Int J Infect. 2015 April; 2(2): e23214. DOI: 10.17795/iji-23214

Published online 2015 April 30. **Research Article**

Antibacterial Activities of the Hydroalcoholic Extract of *Portulaca oleracea* Leaves and Seeds in Sistan Region, Southeastern Iran

Seyed Mohammad Mousavi [1,2]; Gholamreza Bagheri [1,*]; Saeide Saeidi [3,4]

[1]Zabol Medicinal Plant Research Center, Zabol University of Medical Sciences, Zabol, IR Iran
[2]Iranian Academic Center for Education, Culture and Research (ACECR), Zahedan, IR Iran
[3]Department of Biology, Faculty of Sciences, Islamic Azad University, Kerman, IR Iran
[4]Infectious Diseases and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Zahedan, IR Iran

*Corresponding author: Gholamreza Bagheri, Zabol Medicinal Plant Research Center, Zabol University of Medical Sciences, Zabol, IR Iran. Tel: +98-5422230768, Fax: +98-5422230770, E-mail: drgrbagheri@yahoo.com

Received: September 23, 2014; **Accepted:** September 23, 2014

Background: Many efforts have been made by researchers to find new antibiotics which are clinically useful against bacteria and drug resistant microorganisms.
Objectives: We aimed to evaluate the antimicrobial effects of hydroalcoholic extracts of leaves and seeds of the *Portulaca oleracea* plant in traditional medicine of Sistan region, southeast of Iran.
Materials and Methods: In this cross-sectional and descriptive study, from January 2010 to January 2012, we studied the antibacterial activity of *P. oleracea* extract using nine bacteria (Gram-positive and Gram-negative bacterial strains) which were resistant to standard antibiotics such as erythromycin, cefixime, ceftazidime, tetracycline, ampicillin and amikacin. Bacterial strains were obtained from a standard laboratory. Broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).
Results: The hydroalcoholic extract of *P. oleracea L.* leave and seed had antibacterial effects on selected drug resistant bacterial strains.
Conclusions: The leaves and seeds extract of *P. oleracea* has a remarkable antibacterial effect and it can be a good alternative when we are faced with drug resistance bacteria. However, more studies are required.

Keywords: Antibacterial Activity; Antioxidant; *Portulaca oleracea L.*

1. Background

Many plants and their extracts have been used in traditional medicine to treat infectious and noninfectious diseases in the world (1). These plants have a wide range of antibacterial properties (2). Medicinal plants with antibacterial effects do not have any major side effects on human health and are also inexpensive. During the last 40 years, many researchers in the world worked on plants to find new antibiotics which were clinically effective against drug sensitive-and drug-resistant bacteria (1-6). One of these plants which is important in traditional medicine in Sistan region (southeast of Iran) is *Portulaca oleracea*. *P. oleracea* is an annual plant with fresh, thick, and juicy stems and leaves. This plant has green and red stems and small yellow or white flowers and tiny black seeds which consist of many medicinal properties. This plant grows in most parts of the world. *P. oleracea* has a diuretic effect and also reduces the stomach pain and liver discomforts; the leaf extract is helpful in treating kidney pain (3). *P. oleracea* is also used as an analgesic and antitussive agent and can set the blood sugar in diabetic patients (4). In many parts of the world including southern countries of Asia, this plant is used as a green vegetable (5). Other biological properties include anti-inflammation, muscle relaxation and fever suppression (6). *P. oleracea* is a rich source of omega-3 fatty acids and can prevent heart attacks and improve the immune system in human (7). Okafor in 2014 in Nigeria studied the water extract of aerial parts of *P. oleracea* and showed the presence of steroids, protein, and alkaloids (8). Dkhil et al. in 2011 in Egypt investigated the antioxidant properties of the *P. oleracea* extract in male mice (9). Uddin et al. in 2012 in Malaysia determined the mineral composition of *P. oleracea* consisting of calcium, iron, zinc, magnesium and potassium, the level of which increase with plant maturation (10). Another study in 2009 showed that the major constituent in the stems and leaves of this plant was water (90.5%). Barbosa et al. reported that the fat content was different ranging from 0.11 to 0.57% and 27 types of leaf fatty acid in the leaf samples were found (11). Nayaka and Londonkar in 2014 evaluated the antimicrobial effect of hydroalcoholic extract of aerial parts of *P. oleracea* in India (12). In 2011, Londonkar and Nayaka showed the antimicrobial and antifungal properties of ethanol extracts of *P. oleracea* on some bacteria

