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Antibacterial Effect of Ethanolic and Methanolic Extracts of *Plantago ovata* and *Oliveria decumbens* Endemic in Iran Against Some Pathogenic Bacteria

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Abstract: *Plantago ovata* (Plantaginaceae) and *Oliveria decumbens* (Umbliferae) are of important medicinal plants in Iran which have been used in traditional medicine. The aim of present study was to consider antibacterial properties of ethanolic and methanolic extracts of seed husk of *Plantago ovata* and aerial part of *Oliveria decumbens*. For this purpose, the 50 to 400 mg mL^{-1} concentration of these extracts were assayed against six Gram-negative and eight Gram-positive bacteria by disc diffusion method. Synthetic Antibiotic discs were used as control. *Staphylococcus epidermidis* and *Staphylococcus aureus* were the most sensitive species to the ethanolic and methanolic extracts of *Plantago ovata* while *Pseudomonas aeruginosa* was the most resistant to these extracts. Furthermore, *Escherichia coli* and *Proteus mirabilis* have shown resistance to ethanolic extract of this plant. Ethanolic extract of *Oliveria decumbens* was effective against all of tested bacteria and *S. aureus* was the most sensitive strain. In the case of the methanolic extract, *Salmonella typhi*, *P. aeruginosa* and *P. mirabilis* were more resistant than the others. The MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bactericidal Concentration) values for *Oliveria decumbens* extracts against *S. aureus* were same (20 mg mL^{-1}), whereas against *Streptococcus pyogenes* were different. Also, MIC for ethanolic extract of *Plantago Ovata* against *S. aureus* and *Bordetella bronchiseptica* were same (20 mg mL^{-1}), whereas for methanolic extract were 20 and 10 mg mL^{-1}, respectively. The MBC for these two bacteria weren't found (>200 mg mL^{-1}). On the basis of these results it can say that these plants have proper antibacterial effect and can be considered as a new source of antibiotic discovery and development for infectious disease treatment purposes.

Key words: Medicinal plants, *Plantago ovata*, *Oliveria decumbens*, *S. aureus*

INTRODUCTION

The medicinal plants have been used since ancient times. Evidence of using these natural resources (Herbal remedies) in Iran goes back to the history itself and there are lots of scientific documents in this area, Ibn-sina (Avicenna, 980-1037) has wrote many books on a wide range of topics but he is perhaps most famous for his Laws of Medicines which contains sections on the formulation of medicine, general medicine and other subjects that discuss the herbal medicines in details (Lothfipour *et al*., 2008). In recent years, antibiotic resistance has become a global concern and this problem is more important especially in developing countries because infectious diseases are still of important causes of morbidity and mortality among humans in these countries. Plants readily synthesize substances for their defense against insects, herbivores and microorganisms (Aboaba *et al*., 2006), also plants maybe produce secondary antimicrobial metabolites as part of their normal growth and development program or in response to stresses (Mirjana *et al*., 2004). *Plantago ovata* Forssk. (*Plantaginaceae*) and *Oliveria decumbens* Vent. (*Umbliferae*) are traditional medicinal plants which have been used in Behbahan (Khuzestan, South West of Iran). *Plantgo ovata* is a Winter annual plant that primarily inhabits desert regions of the Northern hemisphere between the twenty-sixth and thirty- sixth latitudes (Mayers and Liston, 2008). *Plantago ovata* is commonly referred to *Psyllium*, indeed, *Psylium* is the husk from the seed of *P. ovata*. Researches showed that this plant have hypocholesterolemic (Salas-Salvado *et al*., 2007; Anderson *et al*., 2000), anti-diarrhea (Washington *et al*., 1998), anti-diabetic (Hannan *et al*., 2006) and low anti-oxidant (Souri *et al*., 2008) effects. Also, it has been reported that *plantago ovata* powder causes allergic reaction when inhaled by occupationally exposed person (Bernedo *et al*., 2008). *Oliveria decumbens*, a shrub commonly found in South region of Iran, also has dispersed in South West of Anatolia, Syria and Iraq.

