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Perovskia abrotanoides Kar. as a Promising Source of Antimicrobial Compounds against Foodborne Pathogens

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ABSTRACT

Background and objectives: Foodborne pathogens can significantly affect the public health and cause medical, social, and economic burden. *Listeria monocytogenes*, *Salmonella - enterica, and Yersinia enterocolitica* are important foodborne pathogens that can cause various diseases. Plant-derived compounds are promising bioactive substances with inhibitory effects against bacteria. *Perovskia abrotanoides* Kar. is a medical plant with broad therapeutic activities. In the present study, we aimed to investigate the inhibitory effects of *P. abrotanoides* extracts against some foodborne pathogens.

Methods: Flowering branches of *P. abrotanoides* were collected in 2018 and 2019 from three different habitats in the eastern Alborz Mountains, Iran. The antimicrobial activity of the extracts was evaluated using the agar well diffusion test. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were determined against *L. monocytogenes*, *S. enterica, and Y. enterocolitica*. In addition, the antioxidant activity of the extracts was investigated by the DPPH test.

Results: The lowest MIC (200 µg/ml) and MBC (400 µg/ml) values against *Y. enterocolitica* were related to the ethyl acetate extract of plants collected from habitat 1 in 2019. The lowest MIC (50 µg/ml) and MBC (400 µg/ml) values against *L. monocytogenes* were related to the dichloromethane extract of plants collected from habitat 1 in 2019. All extracts showed antioxidant properties. Results of one-way ANOVA indicated that the DPPH scavenging activity of extracts from plants collected in 2019 was greater than that of those collected in 2018. In most cases, the methanol and ethyl acetate extracts showed more radical scavenging potential.

Conclusion: It seems that *P. abrotanoides* is a rich source of antimicrobial and antioxidant compounds with great potential for use in the pharmaceutical and food industries.

Keywords: Listeria monocytogenes, Yersinia enterocolitica, Salmonella enterica, Antioxidant.

INTRODUCTION

Food contamination with foodborne pathogens cause nutrient deterioration. It also adversely affects the quality and safety of foods by producing poisonous and foul-smelling compounds. According to a report by the Centers for Disease Control and Prevention, bacterially contaminated food causes significant mortality (3,000 deaths) and morbidity (48 million illnesses) in the United States annually (1, 2). *Listeria monocytogenes*, *Salmonella enterica, and Yersinia enterocolitica* are responsible for listeriosis, salmonellosis, and yersiniosis, respectively. They are major foodborne diseases that cause considerable public health concerns in the world. (3). *L. monocytogenes* causes a mortality rate of up to 30%. Major clinical manifestations of *L. monocytogenes*-related infections are gastroenteritis, meningitis, and septicemia. The persistence of *L. monocytogenes* in food processing for a long time, even at low temperatures, and its ability to generate biofilms or integrate into previously developed biofilms by other species, make it very elusive to control (4).

Food contamination with *Salmonella* can occur during the manufacturing and processing steps of livestock feed and food. This bacterium is also resistant to the harsh conditions of food matrices. This facilitates the transmission of this high-infective foodborne pathogen to human beings and enhances the number of outbreaks (5). *Y. enterocolitica* is considered the main species in the genus associated with yersiniosis. Associated symptoms include diarrhea, fever, and abdominal pain (6).

In order to guarantee food safety from foodborne-related illnesses, compounds with inhibitory effects on their growth are critically needed (4). Plants are promising sources of bioactive compounds such as antimicrobials and antioxidants. Herbal antioxidants improve food quality as natural antimicrobial compounds. Natural antioxidants in plants can scavenge free radicals before triggering oxidative chain reactions in the cell membrane or lipid-containing portions (11, 12).

Perovskia is a small genus of medicinal plants from the *Lamiaceae* family, a potential source of phenolic acids (7). Its aromatic shrubs grow wild in arid habitats of central Asia, including Afghanistan, Pakistan, Turkmenistan, and Iran, particularly in the northern, eastern, and central parts of Iran (7). There are three species of this plant in Iran including, *P.abrotanoides* Kar., *P. atriplicifolia* Benth., and *P. artemisoides* Boiss.. *P. abrotanoides*, locally known as brazambal, is an aromatic herb that has been used to treat rheumatic pains and leishmaniasis in Iranian traditional medicine (8). Jaafari and coworkers reported the anti-leishmaniasis activity of phenolic and terpenic constituents of *P. abrotanoides* stems and leaves (9).