and fungi (5). A study by Ji-Hyun Bae in 2004 showed the antimicrobial effect of *P. oleracea* extracts on foodborne pathogens (13). During these years, researchers have not faced with any signs of significant toxicity in connection with this plant (14).

2. Objectives

According to the medicinal properties of *P. oleracea*, there was no comprehensive study in Southeastern Iran; thus, we aimed to study the antibacterial effect of this plant.

3. Materials and Methods

3.1. Preparation Method of the Alcoholic Extract

P. oleracea was prepared from the central part of Zabol in Sistan and Baluchestan province in the southeast of Iran; then, its leaves and seeds were collected and separated. Afterwards, they were dried to obtain the extract from the maceration with soaking method. The *P. oleracea* leaves and seeds powder was prepared; 20 g of the powder was added to 60 mL of 95% ethanol and water; then, they were soaked. The extract was filtered using Whatman paper and rotary vacuum distillation unit and dried at 50-40°C for two days.

3.2. Bacterial Strains and Culture Conditions

Bacterial strains were obtained from a standard laboratory. Antibacterial activities of the plant extracts were investigated using *Streptococcus pyogenes* ATCC® 19615™, *S. pneumoniae* ATCC 49619, *S saprophyticus* ATCC®15305, *Hafnia alvei* ATCC 51873, *Acinetobacter baumannii* ATCC 19606, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659, *Serratia marcescens* ATCC 274, and *Staphylococcus aureus* ATCC® 25923. The typed cultures of bacteria were subcultured on nutrient agar (oxoid) and stored at 4°C until required for study.

3.3. Agar Disc Diffusion Assay for Antibiotics

The susceptibility test of all the antibiotics was carried out using disc diffusion method on Muller-Hinton agar, as recommended by Clinical and Laboratory Standards Institute (CLSI) (11). All the mentioned bacteria plates were grown overnight on blood agar and nutrient agar and colony suspension was prepared using a sterile saline water equivalent to a 0.5 McFarland standard. The suspension (100 μL) was spread over the media plate and the antibiotic disc was transferred aseptically to the surface of the inoculated media plate. The isolated plates were tested with different antibiotics and their concentrations were shown in parenthesis.

3.4. Plant Materials

The leaves and seeds of *P. oleracea L.* were collected from Zabol, southeastern Iran and dried at room temperature. Samples were crashed and transferred into a glass container and preserved until the extraction procedure was performed in the laboratory.

3.5. Preparation of Extracts

Plants were properly dried and pulverized into a coarse powder. Each of 20 g grinded powders was soaked in 60 mL of ethanol 95% separately for one day (shaking occasionally with a shaker). After one day from the dissolving process, the materials were filtered (Whatman No. 1 filter paper). Then, the filtrates were evaporated using rotary evaporator. At last, 0.97 g of the dried extracts were obtained and then, they were stored at 40°C in an air-tight screw-cap tube.

3.6. Minimum Inhibitory Concentration and Minimum Bacterial Concentration of Plant Extracts

Broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC). All the tests were performed in Mueller-Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Serial doubling dilutions of the extract were prepared in a 96-well microtiter plate, ranged from 12.5 ppm to 400 ppm. To each well, 10 μL of indicator solution and 10 μL of Mueller-Hinton broth were added. Finally, 10 μL of the bacterial suspension (106 CFU/mL) was added to each well to achieve a concentration of 104 CFU/mL. The plates were wrapped loosely with cling film to ensure that the bacteria were not dehydrated. Plates were prepared in triplicates and then placed in an incubator at 37°C for 18–24 hours. Afterwards, the color change was assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of the three values was calculated providing the MIC values for the tested extract. MIC was defined as the lowest concentration of the extract at which the microorganism does not demonstrate any visible growth. The microorganism growth was indicated by turbidity. MBC was defined as the lowest concentration of the extracts at which the incubated microorganism was completely killed.

