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Antimicrobial and antioxidant properties of *Anabasis setifera* Moq. and *Caroxylon imbricatum* (Forssk.) Moq.

Mehdi Dehghani Kazemi [1], Zahra Ebrahimnezhad [1], Hamid Beyzaei [2]*

1. Department of Biology, Faculty of Science, University of Zabol, Zabol, Iran
2. Department of Chemistry, Faculty of Science, University of Zabol, Zabol, Iran
* Correspondence: Hamid Beyzaei, Department of Chemistry, Faculty of Science, University of Zabol, Zabol, Iran. Tel: +98-9153255282
Email: hbeyzaei@yahoo.com and hbeyzaei@uoz.ac.ir

Abstract

Background: *Anabasis setifera* and *Caroxylon imbricatum* are two halophytic and xerophytic plant species belonging to the family Chenopoodiaceae *s.str.*, that are distributed widely in dry areas around the world. This study investigated the antioxidant and antibacterial properties of the hydroethanolic extracts of fruit-bearing branches of these species collected from Zabol in the east of Iran.

Methods: The antimicrobial effect was essayed using broth microdilution and streak plate protocols against nine pathogenic microorganisms from different genera, including *Klebsiella*, *Pseudomonas*, *Escherichia*, *Bacillus*, *Staphylococcus*, *Streptococcus*, *Aspergillus*, *Fusarium* and *Candida*. The antioxidant activity was measured by the DPPH free radical scavenging method.

Results: Both extracts could inhibit the growth of all tested bacterial and fungal strains except for *Candida albicans*, which *Caroxylon imbr icatum* didn't exhibit any inhibitory effect against it. The MIC values ranged from 8 to 2048 µg/ml. The IC_{50} values of 76.40 and 154.05 µg/ml were observed with *Anabasis setifera* and *Caroxylon imbricatum* extracts, respectively.

Conclusion: These plant species can efficiently treat infectious and oxidative stress-related diseases due to their broad-spectrum antimicrobial properties and acceptable antioxidant activities.

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Highlights

What is current knowledge?
This study investigated the potent antimicrobial and antioxidant effects of *Anabasis setifera* Moq. and *Caroxylon imbricatum* (Forssk.) Moq. to treat infectious and oxidative stress-related diseases.

What is new here?
The biological properties of *Caroxylon imbricatum* and *Anabasis setifera* collected from Sistan Plain, Iran, have not been studied yet.

Introduction

Microbial infections due to multidrug-resistant bacteria have grown dramatically over the last decades, resulting in millions of deaths (1-4). Unfortunately, the rate of discovering new antibiotics is not keeping pace with resistance development, and many classical antibiotics have lost their efficiency. Antibiotic resistance is a global threat considered as dangerous and deadly as terrorism and global warming (5-7). The scientific community desperately needs antibiotics, especially those with natural origin, and consequently less harmful side effects. Plant organs are one of the most promising sources of natural antibiotics, considering the huge diversity of the plant species and the immense variety of herbal ingredients with various levels of antioxidant and antimicrobial activities. Natural antioxidants are found in plant organs, predominantly in phenolic and flavonoid compounds, scavenging free radicals and preventing oxidative stress and its harmful consequences (8).

The genus *Caroxylon* Thunb. has recently been segregated from the polyphyletic genus *Salsola* L. based on various taxonomical resources, including physiology, morphology, and nuclear and chloroplast markers (9). *Caroxylon* (comes from caro = flesh and xylon = wood) is a predominantly shrubby genus (with only two annual species) in the family Chenopodiaceae *s.str.*, distributed in dry areas of the Old World. Only one introduced species, *S. vermiculata* Botsch., is found in North America (10). *Caroxylon imbricatum* (Forssk.) Moq., (synonym *Salsola imbricata* Forssk.) is a halophytic subshrub (40-100 cm) species distinguished by much-branched woody stems with young branches finely pubescent, tiny succulent leaves, winged fruiting perianth, thin-walled fruit, and vertical seeds. The specific epithet *imbricatum* means overlapping, probably pertaining to the overlapping fleshy leaves. *Caroxylon imbricatum* grows in North Africa, the Arabian Peninsula, and southwestern Asia (11).

