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In vitro assessment of antibacterial activity of *Salicornia herbacea* L. seed extracts against multidrug resistant gram-positive and gram-negative bacteria

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Abstract

In this study, the antibacterial activities of *Salicornia herbacea* L. seed extract against two gram-negative and two gram-positive bacteria were evaluated with the agar disc diffusion and MIC methods. Result showed that inhibition zones of 9.5±0.01, 6.2±0.00, 4±0.00 and 3.5±0.10 mm for *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli*, respectively. Among four bacteria the maximum and minimum inhibition seed ethanolic extract were related to *S. aureus* with inhibition zones of 9.5mm and MIC 189.5 mg/ml and *E. coli* with inhibition zones of 3.5 mm and MIC 420 mg/ml, respectively. The antimicrobial activity of ethanol seed extract of *S. herbacea* is the result of phenolic compounds, fatty acids, osmotic compound (betaine) or synergic and additive effect of several compounds present in it. Our results suggest the possibility of using *S. herbacea* seed, which possesses strong antibacterial activity, in the treatment of diseases caused by the microorganisms tested.

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Introduction

Many gram-positive such as *Staphylococcus aureus, Bacillus subtilis* and gram-negative bacteria such as *Pseudomonas aeruginosa* and *Escherichia coli* cause different diseases in humans (Alfatemi *et al*., 2014). In recently years, discovery of multidrug resistant in bacteria, the effectiveness of some usual antibacterial is unfortunately falling, but herbal medicines can suggest some alternatives (Miri *et al*., 2013).

The Salicornioideae are among the most salt-tolerant land plant and often occur in saline regions connected with coastlines, salt lakes and tidal floodways (Rhee *et al*., 2009). The Salicornioideae family includes nearly 15 genera and 80 species (Rhee *et al*., 2009). Salicornia species are native to North America and are widely distributed in Europe, South Africa, South Asia and in Central and South Iran (Shepherd *et al*., 2005). In a long time, Salicornioideae has been used as a folk medicine for treatment of nephropathy, hepatitis and diarrhea or constipation in the world. *Salicornia herbacea* L. has several active constituents including tungtungmadic acid (3-caffeoyl-4-dihydrocaffeoyl quinic acid), a chlorogenic acid derivative which had higher anti-oxidative activity in the 1,1-diphenyl-2-picrylhydrazyl (DPPH)-free radical scavenging test and in the iron-induced liver microsomal lipid peroxidation assay (Chung *et al*., 2005). Chlorogenic acid, an ester of caffeic acid with quinic acid, is a recognized antioxidant found in several plants (Medina *et al*., 2007). In addition, other active compounds, including β–sitosterol, stigmasterol, uracil, quercetin 3-*O*-β-D-glucopyranoside, and isorhamnetin 3-*O*-β-D-glucopyranoside, were isolated from *S. herbacea* by repeated column chromatography (Lee *et al*., 2007; Park and Kim, 2004).

The search of biologically active ingredient of plants has always been great interest to scientists looking for new sources of useful alternative against diseases. In this research we assessment the antibacterial effect of *S. herbacea* seed extract against multidrug resistant gram-positive and gram-negative bacteria by aiming to serve that as an alternative for antibiotics to avoid the side effect of them on the host cells.

Material and method

Plant material

Fresh seeds of *S. herbacea* were obtained from around Maharlo salt lake in Iran.

Preparation of ethanolic extract

Seeds of mature plants were powdered in a knife mill. Ground sample (20 g) was mixed with 200 ml of 85% ethanol using a shaking water bath for 24 h at room temperature. The extract was separated from the solid concentrate by filtering through Whatman No. 1 filter paper. The remaining residue was re-extracted twice and the extracts were pooled. The solvent was removed under vacuum at 30°C using a rotary vacuum evaporator (Laborota 4000, Heidolph, Germany).

Preparation of Microorganisms

The two gram-positive include *Staphylococcus aureus, Bacillus subtilis* and two gram negative include *Pseudomonas aeruginosa and Escherichia coli* were obtained from the microbiological laboratory of the MRI hospital in Shiraz, Iran. Susceptibility of four reference bacterial strains to antibiotics in nutrient agar summarized in Table 1. The microorganisms were inoculated onto nutrient agar slants at 36°C and maintained at -70°C. These bacteria were isolated from MRI patients that provided written permission to do so.