3.7. Agar Well Diffusion Assay for Extracts

Antibacterial activity of the plant crude extracts was tested using agar well diffusion method. The test inoculums (0.5 McFarland turbidity) were spread into Muller-Hinton agar using a sterile cotton swab. The wells were made by sterile well puncture and 20 μL of the extracts were added to each well and incubated at 37°C for 24 hours. The presence of inhibition zone was regarded as the presence of antimicrobial action. The average diameter of the inhibition zone was measured in millimeter.

Table 1. Antimicrobial Susceptibility and Minimum Inhibitory Concentration/Minimum Bacterial Concentration of Leaf Extract of *Portulaca oleracea L.* for Standard Bacteria [a]

Bacteria	MIC/MBC	Antibiotic Resistance
Staphylococcus aureus	50/100	E,CE,TE,AM
Streptococcus pyogenes	100/200	-
Streptococcuspneumoniae	200/200	E,CE,CF, AM
Hafniaalvei	200/200	E,TE, AM
Staphylococcus saprophyticus	200/400	E,CF,TE, AM
Acinetobacterbaumannii	100/100	CE,TE
Enterococcus faecalis	100/200	E,CE, AM
Proteus mirabilis	50/50	E,TE, AM
Serratiamarcescens	100/100	CE

[a] Abbreviations: E, erythromycin; CE, cefixime; CF, ceftazidime; TE, tetracycline; AM, ampicillin; AN, amikacin; MIC, minimum inhibitory concentration; MBC, minimum bacterial concentration.

Table 2. Antimicrobial Susceptibility and Minimum Inhibitory Concentration/Minimum Bacterial Concentration of Seed Extract of *Portulaca oleracea L.* for Standard Bacteria [a]

Bacteria	MIC/MBC	Antibiotic Resistance
Staphylococcus aureus	50/100	E,CE,TE,AM
Streptococcus pyogenes	50/50	-
Streptococcus pneumoniae	50/50	E,CE,CF, A
Hafniaalvei	50/100	E,TE, AM
Staphylococcus saprophyticus	50/100	E,CF,TE, AM
Acinetobacter baumannii	50/50	CE,TE
Enterococcus faecalis	100/200	E,CE, AM
Proteus mirabilis	50/50	E,TE, AM
Serratiamarcescens	50/100	CE

[a] Abbreviations: E, erythromycin; CE, cefixime; CF, ceftazidime; TE, tetracycline; AM, ampicillin; AN, amikacin; MIC, minimum inhibitory concentration; MBC, minimum bacterial concentration.

Table 3. Antimicrobial Activity of the Plant Crude Extracts as Mean of Inhibition Diameter Zone Against Gram-Positive and Gram-Negative Pathogenic Bacteria

Bacteria	Diameter Zone for Leaf Extract, mm	Diameter Zone for Fruit Extract, mm
Staphylococcus aureus	15	15.5
Streptococcus pyogenes	12	14
Streptococcus pneumoniae	7	16
Hafniaalvei	7	14
Staphylococcus saprophyticus	5.5	13.5
Acinetobacter baumannii	10.5	15
Enterococcus faecalis	9	10
Proteus mirabilis	18	13.5
Serratiamarcescens	8.5	15

