antimicrobial activity of *Otostegia persica* Boiss. extracts

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Abstract

Plants produce a diverse array of secondary metabolites, many of which have antimicrobial activity. *Otostegia persica* Bioss. (Labiatae) grows in south east of Iran. No previous investigation on antimicrobial effects of this plant has been reported. Thus, the aim of this work was to examine the antimicrobial effect of *O. persica* extracts with different polarity on several microorganisms. Aerial parts of *O. persica* were collected from Sistan-Baluchestan province (Iran). Powdered aerial parts of the plant were extracted with ethanol. The extract was concentrated under reduced pressure. The dried extract was extracted with hexane, followed by chloroform and methanol, respectively. Antimicrobial activities of three extracts were tested on several microorganisms using well plate method, MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) methods. *O. persica* extracts showed antimicrobial activity against Gram positive strains including *Listeria monocytogens, Enterococcus fecalis, Staphylococcus aureus*, and *Staphylococcus epidermidis* with MIC values from 0.62 to 20 mg/ml. The MBC values were identical, two, four or eight times higher than MIC values for the corresponding MIC for extracts. The Gram negative strains; *Escherichia coli, Pseudomonas aeruginosa, Salmonella* spp., *Klebsiella* spp., and *Proteus* spp. were not inhibited by *O. persica* polar, semi-polar, and non-polar extracts. It can be concluded that the tested extracts of *O. persica* exhibit significant antibacterial activity. This investigation supports the idea of using *O. persica* extracts as a candidate for further antimicrobial and phytochemical researches.

Key words: Antimicrobial activity, *Otostegia persica*, Plant extracts

INTRODUCTION

Plants are the oldest source of pharmacologically active compounds, and have provided humankind with many medically useful compounds for centuries (1). Screening of antimicrobial plants for new agents possesses an enormous challenge and is important especially with the emergence of drug resistant pathogenic strains.

Plants produce a diverse array of secondary metabolites, many of which have antimicrobial activity. Some of these compounds are constitutive, existing in healthy plants in their biologically active forms. Others such as cyanogenic glycosides and glucosinolates, occur as inactive precursors and are activated in response to tissue damage or pathogen attack (2).

Plant species of Labiatae are reputed for their medicinal uses. Due to their essential oils content, several species of this family show antimicrobial activity (3). It was reported that *Mentha* spp. exhibit antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Salmonella enteritidis* and *Staphylo-*

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coccus aureus (4, 5).

The genus *Otostegia* Bioss. (Labiatae) comprises 20 species, no previous antimicrobial investigation of this genus has been reported, and however there are reports on the anti insect activity of *O. integrifolia* (6). Methanolic extract of *Otostegia persica* has been exhibited strong antioxidant activity (7). Two compounds which were separated from methanolic extract by column and paper chromatography showed significant antioxidant activity. These active compounds were identified as morin and quercetin.

O. persica Bioss. grows in south of Iran in Fars province between Shiraz and Jahrum, also in south east region mainly in Sistaan and Baluchestan (8).

The aim of this work was to examine the effect of the methanolic, chloroform and hexane extracts of *O. persica* on several microorganisms.

MATERIALS AND METHODS

Microorganisms: *E. coli*, *L. monocytogen*, *E. faecalis*, S. *aureus*, *S. epidermidis*, *P. aeruginosa*, *B. subtiltis*, *Salmonella spp*. and *Klebsiella spp*. were obtained from Microbial Collection of Medical School of Isfahan University of Medical Sciences.

Plant materials

O. persica aerial parts were obtained form Research Center of Jihad -E- Agriculture Organization, Sistan province, Iran. Extraction was carried out by maceration of l00 g of powdered dry plant in 900 ml of 70% ethanol (Istelak, Iran) for 48 h at room temperature. Then the macerated plant material was extracted with 70% ethanol solvent using a percolator apparatus (2 liter volume) at room temperature. The extracts were filtered through Whatman filter paper (no. 4), dried under reduced pressure at 40 $^{\circ}$C using rotary evaporator.

The dried extracts of plant were then extracted with n-hexane (350 ml, Merck, Germany), followed by chloroform (350 ml, Merck, Germany) and methanol (350 ml, Merck, Germany), respectively. The concentration of extracts was determined by subtracting the weight of evaporated volume from the primary volume of the extracts.

Well – plate method

This method is based on the diffusion of antibacterial substance in agar. The extracts were weighed and dissolved in ethanol 10% to prepare extract solution of 100 mg/ml. Each microorganism was suspended in sterile saline and diluted at 1.5 x10^6 organisms per ml. They were 'flood-inoculated' onto the surface of Muller Hinton agar plates (Merck, Germany) and then were dried. Six mm diameter wells were cut from the agar using a sterile cork-borer, and 0.1 ml of the plant extract solutions were delivered into the wells. The cell cultures were refrigerated for an hour in order to make herbal extract spread. After incubation at 37 °C overnight, plates were examined for any zones of growth inhibition. Ethanol (10%) served as negative control. Each experiment was repeated five times and the mean of inhibition zones diameter was calculated.