Antibiotic discs and antimicrobial activity

Antimicrobial activity was based on the disc diffusion method (Thitilertdecha *et al*., 2008) using a cell suspension of microorganisms. The concentration of the cell suspension was equilibrated to a 0.5 McFarland standard and 50 µl of each microorganism's suspension was spread on a Mueller-Hinton agar plate. In addition, 50 µl of diluted seed extract was pipetted onto sterile paper discs (5 mm in diameter), which was allowed to dry in an open sterile petri dish in a biological laminar flow bench. Discs were placed on the surface of inoculated plates and incubated at 36°C for 24 h. Diameters

(mm) of the zones of bacterial inhibition minus the discs diameter were recorded (Ilçim *et al*., 1998). The surfaces of the media were inoculated with bacteria from a broth culture. High potency bio-discs (Himedia) were placed on the agar. After 18 h of incubation at a 37°C, the plates were examined and the diameters of the inhibition zones were measured to the nearest millimeter.

Minimum inhibitory concentration (MIC) assay

The minimum inhibitory concentration (MIC) is defined as the lowest concentration at which the microorganism does not demonstrate visible growth. The MIC values were also studied for the microorganisms, which were determined as sensitive to the extracts in the disk diffusion assay. The inoculum (100 µl), initially adjusted to the density cited above, was spread onto 25 ml Mueller–Hinton agar supplemented with the seed at concentrations ranging from 3–10 µl/ml in Petri dishes, with each one of its equivalent in 10% dimethylsulfoxide (DMSO). These serially diluted cultures were then incubated at 36±1°C for 24 h. The MIC is defined as the lowest concentration at which the microorganism does not demonstrate visible growth. As control, 10% DMSO was used (Mazari *et al*., 2010).

Statistical analysis

Results were expressed as mean±SE of three independent experiments. Statistical significant differences were considered at $p < 0.05$ using one-way analysis of variance (ANOVA) with stat graphics centurion.

Result

The antibacterial activities of seed extract of *S. herbacea* were assayed in vitro by agar disc diffusion methods against four multidrug resistant gram-positive and gram-negative bacteria. Result showed that, seed extracts of plants recorded different degrees of antibacterial activity against multi-drug resistant bacteria as evidenced by the zone of inhibition (Table 2). Result showed that inhibition zones of 9.5±0.01, 6.2±0.00, 4±0.00 and 3.5±0.10 mm for *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli,* respectively. Result of minimum Inhibitory Concentration (MIC) of the seed against multidrug resistant gram-positive and gram-negative bacteria showed in Fig 1. Result showed that MIC of 189.5, 270, 350 and 420 mm for *S. aureus, B.subtilis, P. aeruginosa and E. coli,* respectively. Among four bacteria the maximum and minimum inhibition seed ethanolic extract were related to *S. aureus* with inhibition zones of 9.5mm and MIC 189.5 mg/ml and *E. coli* with inhibition zones of 3.5 mm and MIC 420 mg/ml, respectively.

Table 1. Susceptibility of four reference bacterial strains to antibiotics in nutrient agar.

Diameter of the inhibitory zones (mm)				
Antibiotics (µg/ml)	*S. aureus*	*B.subtilis*	*P.aeruginosa*	*E.coli*
Ampicillin (20)	00	28	19	00
Amikacin (20)	19	13	24	11
Cotrimoxazole (20)	16	28	00	12
Ciprofloxacin (10)	00	20	06	00
Cloxacillin (25)	00	00	00	00
Cefadroxil (20)	00	00	00	00
Cefuroxime (20)	00	00	00	00
Doxycycline (20)	10	12	23	11
Erythromycin (10)	00	26	00	00
Gentamycin (10)	00	14	21	15
Kanamycin (20)	00	26	17	12
Nalidixic acid (20)	18	00	00	00
Norfloxacin (10)	07	00	16	11
Penicillin-G (10)	00	00	00	00
Sparfloxacin (10)	00	14	22	00
Tobramycin (10)	15	28	18	14
Tetracyclin (25)	12	27	00	20