Previous phytochemical investigations on the genus *Perovskia* indicate that this plant contains flavonoids, phenolics, and anthocyanins with leishmanicidal, anti-plasmodial, cytotoxic, anti-inflammatory, anti-diabetic, anti-lipidemic, and anthelmintic activities (8, 15-19). It also has structurally unique diterpenoids possessing abietane and rearranged icetexane scaffolds. The aerial parts and roots of *P. abrotanoides* contain isoprenoids and tanshinones, respectively (20, 21).

In this study, we aimed to determine the antimicrobial activity of extract from *P. abrotanoides* plants collected from three different parts of Iran and two different periods against *P. abrotanoides*, *L. monocytogenes*, *Salmonella spp, and Y. enterocolitica*. Given the great diversity of climate in Iran, plants grown under different conditions may have different antioxidant capacities (13, 14). Hence, we investigated the antioxidant activity of *P. abrotanoides* collected from different habitats.

MATERIALS AND METHODS

Chemical materials including hexane, dichloromethane, ethylacetate, methanol, dimethyl sulfoxide (DMSO), phosphate buffer saline (PBS), agar, thin-layer chromatography (TLC) plates, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Merck Co., Germany. Culture media including nutrient broth, brain heart infusion, tryptic soy broth, Mueller-Hinton agar, and tryptic soy agar were purchased from Ibresco, Iran. Gram-negative and Gram-positive bacterial species were obtained from the Iranian Biological Resource Center (Tehran, Iran).

The medicinal plant *P. abrotanoides* Kar. was collected from the mountain regions of eastern Alborz (with considerably different climates from Semnan and Mazandaran provinces)

(Table 1). The flowering branches were properly rinsed with distilled water, shade-dried, and grounded into fine powder. The extraction of plant material was done according to a previous study with minor modifications (22). Briefly, 5 g of powdered branches were added into organic solvents (100 ml) with a different polarity index (PI), including hexane (PI=0), dichloromethane (PI=3.1), ethylacetate (PI=4.4), and methanol (PI=5.1). The mixture was heated at 40 ºC on a hotplate with continuous stirring for 2 hours. The resulting solution was filtered using a Whatman filter paper and then air-dried to obtain the extracts.

The antimicrobial potential of the extracts (400 µg/ml) was evaluated using the agar well diffusion test. Antibiotic discs (6 mm diameter) and diluted DMSO were used as the positive and negative controls, respectively. Bioactive compounds were integrated into the medium, and culture plates were incubated at 37 °C for 24 hours. Inhibitory properties were assessed by measuring the diameter of inhibition zone (mm) around the wells and antibiotic disk. The test was repeated three times, and the results were reported as mean ± standard deviation (SD) after three repeats (23).

The minimal inhibitory concentration (MIC) of the extracts was determined according to the Clinical and Laboratory Standards Institute (CLSI), protocol M7-A6 (2012) (24). The lowest concentration of each extract that inhibited bacterial growth was determined as MIC. The wells without extracts and bacterial inoculum were considered as growth and sterility controls, respectively. To determine the minimal bactericidal concentration (MBC), 10 µl of the sample from each well of MIC microtiter plate were directly transferred to Mueller-Hinton agar or tryptic soy agar plates and incubated for 24 hours at 37 ºC. The MBC was determined as the lowest concentration of the extract in which no detectable growth was observed (25).

The antioxidant activity of extracts was evaluated qualitatively by the TLC-DPPH method. For this purpose, the crude extracts were developed on TLC silica gel 60 F254 plates (Mecherey-Nagel) in dichloromethane and methanol (93:7 v/v) mixture as solvent systems. Then, to recognize the radical scavenging activity of the extracts, the plates were sprayed with DPPH reagent solution (0.05% DPPH/methanol). The plates were incubated at room temperature in the dark for 30 minutes. The formation of yellow spots against a purple background, following spraying DPPH occurred for bonds containing compounds with antioxidant activity (26).

The radical scavenging activity of the extracts (800 µg/ml) was assessed according to the DPPH method in a 96-well microtitre plate as described previously (27). The antioxidant activity of the extracts was further evaluated at 400, 200, 100, and 50 µg/ml. Triplicate measurements were made and the ability to scavenge the DPPH radical was calculated by the following formula, where A_{blank} is the absorbance of the control and A_{sample} is the absorbance of the sample:

Antioxidant activity%= (A_{blank}- A_{sample}/ A_{blank}) × 100.