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Oliveria decumbens is a relatively less explored plant. The limited researches about therapeutic property of this plant are reported antibacterial (Mahboubi *et al*., 2008) and antifungal (Mahboubi and Feizabadi, 2008) activity of its essential oil, but about of its extracts there isn't any reports. The aim of this study was to screen antibacterial activity of ethanolic and methanolic extracts of *O. decumbens* aerial parts and *P. ovata* seed husk against some clinical pathogens.

MATERIALS AND METHODS

Plant collection and identification: The plants used in this study were collected from hills around Behbahan (south east of Khuzestan, Iran) in May, 2008. The taxonomic identification of these plants was done by Herbarium in faculty of agriculture, Shahid Chamran University.

Plant extraction preparation: The seeds of *P. ovata* and aerial parts of *O. decumbens* were shade dried at room temperature for ten days and then were ground to a fine powder. One gram of powder was extracted by using 10 mL of alcohol (ethanol-or-methanol)-distilled water solution (8:2 v/v), centrifugation (3000 rpm) for 15 min and collecting the supernatants. This process was repeated for three times. Solvents then removed by evaporation (Seyyednejad *et al*., 2001; Moazedi *et al*., 2007).

Bacterial strains: Fifteen bacterial species were used in this study. The Gram-positive species were *Bacillus anthracis*, *Bacillus Pumilus*, *Bacillus cereus*, *Bacillus licheniformis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Listeria monocytogenes*, *Streptococcus pyogenes and Corynebacterium renale*. But *S. pyogenes* and *C. renale* were used only for *O. decumbens* and *P. ovata*, respectively. Gram-negative species were *Pseudomonas aeruginosa, Bordetella bronchiseptica*, *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis and Klebsiella pneumoniae*. These species were originally isolated from clinical specimens and identified by standard biochemical reactions.

Antibacterial susceptibility testing: The isolates were grown in Muller Hinton Broth (MHB, Merck) medium at 37°C for 22 h. Final inoculum bacterial number were adjusted to 10^8 cfu mL^{-1} with reference to the Mc Farland turbidometry (Burt and Reinders, 2003; Zuraini *et al*., 2007). A lawn culture was prepared by pouring 0. 1 mL of bacterial suspension on Muller Hinton Agar (MHA Merck) and dispersed by a sterile cotton swab and allowed to remain in contact for 1 min. Four concentrations of ethanolic and methanolic extracts (50, 100, 200, 400 mg mL^{-1}) of both plants were prepared. The sterile filter paper discs (6 mm diameter) (Cermelli *et al*., 2008; Hsieh *et al*., 2001) were saturated through adding 50 μL of different concentrations of both extracts. Then, the discs were placed on lawn cultures. The Petri dishes were subsequently incubated at 37°C for 24 h and the inhibition zone around each disc was measured in mm. As positive controls, discs containing different concentrations of seven antibiotics including nafcillin 1 mcg, colistin 10 mcg, doxycycline 30 mcg, novobiocin 30 mcg, carbenicillin 100 mcg, methicillin 5 mcg and oxacillin 1 mcg were used. All these synthetic antibiotics were produced by Difco. Discs impregnated with 80% of ethanol and methanol was also included to test if they have inhibitory effect on the test bacteria in this study.

MIC and MBC determination: MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bactericidal Concentration) of ethanolic and methanolic extracts of these two plants were determined against two bacterial species (somewhat important and more sensitive bacteria) *S. aureus* and *Str. pyogenes* for *O. decumbens* and *S. aureus* and *B. bronchiseptica* for *P. ovata* were determined. MIC was determined by macro broth dilution assay method (Forbes *et al*., 1998). In the tube dilution assay, standard bacterial suspension was added to tubes containing 1 mL MHB (Muller Hinton Broth) and different concentrations of extracts (5, 10, 20, 40, 80, 160, 200 mg mL^{-1}). The tubes were incubated at 37°C for 24 h. The first tube in the above series with no sign of visible growth was reported as the MIC. MBC was determined by culturing one standard loop of the tubes showing no apparent growth on MHA and subsequent incubation at 37°C for 24 h. The least concentration that was inhibited no colony formation on agar assumed as MBC for these extracts.