Various organs of *Caroxylon imbricatum* contain various bioactive compounds, including phenols, flavonoids, saponins, tannins, terpenoids, cardiac glycosides, and alkaloids. The plant is widely used in various areas, like camel food, contraceptive, expectorant, anthelmintic, antidiarrheal, anti-inflammatory, analgesic, antipyretic, and lightening itchy skin (12-16). Other biological activities reported for *Caroxylon imbricatum* in the scientific literature are antioxidant and antibacterial activities. Phytochemical evaluation of the root of *C. imbricatum* resulted in isolating two new bioactive compounds of biphenylpropanoids and biphenylsalsonoid with moderate activity against DPPH radicals and significant antibacterial activity against both gram-positive and gram-negative bacterial strains (17). The triterpenes Salsolins A and B, isolated from *C. imbricatum*, have also shown significant antioxidant activity (18). Moreover, the methanol extract of the bark of *C. imbricatum* has shown remarkable antibacterial activity against *Bacillus subtilis* (inhibition zone = 40 mm) (19). However, little research has been conducted on the phytochemical profiles and biological properties of *C. imbricatum*, especially in Iran.

Moreover, the genus *Anabasis* L. is a member of Chenopodiaceae *s.str.*, encompassing Perennial to shrub species with articulate and often succulent stems, opposite, fleshy leaves, winged perianth, and vertical seeds. *Anabasis setifera* Moq. (*Anabasis* is derived from a Greek word meaning ascent, and *setifera* means bearing a seta, referring to a seta at the end of the leaves) is a richly branched subshrub up to 50 cm with fleshy, 4-angled young stems and cylindrical leaves bearing a terminal caducous bristle. The plant is distributed from Egypt eastwards to NW India (11).

Studies have shown that the aerial parts of *A. setifera* contain various bioactive compounds and good antioxidant capacity (20). However, no scientific report is available regarding the antimicrobial effects of this plant species in the literature.

This study aimed to to explore the antioxidant and antimicrobial properties of aerial parts of *Anabasis setifera* and *Caroxylon imbricatum* using hydroalcoholic extracts of these environmentally valuable inhabitants of arid regions to discover their potential as future nutritional or pharmacological sources for human beings and animals.

Methods

Plant material collection and extract preparation

The fruit-bearing branches of *Caroxylon imbricatum* and *Anabasis setifera* were collected in October 2022 from Sistan plain (coordination: 31.056111° lat., 61.265278° long., 457m alt.) in the east of Iran. The first author at the University of Zabol Herbarium identified the plant species. The herbarium voucher specimens were deposited in the same herbarium under voucher codes UOZH 1505 for *Caroxylon imbricatum* and UOZH 1506 for *Anabasis setifera*. (Figure 1) shows the examined species' location, habitat, habit, and morphological characteristics.

The aerial parts of plant species were washed with distilled water and then dried separately away from direct sunlight at room temperature and humidity (about 25 °C and 10-15% relative humidity). Since the plant organs were succulent, the air-drying process took 12 days. Then, using an electronic grinder, the dried plant materials were ground into fine powders separately and were kept in the refrigerator for further use. The maceration method extracted the plants' ingredients by soaking 10 g of each sample into 100 ml of hydroethanolic solvent (distilled water and ethanol 1:1) with continuous shaking on a shaker device for 24 h at room temperature in the dark. The mixtures were then passed through a Whatman No. 1 filter paper. The filtrates were allowed to evaporate using a rotary evaporator at 37 °C (21,22).