Data were expressed as means ± SD and analyzed using one-way ANOVA. All analyzes were carried out in SPSS (version 18) and at a statistical significance of 0.05.

Table 1- The geographical location of *P. abrotanoides* habitats

Habitat	Province	Height above sea level	Longitude	latitude	Collection year	Sample name
1	Semnan	1504m	N 36°16′22.46″	E 54°5′3.24″	2018	1-2018
					2019	1-2019
2	Mazandaran	1672m	N 36°14′44.48″	E 53°43′47.3″	2018	2-2018
					2019	2-2019
3	Semnan	1285m	N 36°21′42.47″	E 54°53′19.5″	2018	3-2018
					2019	3-2019

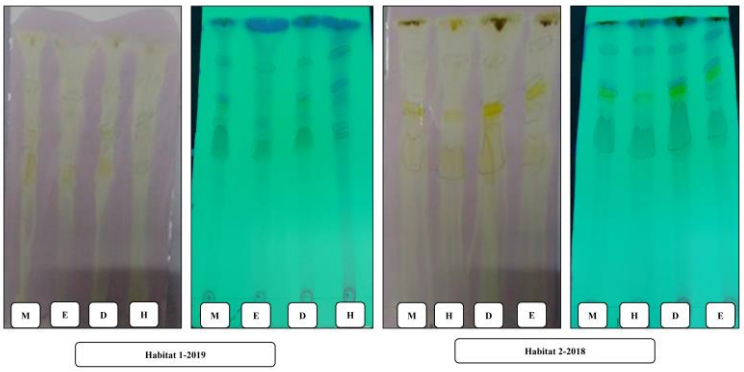

Figure 1- Antioxidant activity of the extracts derived from *P. abrotanoides* collected from habitat 1 (2019) and habitat 2 (2018) based on the TLC-DPPH assay. M, E, D, and H indicate methanol, ethyl acetate, dichloromethane, and hexane extracts, respectively.

RESULTS

Crude extracts of *P. abrotanoides* were obtained using solvent extraction. According to the weight of the obtained extracts, hexane and ethyl acetate had a higher capacity to extract compounds compared with dichloromethane and methanol (Table 2).

The crude extracts of *P. abrotanoides* showed significant inhibitory effects on *L. monocytogenes*. In this regard, extracts of samples 3-2018 and 3-2019 showed the highest and lowest anti-*Listeria* activities, respectively (Table 3). The extracts showed a lower antimicrobial activity against *Y. enterocolitica* than *L. monocytogenes*.

In most cases, significant antimicrobial activity was related to the ethyl acetate or methanolic extracts.

The ethyl acetate extract collected from habitats 1 and 2 in 2019 showed the highest antimicrobial activity against *Y. enterocolitica* (Table 3).

Finally, the extracts of *P. abrotanoides* showed minimal antimicrobial activity against *S. enterica* (Table 3).

Table 2- The dry weight of crude extracts of *P. abrotanoides*

Habitat	**1**		**2**		**3**	
Year	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
Dry weight (g) of *P. abrotanoides*	**2.5**	**4.8**	**2.5**	**6**	**5.8**	**6**
			Solvent			
Hexane	**0.59**	**0.89**	**0.77**	**1.16**	**1.11**	**0.93**
Dichloromethane	**0.2**	**0.37**	**0.3**	**1.05**	**0.3**	**0.86**
Ethyl acetate	**1.17**	**0.89**	**0.76**	**1.2**	**1.32**	**0.9**
Methanol	**0.21**	**2.16**	**0.21**	**0.59**	**0.98**	**1.37**

The extracts showed a lower antimicrobial activity against *Y. enterocolitica* than *L. monocytogenes*. In most cases, significant antimicrobial activity was related to the ethyl acetate or methanolic extracts. The ethyl acetate extract collected from habitats 1 and 2 in 2019 showed the highest antimicrobial activity against *Y. enterocolitica* (Table 3). Finally, the extracts of *P. abrotanoides* showed minimal antimicrobial activity against *S. enterica* (Table 3).

and MBC values of the extracts with considerable antimicrobial activity in the agar well diffusion MIC test were determined via broth microdilution and culturing methods. The lowest MIC (200 µg/ml) and MBC (400 µg/ml) values against *Y. enterocolitica* belonged to the ethyl acetate extract of 1-2019 (Table 4). The lowest MIC (50 µg/ml) and MBC (400 µg/ml) values on *L. monocytogenes* belonged to the dichloromethane extract of 1-2019 (Table 4).