RESULTS

The results indicate that ethanolic and methanolic extracts of these plants have antibacterial activities against both Gram-positive and Gram-negative bacteria. Table 1 show that ethanolic extract of *O. decumbens* was effective against all of the test bacteria even at one concentration. The methanolic extract of this plant have antibacterial activity against the majority of bacterial species, but *S. typhi*, *P. mirabilis* and *K. pneumoniae* were resistant. However, *S. aureus* and *B. bronchiseptica* were the most sensitive isolates to ethanolic and methanolic extracts, respectively. Furthermore, inhibitory effect of both extracts of *O. decumbens* against *B. cereus*

Table 1: Inhibition zones (mm)* of ethanolic and methanolic extracts of *Oliveria decumbens* against some pathogenic bacteria

	Different concentrations of extract (mg mL^{-1})							
	Ethanolic				Methanolic			
Bacterial species	50	100	200	400	50	100	200	400
Gram-positive bacteria								
B. pumilns	7	8	9	11	9	9	10	11
B. anthracis	8	9	10	13	9	11	12	16
B. licheniformis	R	7	11	13	R	R	8	11
B. cereus	R	R	R	9	R	R	R	8
S. aureus	12	14	17	19	11	13	16	18
S. epidermidis	11	13	14	16	10	11	13	15
Str. Pyogenes	11	12	13	16	9	10	12	14
L. monocytogenes	R	7	9	10	R	R	7	7
Gram-negative bacteria								
E. coli	7	7	9	10	R	7	8	8
S. typhi	R	7	9	10	R	R	R	R
P. mirabilis	7	7	7	9	R	R	R	R
B. bronchiseptica	10	12	14	16	12	15	18	21
K. pneumoiae	R	R	R	7	R	R	R	7
P. aeruginosa	R	R	8	9	R	R	R	7

*(6 mm) diameter disc. R: Resistant

Table 2: Inhibition zones (mm)* of ethanolic and methanolic extracts of *Plantago ovata* against some pathogenic bacteria

	Different concentrations of extract (mg mL^{-1})							
	Ethanolic				Methanolic			
Bacterial species	50	100	200	400	50	100	200	400
Gram-positive bacteria								
B. pumilns	7	8	9	11	9	9	10	11
B. anthracis	8	9	10	13	9	11	12	16
B. licheniformis	R	7	11	13	R	R	8	11
B. cereus	R	R	R	9	R	R	R	8
S. aureus	12	14	17	19	11	13	16	18
S. epidermidis	11	13	14	16	10	11	13	15
Str. pyogenes	11	12	13	16	9	10	12	14
L. monocytogenes	R	7	9	10	R	R	7	7
Gram-negative bacteria								
E. coli	7	7	9	10	R	7	8	8
S. typhi	R	7	9	10	R	R	R	R
P. mirabilis	7	7	7	9	R	R	R	R
B. bronchiseptica	10	12	14	16	12	15	18	21
K. pneumoiae	R	R	R	7	R	R	R	7
P. aeruginosa	R	R	8	9	R	R	R	7

*(6 mm) diameter disc. R: Resistant

observed only at 400 mg mL^{-1}. The antibacterial activities of both extracts from *O. decumbens* were decreased in lower concentrations. Table 2 revealed that the *S. epidermidis* and *S. aureus* were the most sensitive strains to ethanolic and methanolic extracts of *P. ovata*, respectively. *P. aeruginosa* was resistant to both extracts of this plant; also *E. coli*, *L. monocytogenes* and *P. mirabilis* were resistant to ethanolic extract of *P. ovata* even at highest concentration. Antibacterial activity of ethanolic and methanolic extracts of *P. ovata* against all of sensitive isolates was decreased at lower concentrations. Exceptionally, the effect of methanolic extract from this plant against *E. coli* was increased with dilution of the extract. Among tested Bacillus species,

Table 3: Inhibition zones (mm)* of antibiotic discs against some pathogenic bacteria