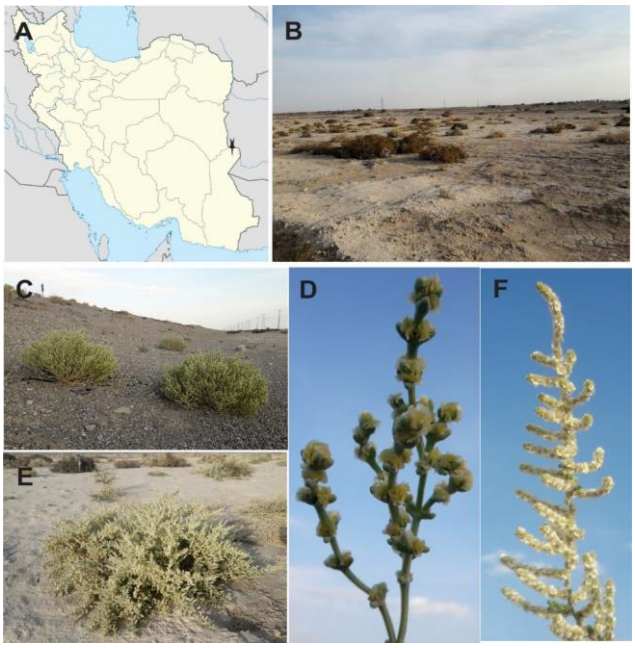

Figure 1. A. the geographical position of the studied plant species. B. the habitat of the species, C. *Anabasis setifera* habit, D. *Anabasis setifera* fruiting branch, E. *Caroxylon imbricatum* habit, F. *Caroxylon imbricatum* fruiting branch

Antimicrobial tests

The antimicrobial activity of plant extracts was investigated against three gram-positive and three gram-negative pathogenic bacteria, including *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and three fungi including *Candida albicans*, *Fusarium oxysporum,* and *Aspergillus fumigatus* from the Persian Type Culture Collection (PTCC), Karaj, Iran. The bacteria and the fungi were inoculated onto nutrient agar and Sabouraud agar, respectively, followed by incubation at 37 °C for 24 h. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) of the extracts were determined using the broth microdilution and streak plate methods, following the M07-A9, M27-A2, M38-A2, and M26-A CLSI (Clinical and Laboratory Standards Institute) protocols (23,24). Each dried extract was dissolved in DMSO at an initial concentration of 40960 µg/ml and two-fold serially diluted to final concentrations 4096, 2048, 1024, 512, 256, 128, 64, 32, 16, 8, 4, and 2 µg/ml in a 96-well microtiter. The microorganism suspensions and broth culture media were added to each microplate well and incubated at 37 °C for 24 h. Finally, the turbidity of the cultures was examined using a plate-reading ELISA reader ELX800 (BioTek Instruments), and the lowest concentration at which the culture was transparent was reported as MIC. Additionally, to determine the MBC and MFC, 10 microliters of the content of each clear well were transferred to Mueller Hinton Agar and Sabouraud agar media, respectively, and incubated for 24 h at 37 °C. The plant extract concentration at which all microorganisms were eliminated was regarded as MBC or MFC. The results were presented as the average of three independent experiments. All values were the same in all three replicates, and thus they have no deviation from the averages. Amoxicillin, gentamicin antibiotics, and terbinafine antifungals were positive controls for the bacteria and fungi.

DPPH free radical scavenging activity

The scavenging activity of the plant extracts on stable 2.2-diphenyl-1-picryl hydrazylradical (DPPH) was assayed following our previous publications (23-25). Plant extracts were prepared at 125, 250, 500, and 1000 µg/ml concentrations. A methanolic 0.004% DPPH was also provided. Then, 1 ml of each hydroalcoholic extract was mixed with 4 ml of DPPH solution in separate tubes and incubated in a dark place for 30 min. The absorbance readings were recorded at 517 nm, where a solution of 4 ml DPPH and 1 ml methanol was used as blank. The absorbance readings took place in three replications for each sample. The inhibition percentage (I%) was calculated as I% = (B-S)/B×100, where B equals the absorbance of the blank sample and S equals the absorbance of tested samples at 517 nm. The IC_{50} values of extracts and vitamin C were determined using the graph of inhibition percentage versus concentration. All tests were repeated three times independently and given as average ± standard deviation.