Table 3- Antimicrobial activity of the *P. abrotanoides* extracts against the tested pathogens.

Solvent	Dose	Habitat 1 Year 2018	Habitat 1 Year 2019	Significance	Habitat 2 Year 2018	Habitat 2 Year 2019	Sig. value	Habitat 3 Year 2018	Habitat 3 Year 2019	Significance
					L. monocytogenes					
Hexane	400 µg/ml	11±0.1	12±0.3	NS	19±0.6	12±0.5	*	20±0.2	0±0	*
Dichloromethane		11±0.2	20±0.4	*	12±0.2	16±0.3	*	21±0.7	0±0	*
Ethyl acetate		11±0.2	11±0.3	NS	20±1	20±0.8	NS	16±0.5	0±0	*
Methanol		13±0.5	16±0.7	*	15±0.06	15±0.1	NS	20±0.4	0±0	*
					Y. enterocolitica					
Hexane	400 µg/ml	0±0	0±0	NS	0±0	0±0	NS	0±0	0±0	NS
Dichloromethane		0±0	12±0.3	*	0±0	11±0.5	*	0±0	0±0	NS
Ethyl acetate		12±0.45	16±0.7	*	12±0.6	16±0.4	*	13±0.4	0±0	*
Methanol		12±0.5	12±0.8	NS	9±0.2	13±0.3	NS	11±0.1	0±0	*
					S.enterica					
Hexane	400 µg/ml	12±0.3	0±0	NS	0±0	0±0	NS	0±0	0±0	NS
Dichloromethanee		12±0.4	0±0	*	0±0	0±0	*	0±0	0±0	NS
Ethyl acetate		0±0	0±0	*	0±0	0±0	*	0±0	0±0	*
Methanol		0±0	0±0	NS	0±0	11±0.3	NS	0±0	9±0.1	*
Positive controls			*L. monocytogenes*			*Y. enterocolitica*			*S. enterica*	
Trimethoprim	5 µg/*disk*		40±2			17±0.3			27±0.5	
Trimethoprim + sulfamethoxazole	1.25 mg 23.75 mg/disk		44±0.7			23±0.9			25±0.6	
Gentamicin	10µg/disk		34±0.6			25±0.5			30±0.8	
Penicillin	10µg/disk		0±0			0±0			0±0	

*** Statistically significant difference between the groups based on t-test.**
NS: not significant

Table 4- MIC and MBC values of the extracts of *P. abrotanoides* against *Y. enterocolitica* and *L. monocytogenes* (31)

Solvent	Habitat	Year	MIC (µg/ml)	MBC (µg/ml)	Mode of action	Antimicrobial activity
			Y. enterocolitica			
Ethyl acetate	3	2018	400	800	Bactericidal	Moderate
Ethyl acetate	2	2019	800	1600	Bactericidal	Weak
Methanol	2	2019	800	1600	Bactericidal	Weak
Methanol	1	2018	800	1600	Bactericidal	Weak
Ethyl acetate	1	2019	200	400	Bactericidal	Moderate
Ethyl acetate	1	2018	800	1600	Bactericidal	Weak
			L. monocytogenes			
Dichloromethane	3	2018	1600	3200	Bactericidal	Inactive
Ethyl acetate	3	2018	400	3200	Bacteriostatic	Moderate
Methanol	3	2018	200	3200	Bacteriostatic	Moderate
Hexane	3	2018	800	3200	Bactericidal	Moderate
Methanol	1	2019	100	800	Bacteriostatic	Moderate
Dichloromethane	1	2019	50	400	Bacteriostatic	Good
Methanol	2	2018	1600	3200	Bactericidal	Inactive
Hexane	2	2018	800	3200	Bactericidal	Moderate
Ethyl acetate	2	2018	800	3200	Bactericidal	Moderate

All investigated extracts showed antioxidant properties in the TLC-DPPH assay (Figure 1). Results of the one-way ANOVA indicated that the antioxidant activity of the extracts derived from plants collected in 2019 was higher than 2018 and 2-2019, respectively). In most cases, the methanol and ethyl acetate extracts showed greater antioxidant activity compared with the hexane and dichloromethane extracts (Figure 2a).

that of those collected in 2018 (except for the dichloromethane and hexane extracts of 3- The antioxidant activity of the extracts increased in a dose-dependent manner. The most radical scavenging activity was related to the ethyl acetate extract (400 µg/ml) of 3-2019. Also, the weakest DPPH scavenging activity was related to the ethyl acetate (50 µg/ml) and methanol (100 µg/ml) extracts of plants collected from 3-2019.