Discussion

Many plants or plant parts have been studied for their antimicrobial activity. In the present study seed of *S. herbacea* ethanol extract was screened for its antimicrobial activity by disc diffusion method. To the best of our knowledge, a very little study has carried out the antimicrobial activity of *S. herbacea* plant (Lellau TF and Liebezeit, 2003). Result showed that the ethanol extract of *S. herbacea* exhibited an antibacterial effect with all strains. The gram positive bacteria were significantly more susceptible to the extract ($p< 0.05$) and showed greater inhibition zone than the gram negative bacteria, which have recently greatly been reported in the literature (Jayalakshmi *et al.*, 2011).

Table 2. Antibacterial activity of the ethanolic seed extracts of plants multidrug resistant gram-positive and gram-negative bacteria.

Diameter of the inhibitory zones (mm)				
Microorganisms	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*E. coli*
Seed extract	9.5±0.01	6.2±0.00	4±0.00	3.5±0.10

Knowledge of the phytochemical components of plants is worthwhile, not only for the finding of therapeutic factors, but also because such information may be of value in revealing new origin of such economic materials as tannins, oils, gums, flavonoids, saponins, essential oils precursors for the synthesis of complex chemical material (Rad *et al.*, 2013; Viji and Murugesan, 2010). This antibacterial activity is related to the presence of phenols (flavones and the related flavonoids) and polysaccharides compounds found in *S. herbacea* extract (Essaidi *et al.*, 2013). Phenolic substances tend to be water soluble, since they most often combined with sugar as glycosides and they are commonly located in the cell vacuole (Essaidi *et al.*, 2013).

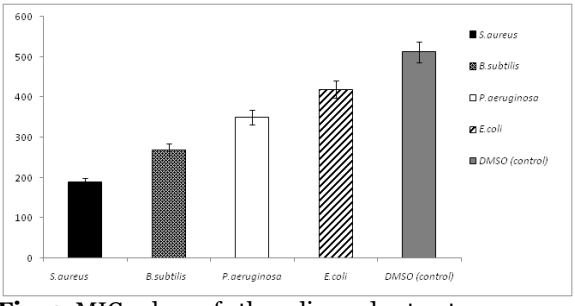

Fig. 1. MIC values of ethanolic seed extract.

The previous studies showed that results of *S. herbacea* stem extract have several phenolic compounds which could have antimicrobial activity (Essaidi *et al.*, 2013). However, this activity was not only related to phenols but also to other components such as fatty acids and the osmotic compound (betaine), which has been reported by Chandrasekaranm *et al.* (Chandrasekaran *et al.*, 2008) and Kim *et al.* (15) respectively. Therefore, we can suggest that the antimicrobial activity of ethanol extract of *S. herbacea* is the result of a synergic or additive effect of several compounds present in this plant. Also we suggest that more work should be encouraged to explore the nutritional and pharmaceutical values of *S. herbacea* through extensive study methods, including *various in vivo* designs based on these *in vitro* results. In conclusion, the results of this study suggest the possibility using the *S. herbacea* plant seed which possess antibacterial activity. The result indicated that the *S. herbacea* seed may be used in the treatment of diseases caused by the micro-organisms tested.

References

Alfatemi SMH, Rad JS, Rad MS, Mohsenzadeh S, da Silva JAT. 2014. Chemical composition, antioxidant activity and in vitro antibacterial activity of *Achillea wilhelmsii* C. Koch essential oil on methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* spp. 3 Biotech, 1-6. http://dx.doi.org/10.1007/s13205-014-0197-x

Chandrasekaran M, Kannathasan K, Venkatesalu V. 2008. Antimicrobial activity of fatty acid methyl esters of some members of Chenopodiaceae. Zeitschrift für Naturforschung B **63c,** 331e336.