Minimum inhibitory concentration test

The micro dilution minimum inhibitory concentration (MIC) provides a quantitative measurement of the lowest concentration of antimicrobial agent that inhibits the growth of a bacterium.

Serial dilutions of extract were prepared in 1.0 ml volumes of sterile tryptic soya broth (TSB, Oxoid, UK) to give a concentration range from 100 to 0.78 mg/ml. After preparation of suspensions of the tested microorganisms (1.5 × 10^6 organisms/ml), 1mm of suspension was added to the extract / broth dilutions. The first tube containing plant extract and the tenth tube containing TSB served as negative control and positive control, respectively. After 16-20 h incubation at

35 °C, the tubes were examined for growth. The MIC of extract was taken as the lowest concentration that showed no growth.

Minimum bactericidal concentration test

For minimum bactericidal concentration (MBC) test, aliquots (0.1 ml) of broth from test tubes containing no growth were plated on to Muler Hinton agar and again incubated overnight at 37 °C. The highest dilution in which no survivor existed was recorded as MBC.

RESULTS

The results of antimicrobial activity of methanolic, chloroform, and hexane extracts of *O. persica* on tested micro-organisms using well plate method, MIC and MBC methods are presented in tables 1, 2, 3, and 4, respectively. Methanolic extract was active against *S. aureus, S. epidermidis, E. fecalis,* and *L. monocytogenes*. Chloroform and hexane extracts were active against *S. aureus, S. epidermidis,* and *E. fecalis.* No activity was observed against five Gram-negative microorganisms (*E. coli, Klebsiella, Proteus, Salmonella* and *P. aeruginosa*) and *B. subtilis* as a Gram-positive microorganism.

DISCUSSION

The antimicrobial activities of *O. persica* methanolic, chloroform and hexane, extracts against microorganisms examined in the present study and their activity were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and zone diameter, MIC and MBC values.
At concentration of 100 mg/ml, methanolic extract was active against *S. aureus, S. epidermidis, E. fecalis, and L. monocyto-genes*. Methanolic extract, as a polar extract, exhibited antimicrobial activity at a range of 100 mg/ml to 3.12 mg/ml. Similarly, the methanolic extract of the aerial parts of *Satureja khuzistanica* (Labiatea) showed strong activity against *S. aureus, and S. epidermidis* (9). The results of methanolic extract indicate that *S. aureus* is the most sensitive tested microorganism, with the lowest MIC values (3.12 mg/ml) in the presence of the extracts. *E. fecalis, S. epidermidis* and *L. monocytogens* were other sensitive ones to the extracts with an MIC value at 6.25 mg/ml, 12.5 mg/ml and 12.5 mg/ml, respectively.

The rational for this effect may be based on the ability of polar extract permeability through the plasma membrane. It seems hydrophobicity is not the sole determinant factor for the active stability of a membrane structure. Many other molecular mechanisms other than hydrophobicity are involved such as protein flexibility (10), and solubility (11). There may be an electrostatic interaction between the positively charged groups of polar compound and negatively charged head groups of phospholipids, leading to localization of polar compound in the vicinity of the site of action (9). Preliminary phytochemical screening of *O. persica* has shown the presence of polyphenolic polar compounds such as flavonoids and tannins (12). Comparison of the tested micro-organisms' sensitivity to the methanolic extracts showed that *S. aureus* was the most sensitive, while *L. monocytogens* and *E. fecalis* were the most resistant micro-organism. Their MIC values were 3.12, 12.5 and 12.5 mg/ml, respectively. *L. monocytogens* and *E. fecalis* showed almost same sensitivity against the methanolic extracts.

The activity of methanolic extracts against *S. aureus*, a human pathogen, qualify the polar extracts for further investigation on their bioactive compounds.

Chloroform extracts exhibited antimicrobial activity at a range of 40 to 2.5 mg/ml. As indicated in table 1, chloroform extracts showed strong antibacterial activity against Gram-positive *S. aureus, S. epidermidis, and E. fecalis* and produced zone of

inhibition between 11 to 15 mm. The methanolic extracts showed comparatively same antibacterial activity on these bacteria. *Listeria monocytogens* was not susceptible to the effect of chloroform and hexane extract, however, methanolic extracts showed an inhibition zone about 12 mm against this bacteria and proved to be more active than chloroform and hexane extracts.

Comparison of tested microorganisms' sensitivity to chloroform extract showed that *E. fecalis* was the most sensitive while *S. epidermidis* was the most resistant. Their MIC values were 0.62 and 5 mg/ml, respectively.

As indicated in table 4, hexane extracts, exhibited antimicrobial activity against *S. aureus, S. epidermidis,* and *E. fecalis* at a range of 80 to 5 mg/ml. Comparison of tested micro organisms' sensitivity to hexane extracts showed that *E. fecalis* was the most resistant while *S. epidermidis* was the most sensitive. Their MIC values were 20 and 5 mg/ml, respectively.