According to the results, the antioxidant activity of methanol and dichloromethane extracts of *P. abrotanoides* collected from habitat 3 in 2018 showed negligible differences in various concentrations (Figure 2b).

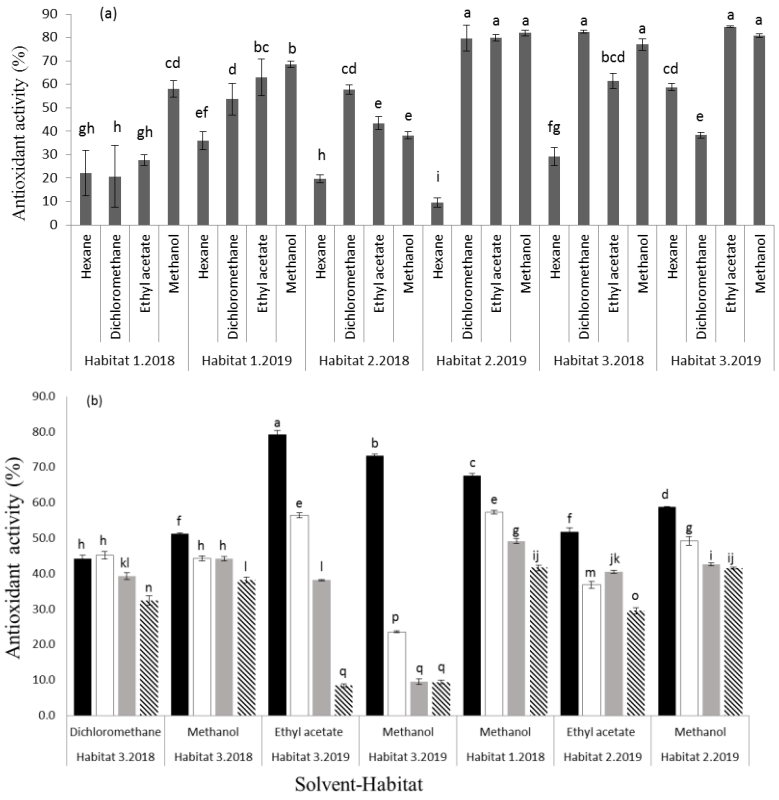

Figure 2- Antioxidant activities of extracts of *P. abrotanoides* (400 µg/ml) from different habitats based on the DPPH test. Antioxidant activity of *P. abrotanoides* extracts [400 (black column), 200 (white column), 100 (gray column), and 50 µg/ml (crosshatched column) concentrations]

DISCUSSION

Microbial food contamination is an important aspect of both pharmaceutical and food industries (28). *P. abrotanoides* is a promising medicinal plant with antimicrobial properties. It is locally known as hoosh, visk, brazambal, domou, and gevereh, which grows in mountainous areas of Iran, including the Semnan, Golestan, Isfahan, Khorasan, and Mazandran provinces (18, 29). The composition of *P. abrotanoides* essential oil and its bioactivities such as antifungal, antibacterial, anti-helminthic, anti-nociceptive, antiprotozoal, insecticidal, wound healing, and antioxidant effects have been demonstrated previously. Research has shown the presence of phenolic, flavonoid, and terpenoid components such as thymol, menthol, carvacrol, γ-terpinene 4-ol, and p-cymene in the essential oil of *Perovskia*. These compounds can be responsible for the favorable antioxidant and anti-microbial activities of this plant. Hozoorbakhsh et al. reported the inhibitory effects of *P. abrotanoides* essential oil against *Mycobacterium tuberculosis* (8, 15-18). Another study also showed that the essential oils of *P. abrotanoides* exert antimicrobial activity against *Microsporum gypseum*, *Candida albicans*, *Aspergillus fumigatus*, and *Salmonella typhi* (30).

In a study by Mahboubi and Kazempour, essential oil from leaves of *P. abrotanoides* (15.2 mm on *B. cereus*) showed higher antimicrobial activity compared with the fixed

oil from the stem (8.34 mm on *S. aureus*) and leaves (11.2 mm on *S. aureus*). The study also reported the antimicrobial activity of the essential oil against *C. albicans* and Gram-positive bacteria. In the mentioned study, *Aspergillus niger* and Gram-negative bacteria were the least susceptible tested microorganisms (17).