	Antibiotic discs						
Bacterial species	NF	CB	NB	DX	CL	MT	OX
Gram-positive bacteria							
B. pumilns	R	30	24	30	9	23	R
B. anthracis	R	28	20	32	R	23	R
B. licheniformis	R	32	38	36	R	16	R
B. cereus	R	7	18	18	R	R	R
S. aureus	R	13	31	15	R	R	R
S. epidermidis	R	36	29	21	R	R	R
L. monocytogenes	25	19	28	20	12	R	R
Str. pyogenes	-	-	-	-	-	-	-
C. renale	-	-	-	-	-	-	-
Gram-negative bacteria							
E. coli	R	R	17	11	R	R	R
S. typhi	R	27	34	30	R	R	R
P. mirabilis	R	15	14	R	R	R	R
B. bronchiseptica	R	R	24	31	27	40	R
K. pneumoiae	R	R	11	R	11	R	R
P. aeruginosa	R	R	16	R	15	R	R

*(6 mm) diameter disc, R: Resistant, -: Not used

Table 4: MIC and MBC of ethanolic and methanolic extracts of *O. decumbens* aerial part and of *P. ovata* seed husk against pathogenic bacteria

			MIC	MBC
Plant	Extract	Bacterial species	------(mg mL^{-1})-------	
O. decumbens	Methanolic	*S. aureus*	20	20
O. decumbens	Ethanolic	*S. aureus*	20	20
O. decumbens	Methanolic	*Str. pyogenes*	20	40
O. decumbens	Ethanolic	*Str. pyogenes*	10	20
P. ovata	Methanolic	*S. aureus*	20	>200
P. ovata	Ethanolic	*S. aureus*	20	>200
P. ovata	Methanolic	*B. bronchiseptica*	10	>200
P. ovata	Ethanolic	*B. bronchiseptica*	20	>200

B. anthracis and *B. licheniformis* had most sensitivity to *O. decumbens* and *P. ovata*, respectively. In addition, results showed that the extracts of studied plants had good anti-Staphylococcocal activity. The results of antibacterial activity of standard antibiotic discs are shown in Table 3. All of the tested bacteria were resistant to oxacillin and most of them presented resistance to colistin, nafcillin and methicillin. MIC and MBC results are shown in Table 4. MIC and MBC values for extracts of *O. decumbens* against *S. aureus* were 20 mg mL^{-1}. MICs for ethanolic and methanolic extracts of this plant against *S. pyogenes* were 10 and 20 mg mL^{-1} and MBC values were 20 and 40 mg mL^{-1}, respectively. About *P. ovata*, MIC values for both extracts against *S. aureus* were the same (20 mg mL^{-1}). The MICs for methanolic and ethanolic extract of this plant against *B. bronchiseptica* were 10 and 20 mg mL^{-1}, respectively. But MBCs for both extracts of *P. ovata* against these two bacteria weren't found (>200 mg mL^{-1}). Discs containing 80% ethanol and methanol did not have a zone of inhibition probably due to the volatile nature of alcohol, so it was not considered as a factor that might affect the results.