4. Results

4.1. Antimicrobial Activity of Leaf and Seed Extract of Portulaca oleracea L.

The highest MIC for the *P. oleracea* leave was 200 ppm and three strains were inhibited at this concentration, while the highest MIC for the *P. oleracea* seed was 100 ppm. The lowest MIC concentration for *P. oleracea* seed and leave was 50 ppm and eight strains were inhibited at this level. The highest MBC for *P. oleracea* leave was 400 ppm and *S. saprophyticus* was lost at this level, while the lowest MBC was 50 ppm and *P. mirabilis* was destroyed at this concentration. The highest MBC for seed was 200 ppm and *E. faecalis* was destroyed at this level. The maximum diameter of inhibition zone (mm 18) for *P. oleracea* leaves was observed against *P. mirabilis*, while the highest inhibition zone (16 mm) for seed of *P. oleracea* were observed against *S. pneumonia*. All the results are shown in Tables 1 to 3.

5. Discussion

Medicinal plants with therapeutic properties due to different compounds have protean effects on human health (15). Our results showed that *P. oleracea* had antibacterial effect on drug-resistant bacteria. The highest MIC for *P. oleracea* leave was 200 ppm, while the highest MIC for *P. oleracea* seed was 100 ppm. The lowest MIC was 50 ppm for leave and seed and the highest inhibition zone diameter for *P. oleracea* leave against *P. mirabilis* was 18 mm, while the highest zone of inhibition for *P. oleracea* seed against *S. pneumonia* was 16 mm. Many studies have reported the antibacterial and antioxidant effects of *P. oleracea*. Nayaka and Londonkar in 2014 showed the antibacterial activity of hydroalcoholic extract of some parts of *P. oleracea* by five pathogenic bacteria, including *Salmonella typhimurium* and *P. mirabilis*. The highest inhibition zones for *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Enterobacter aerogenes* were 14.56 ± 0.21 and 11.68 ± 0.13 mm, respectively (12). Londonkar and Nayaka, in 2011 studied the ethanol extract of the aerial parts of *P. oleracea*, and showed its inhibitory effect against strains of *St. aureus*, *K. pneumonia*, *Bacillus cereus* and *Aspergillus fumigates* (5). The Bae study in 2004 showed that the ethyl acetate extract of *P. oleracea* had the highest antimicrobial activity against *S. aureus* and *Shigella dysenteriae* (13). In our study, the antibacterial effect of the mentioned plant was detected in nine bacterial strains including *S. pyogenes*, *S. pneumoniae*, *S. saprophyticus*, *H. alvei*, *A. baumannii*, *E. faecalis*, *P. mirabilis*, *S. marcescens*, and *S. aureus*, which were resistant to erythromycin, cefixime, ceftazidime, tetracycline, ampicillin, and amikacin. In addition, with increasing the extract concentration, the percentage of free radical scavenging increased and when compared, the antioxidant properties of leaves were higher than those of seed. Hyun Kim in 2009 showed that with

increasing the extract concentration of the aqueous extract of *P. oleracea*, the percentage of free radical scavenging increased (16). A study by Radhakrishnan et al. detected that the 10% ethanolic extract of this plant restricted movement in animals during routine screening studies (17). Behravan et al. (18) showed that the aqueous extract of *P. oleracea* significantly inhibited DNA damage, while there was no such effect for the ethanolic extract. They concluded that the aqueous extract of *P. oleracea* can prevent oxidative DNA damage to human lymphocytes, which was likely due to antioxidant constituents in the extract.

As a conclusion, the leaves and seeds extract of *P. oleracea* has a remarkable antibacterial effect and it can be a good alternative agent when we are faced with drug-resistant bacteria. However, we need to more studies.

Acknowledgements

We thank all the staff of Zabol Laboratory who helped us in this study.

Authors' Contributions

Seyed Mohammad Mousavi, Gholamreza Bagheri and Saeide Saeidi wrote the manuscript. All the authors had equal roles in design and writing of the manuscript.

Funding/Support

This study was supported by Zabol Medicinal Plant Research Center, Zabol, Iran.