Chung YC, Chun HK, Yang JY, Kim JY, Han EH, Kho YH, Jeong HG. 2005. "Tungtungmadic acid, a novel antioxidant, from *Salicornia herbacea*". Archives of Pharmacal Research **28,** 1122-1126. http://dx.doi.org/ 10.1007/BF02972972

Essaidi I, Brahmi Z, Snoussi A, Koubaier HBH, Casabianca H, Abe N, Omri AE, Chaabouni MM, Bouzouita N. 2013. Phytochemical investigation of Tunisian *Salicornia herbacea* L., antioxidant, antimicrobial and cytochrome P450 (CYPs) inhibitory activities of its methanol extract. Food Control **32,** 125e133. http://dx.doi.org/10.1016/j.foodcont.2012.11.006

Ilçim A, Dıgrak M, Bagci E. 1998. The investigation of antimicrobial effect of some plant extract. Turkish Journal of Biology **22,** 119-125.

Jayalakshmi B, Raveesha KA, Amruthesh KN. 2011. Phytochemical investigations and antibacterial activity of some medicinal plants against pathogenic bacteria. Journal of Applied Pharmaceutical Science **01(05),** 124e128.

Mazari K, Bendimerad N, Bekhechi C, Fernandez X. 2010. Chemical composition and antimicrobial activity of essential oils isolated from Algerian *Juniperus phoenicea* L. and *Cupressus sempervirens* L. Journal of Medicinal Plants Research. **4(10),** 959-964. http://dx.doi.org/10.5897/JMPR10.169

Kim JH, Song JY, Lee JM, Oh SH, Lee HJ, Choin HJ, Go JM, Kim YH. 2010. A study on physiochemical property of *Salicornia herbaciea* & *Suaeda japonica*. Journal of Food Hygiene and Safety **25(2),** 170e179.

Lee CH, Kim IH, Kim YE, Oh SW, Lee HJ. 2004. "Determination of betaine from *Salicornia herbacea* L.". Journal of the Korean Society of Food Science and Nutrition **33,** 1584-1587.

Lellau TF, Liebezeit G. 2003. Activity of ethanolic extracts of salt marsh plants from the lower Saxonian Wadden Sea coast against microoraganisms marine biodiversity. Senckenbergiana maritima **32(1e2),** 177e181. http://dx.doi.org/10.1007/BF03043093

Medina I, Gallardo JM, Gonzalez MJ, Lois S, Hedges N. 2007. "Effect of molecular structure of phenolic families as hydroxycinnamic acids and catechins on their antioxidant effectiveness in minced fish muscle". Journal of Agricultural and Food Chemistry **55,** 3889-3895. http://dx.doi.org/10.1021/jf063498i

Miri A, Rad JS, Alfatemi SMH, Rad MS. 2013. A study of Antibacterial potentiality of some plants extracts against multidrug resistant human pathogens. Annals of Biological Research **4(8),** 35-41.

Park SH, Kim KS. 2004. "Isolation and identification of antioxidant flavonoids from *Salicornia herbacea* L.". Journal of the Korean Society for Applied Biological Chemistry **47,** 120-123.

Rad JS, Alfatemi MH, Rad MS, Sen DJ. 2013. Phytochemical and Antimicrobial Evaluation of the Essential Oils and Antioxidant Activity of Aqueous Extracts from Flower and Stem of *Sinapis arvensis* L. American Journal of Advanced Drug Delivery **1(1),**001-010.

Rhee MH, Park HJ, Cho JY. 2009. *Salicornia herbacea*: Botanical, chemical and pharmacological review of halophyte marsh plant. Journal of Medicinal Plants Research **3(8),** 548-555.

Shepherd KA, Macfarlane TD, Colmer TD. 2005. Morphology, anatomy and histochemistry of Salicornioideae (Chenopodiaceae) fruits. Annals of Botany **95,** 917-933. http://dx.doi.org/10.1093/aob/mci101

Thitilertdecha N, Teerawutgulrag A, Rakariyatham N. 2008. Antioxidant and

antibacterial activities of *Nephelium lappaceum* L. extracts. LWT – Food Science and Technology **41,** 2029-2035.
http://dx.doi.org/10.1016/j.lwt.2008.01.017

Viji M, Murugesan S. 2010. Phytochemical analysis and antibacterial activity of medicinal plant *Cardiospermum halicacabum* Linn. Journal of Phytology Phytopharmacology **2(1),** 68e77.