DISCUSSION

Nowadays, multiple drug resistant strains have been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used for infectious diseases treatment. Unfortunately, bacteria have the genetic ability to transmit and acquire resistance to drugs and chemicals (Nascimento *et al*., 2000). Beyond the increasing prevalence of antibiotic resistance among pathogenic bacteria, undesirable side effects of some synthetic antibiotics add urgency to the search for new infection-fighting strategies, as well. Scientists and pharmaceutical industries consider medicinal plants as a good choice, because these natural resources have ordinary fewer side effects, are costless and effective against broad spectrum of antibiotic resistant bacteria. In many parts of the world, the extracts of medicinal plants are used for their antibacterial, antifungal and antiviral properties (Hassawi and Kharma, 2006). Plant species used in folk medicineare are potential for discovering extracts with active biological compounds that have antibacterial activity. *O. decumbens* and *P. ovata* are among the most important plants extensively used in traditional medicine in South regions of Iran. Based on the results of the antibacterial studies, shown in Table 1 and 2, the extracts of both plants had antibacterial effect on both Gram-positive and Gram-negative bacteria. Also, ethanolic extract of *O. decumbens* and methanolic extract of *P. ovata* were the most effective extracts. Maximum activity of these extracts frequently observed at the 200 and 400 mg mL^{-1} concentrations so that at 400 mg mL^{-1}, ethanolic and methanolic extracts of *O. decumbens* had antibacterial activity against 100 and 86% of strains, respectively; while at this concentration ethanolic and methanolic extracts of *P. ovata* had inhibited 71 and 91% of isolates, respectively. Inhibitory activity of methanolic extract of *P. ovata* against *E. coli* was increased along side with decreasing concentrations; this may be due to the nature and number of available bioactive compounds in the extract, cell membrane permeability or other factors. In general, comparison of the inhibition zones diameter showed that extracts of both plants are more effective against Gram-positive than Gram-negative bacteria. This difference may be due to several possible reasons such as permeability barrier provided by presence of cell wall with multilayer structure in Gram-negative bacteria or the membrane accumulation mechanisms or presence of enzymes in periplasmic space which are able to break down foreign molecules introduced from outside (Parekh and Chanda, 2007; Abu-Shanab *et al*., 2004; Holetz *et al*., 2002). The diameter of inhibition zones around of the more active extracts in particular ethanolic and methanolic extracts of *O. decumbens* and *P. ovata*, respectively were comparable with standard antibiotics (Table 3). All of the Gram-positive and Gram-negative species were resistant to oxacillin; and all of the tested bacteria except *L. monocytogenes* were resistant to nafcillin. Furthermore, methicilin had no growth inhibition on 70% of tested isolates and more of the bacterial species were resistant to colistin. *S. aureus* was resistant to methicillin and some of the other antibiotics, while the extracts from both plants had noticeable activities against this bacterium. This suggest new hopes for searching and discovering effective antibiotic against MRSA (Methicillin Resistant *Staphylococcus Aureus*) strains which will be a serious problem in future and can cause fatal infections. The MIC and MBC (Table 4) for ethanolic and methanolic extracts of *O. decumbens* were 20 mg mL^{-1}, it is reported that for bactericidal antimicrobials the MIC and MBC are often near or aquiline values (Reuben *et al*., 2008) so we can say that extracts of *O. decumbens* have bactericidal effect on *S. aureus*. Extracts from *P. ovata* were unable to show bactericidal activity against *S. aureus* and *B. bronchiseptica* even at 200 mg mL^{-1} (MBC>200 mg mL^{-1}); this result may be caused by high bacteriostatic effect of *P. ovata* extracts, we can call these extracts as bacteriostatic agents which can inhibit bacterial growth but generally do not kill them (Forbes *et al*., 1998). Researches showed that the main components of the *O. decumbens* essential oil are thymol (22%), carvacrol (22%) and p-cymene (19%); also carvacrol is more effective against *S. aureus* than the others (Mahboubi *et al*., 2007). Phytochemical investigations of Plantago species revealed their high potential to produce a wide array of secondary bioactive metabolites, i.e., iridoids, phenols, polysaccharides, sterols, alkaloids and cumarines that have utilities as supplemented foods and as drugs to treat human diseases (Fons *et al*., 2008). Moreover, considering the extracts of *P. ovata* especially methanolic, it was more effective against Gram-positives while extracts of *O. decumbens* especially ethanolic extract was more effective against Gram-negative and Gram-positive bacteria, it can be concluded that perhaps these two plants have two different mechanisms and target locations and can be used to treat infection as synergism. *In vitro* assays may provide a guideline to select the highly active plant extracts for subsequent isolation and identification of potentially useful compounds, so isolation of bioactive constituents of active extracts from studied plants can be a subject for next researches. Also, further studies can evaluate *in vivo* efficacy of active extracts.

CONCLUSION

Finally, the extracts of both plants had antibacterial effect on both Gram-positive and Gram-negative bacteria. Particularly, the extracts of studied plants had good anti-staphylococcocal activity. Considering synergistic the effect of essential oil of *O. decumbens* with vancomycin against *S. aureus* (Mahboubi *et al*., 2007) and probable bactericidal activity of its extracts on this bacterium, it seems that study of *in vivo* efficacy of *O. decumbens* against *S. aureus* is important and suggested for further researches.

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