References

1. Li C, Xie B. Evaluation of the antioxidant and pro-oxidant effects of tea catechin oxypolymers. *J Agric Food Chem.* 2000; **48**(12):6362–6.
2. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, et al. Antioxidant activity of plant extracts containing phenolic compounds. *J Agric Food Chem.* 1999;**47**(10):3954–62.
3. Sharifi-Mood B, Shafaghat M, Metanat M, Saeidi S, Rad NS. The Inhibitory Effect of Ajowan Essential Oil on Bacterial Growth. *Int J Infect.* 2014;**1**(2).
4. Shin JK, Kim GN, Jang HD. Antioxidant and pro-oxidant effects of green tea extracts in oxygen radical absorbance capacity assay. *J Med Food.* 2007;**10**(1):32–40.
5. Londonkar R, Nayaka HB. Phytochemical and Antimicrobial Activities of Portulaca Oleracea L. *J Pharm Res.* 2011;**4**(10).
6. Shirmohammadi E, Saeidi S, Mohasseli T, Boogar AR. Antibacterial Effects of Silver Nanoparticles Produced by Satureja hortensis Extract on Isolated Bacillus cereusfrom Soil of Sistan Plain. *Int J Infect.* 2014;**1**(3).
7. Simopoulos AP. Omega-3 fatty acids and antioxidants in edible wild plants. *Biol Res.* 2004;**37**(2):263–77.
8. Okafor A, Ezejindu N. Phytochemical Studies on Portulacaoleracea L. *Plant .* 2014;**3**(1):132–6.
9. Dkhil MA, Abdel Moniem AE, Al-Quraishy S, Saleh RA. Antioxidant effect of purslane (Portulaca oleracea) and its mechanism of action. *J Med Plant Research.* 2011;**5**:1589–63.
10. Uddin MK, Juraimi AS, Ali ME, Ismail MR. Evaluation of Antioxidant Properties and Mineral Composition of Purslane (Portulaca oleracea L.) at Different Growth Stages. *Int J Mol Sci.* 2012;**13**(8):10257–67.
11. Barbosa PB, de Oliveira JM, Chagas JM, Rabelo LM, de Medeiros GF, Giodani RB, et al. Evaluation of seed extracts from plants found in the Caatinga biome for the control of Aedes aegypti. *Parasitol Res.* 2014;**113**(10):3565–80.
12. Nayaka HB, Londonkar RL, Umesh MK, Tukappa A. Antibacterial Attributes of Apigenin, Isolated from Portulaca oleracea L. *Int J Bacteriol.* 2014;**2014**.
13. Bae JH. Antimicrobial effect of Portulaca oleracea extracts on food-borne pathogens. *J Food Sci Nut.* 2004;**9**(4):306–11.
14. Miladi-Gorji H, Vafaei AA, Bageri A. To investigate the effect of Portulaca oleracea L. and Melissa officinalis L. extract on sleeping time in mice Persian. *J Med Plants.* 2011;**10**(38):95–101.
15. Bameri11 Z, Amini-Boroujeni N, Saeidi S, Bazi S. Antibacterial activity of Cassia angustifolia Extract Against Some Human Pathogenic Bacteria. *J Novel Appl Sci.* 2013.
16. Hyun K, Park P, Choe S, Kim T, Jeong J, Lee K, et al. Anti-Oxidative and anti-filammatory Effects Of Portulaca oleraceae L. On the Lps-stimulated AGS Cells. *Korean J Oriental Physiol Pathol.* 2009;**23**(2):488–93.
17. Radhakrishnan R, Zakaria MN, Islam MW, Chen HB, Kamil M, Chan K, et al. Neuropharmacological actions of Portulaca oleraceae L v. sativa (Hawk). *J Ethnopharmacol.* 2001;**76**(2):171–6.
18. Behravan J, Mosafa F, Soudmand N, Taghiabadi E, Razavi BM, Karimi G. Protective effects of aqueous and ethanolic extracts of Portulaca oleracea L. aerial parts on H2O2-induced DNA damage in lymphocytes by comet assay. *J Acupunct Meridian Stud.* 2011;**4**(3):193–7.