As tables 2, 3 and 4 show, chloroform extract at the concentrations of 2.5 mg/ml is more active than methanolic and hexane extracts against *S. aureus.* Chloroform as a semi-polar solvent is able to extract polar and non-polar constituents of the plant. This property may explain its higher activity against certain bacteria. It was reported that, varying activities of different extracts could be attributed to the presence of several types of compounds belonging to different classes, such as oleoresins in hexane extract (13), sterols and their derivatives, flavones and flavonoids in semi-polar extract (14), and more polar phenolics in hydrophobic methanol extract (15). However, the presence of flavonoids for which the activity has been previously reported, should not be neglected, nor the possibility of synergistic or antagonistic interactions. It is interesting to note that Gram-positive bacteria, such as *B. subtilis,* was not sensitive to the tested extracts; such differences in sensitivity of bacteria have been noted earlier (16).

Results reported here contribute to the knowledge of antibacterial activities of Labiatea family plants, some of which, those of *Thymus* and *Stachys,* showed similar activities on *S. aureus, Entrococus* and *S. epidermidis* (17, 2). It is interesting to be noted that some Gram-positive bacteria were sensitive but not Gram-negatives. As emphasized elsewhere, Gram-positive bacteria are more sensitive to plant oil and extracts than Gram-negative bacteria (18, 19). Among all the bacterial strains tested, the Gram-negative strains; *E. coli, P. aeruginos, Salmonella* spp., *Klebsiella* spp., and *Proteus* spp. were not inhibited by *O. persica* polar, semi-polar, and non-polar extracts. Similarly, it was reported that some plant extracts were not inhibited Gram-negative strains (20). The reason for different sensitivity between Gram-positive and Gram-negative bacteria could be ascribed to the morphological differences between these microorganisms. Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da (21).

Table 1. Antimicrobial activity of *Otostegia persica* extracts against tested microorganisms using well plate method

Microrganisms	Growth Inhibition diameter (mm)		
	Methanol extract	Chloroform extract	Hexane extract
S. aureus	15.6 ± 0.8	15.4 ± 0.5	11.4 ± 0.5
E. fecalis	13.2 ± 1.5	13.4 ± 1.4	11.4 ± 1.5
S. epidermidis	12.0 ± 0.9	11.0 ± 0.0	10.2 ± 1.2
L. monocytogens	12.0 ± 1.0	6.0	6.0
E. coli	6.0	6.0	6.0
Klebsiella spp.	6.0	6.0	6.0
Proteus spp.	6.0	6.0	6.0
P. aeruginosa	6.0	6.0	6.0
Salmonella spp.	6.0	6.0	6.0
B. subtilis	6.0	6.0	6.0

The data are expressed as mean ± SD, (n = 5). The diameter of well plate was 6 mm.

The Gram-positive bacteria should be outer peptidoglycan layer which is not an effective permeable barrier (22).

As far as our literature survey could ascertain, there is no report on the antibacterial activity of *O. persica* various extracts prepared by using solvents of varying polarity. It can be concluded that the tested extracts of *O. persica* exhibit significant anti-bacterial activity against the tested organisms. This property may support the taxonomic relation of this plant to Labiatae family and can be considered as a component of broad spectrum anti-microbial agents.

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Table 2. MIC and MBC of *Otostegia persica* methanolic extracts against tested microorganisms

Concentration (mg/ml) Microorganism	Test	100	50	25	12.5	6.25	3.12	1.56	0.78	Negative control	Positive control
S. aureus	MIC	-	-	-	-	-	-	+	+	-	+
	MBC	-	-	-	+	+	+	+	+	-	+
S. epidermidis	MIC	-	-	-	-	-	+	+	+	-	+
	MBC	-	-	-	+	+	+	+	+	-	+
E. fecalis	MIC	-	-	-	-	+	+	+	+	-	+
	MBC	-	-	-	-	+	+	+	+	-	+
L. monocytogenus	MIC	-	-	-	-	+	+	+	+	-	+
	MBC	-	-	-	+	+	+	+	+	-	+

Table 3. MIC and MBC of *Otostegia persica* chloroform extracts against tested microorganisms

Concentration (mg/ml) Microorganism	Test	40	20	10	5	2.5	1.25	0.62	0.31	Negative control	Positive control
S. aureus	MIC	-	-	-	-	-	-	+	+	-	+
	MBC	-	-	-	-	-	-	+	+	-	+
S. epidermidis	MIC	-	-	-	-	+	+	+	+	-	+
	MBC	-	-	-	-	+	+	+	+	-	+
E. fecalis	MIC	-	-	-	-	-	-	-	+	-	+
	MBC	-	-	-	-	-	+	+	+	-	+

Table 4. MIC and MBC of *Otostegia persica* hexane extracts against tested microorganisms

Concentration (mg/ml) Microorganism	Test	80	40	20	10	5	2.5	1.25	0.62	Negative control	Positive control
S. aureus	MIC	-	-	-	-	+	+	+	+	-	+
	MBC	-	-	-	+	+	+	+	+	-	+
S. epidermidis	MIC	-	-	-	-	-	+	+	+	-	+
	MBC	-	-	-	-	+	+	+	+	-	+
E. fecalis	MIC	-	-	-	+	+	+	+	+	-	+
	MBC	-	-	-	+	+	+	+	+	-	+

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