Phytochemical studies have indicated the presence of monoterpenes and sesquiterpenes such as α-cadinol, 1,8-cineole (eucalyptol), myrcene, pinene, camphor, caryophyllene, humulene, camphene, and bisabolol in high concentration (31). It has been shown that n-hexane and ethyl acetate extracts of *P. abrotanoides* aerial parts have considerable inhibitory effects on the growth of *Leishmania donovani* and *Trypanosoma brucei rhodesiense* (8). In another study, the ethanol extract of *P. abrotanoides* exhibited moderate to high levels of antibacterial activity against *C. albicans, S. aureus, Staphylococcus epidermidis, Bacillus cereus,* and *Enterococcus faecalis* with MIC values of 58, 45, 53, 63, and 85 mg/ml, respectively (32).

In the present study, the highest antimicrobial activity against *Y. enterocolitica* was related to the ethyl acetate (MIC:200, MBC:800) and methanol (MIC:400, MBC:1600) extracts of *P. abrotanoides* collected from habitat 1 in 2019. It seems that these antimicrobial compounds were semi-polar and polar. Since the MBC/MIC value of these extracts was lower than the other extracts, it can be concluded that they may have bactericidal activity (33). In a similar study, MIC values below 100 µg/ml, from 100 to 500 µg/ml, from 500 to 1000 µg/ml, and over 1000 µg/ml indicated good, moderate, weak, and no antimicrobial activity, respectively (31).

The best inhibitory effects on *L. monocytogenes* were caused by the dichloromethane (MIC: 50, MBC:400) and methanol (MIC:100, MBC:800) extracts of *P. abrotanoides* collected from habitat 1 in 2019. These extract act as bacteriostatic antimicrobial compounds (MBC/MIC>4). According to the results, *P. abrotanoides* collected from habitat 1 in 2019 had more antibacterial potential compared with *P. abrotanoides* collected from habitat 2 and 3 in 2018 and 2019, or even habitat 1 in2018. Aoyagi et al. Showed that methanol extract of *P. abrotanoides* had various MIC values (ranging from 78-250 µg/ml) against various pathogens (31). The presence of several known abietane diterpenoids and 11-*O*- and 12-*O*-acetylcarnosic acids in the methanol extract of *P. abrotanoides* has been confirmed (34).

It seems that hexane is not a suitable solvent for extracting compounds with antioxidant activity. In our study, hexane extracts (except for hexane extract of plants collected from habitat 3 in 2019) had weaker antioxidant activity. Overall, extracts of *P. abrotanoides* collected in 2019 had a significantly higher antioxidant potential compared with those from plants collected in 2018 (except for the hexane and dichloromethane extracts of *P. abrotanoides* collected from habitat 2 and 3 in 2018, respectively). It seems that both polar and non-polar compounds in *P. abrotanoides* extracts possess antioxidant activity. However, it is suggested that the number or concentration of polar compounds is more than non-polar compounds since the methanol and ethyl acetate extracts showed a significant DPPH scavenging activity. Similar to our findings, Ghafourian and Mazandarani study reported that *P. abrotanoides* extract had good antioxidant activity, especially in the DPPH method (32). In our study, the extracts of *P. abrotanoides* collected from habitat 3 in 2018 and 2019 showed a greater antioxidant activity than those obtained from plants collected from the other habitats.

Many studies have been carried out on the relationship of plant chemical contents with biological activity and environmental variables in natural and cultivated plant species (35-37). This valuable information has been used to determine the medicinal significance and economic importance of plant products (38). Today, it is known that exposure of plants to environmental stress such as salinity may increase the production of reactive oxygen species, which can lead to cell damage (39, 40). Salt-tolerant plants generally have a better defense mechanism against oxidative stress through antioxidant compounds, which can scavenge reactive oxygen species (41, 42). In the present study, plants from habitat 3 had the highest anti-salinity and antioxidant activity (43), indicating a possible direct relationship between antioxidant capacity and salt tolerance.

CONCLUSION

Our results confirm the antimicrobial and antioxidant activities of various extracts of *P. abrotanoides* grown in three different habitats. It can be concluded that both environmental and genetic factors can affect the quantity and quality of medicinal plants.

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DECLARATIONS

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Ethics approvals and consent to participate

Not applicable since the study did not involve human or animal samples.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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