Results

The inhibitory and lethal potentials of *Anabasis setifera* and *Caroxylon imbricatum* hydroethanolic extracts were determined and compared with those of amoxicillin, gentamicin, and terbinafine (Figure 2).

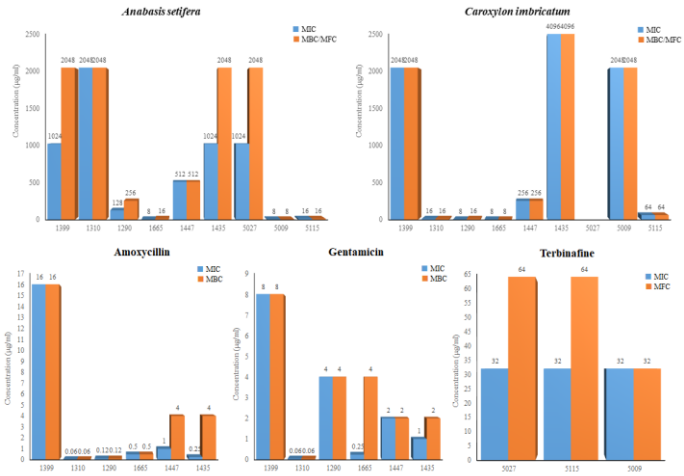

Figure 2. MIC, MBC, and MBC values of extracts and drugs; 1399: *E. coli*, 1310: *P. aeruginosa*, 1290: *K. pneumoniae*, 1665: *B. cereus*, 1447: *S. pyogenes*, 1435: *S. epidermidis*, 5027: *C. albicans*, 5115: *F. oxysporum*, 5009: *A. fumigatus*.

Anabasis setifera extract could inhibit the growth of all tested pathogens in the MICs ranging between 8-2048 µg/ml. All microorganisms except *C. albicans* (PTCC 5027) were inhibited with *Caroxylon imbricatum* extract (MICs of 8-4096 µg/ml). Significant antibacterial effects were observed with *Anabasis setifera* extract against the *B. cereus* (PTCC 1665) strain. These effects were notable in *Caroxylon imbricatum* extract on *P. aeruginosa* (PTCC 1310), *K. pneumoniae* (PTCC 1290), and *B. cereus* (PTCC 1665) strains. *Anabasis setifera* was successful in controlling *A. fumigatus* (PTCC 5009) and *F. oxysporum* (PTCC 5115) at low concentrations, while acceptable antifungal effects of *Caroxylon imbricatum* were seen against only *F. oxysporum* (PTCC 5115).

The antioxidant effects of both extracts were investigated on DPPH free radicals and are shown as IC_{50} values in (Table 1).

Table 1. Radical scavenging effects of extracts and positive control

	Extracts		Control
	Anabasis setifera	*Caroxylonimbricatum*	Vitamin C
IC_{50}(µg/ml)±SD[a]	76.40±1.27	154.05±2.62	3.94±0.33

[a] Standard deviation.

Anabasis setifera showed an increase of more than 50% in antioxidant properties compared to *Caroxylon imbricatum*. Both extracts showed much weaker antioxidant effects than vitamin C.

Discussion

n-Hexane, chloroform, ethyl acetate, and *n*-butanol extracts of *Anabasis setifera* (whole organs) collected from Egypt were assayed for their possible antimicrobial properties (26). Inhibitory activities of extracts at concentrations of 5 and 10 mg/ml for bacteria and fungi, respectively were evaluated against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Candida albicans*, *Aspergillus fumigatus*, *Penicillium italicum*, *Fusarium oxysporum*, *Fusarium s. cucurbitae*, *Fusarium niveum* and *Botrytis cinerrea* strains. The inhibition zone diameters

ranged from 8 to 20.9 mm. The *n*-hexane extract was effective on all tested strains except *P. aeruginosa* and *B. cinerrea*. Chloroform and ethyl acetate extracts could only inhibit the growth of *S. aureus* and *B. subtilis*, respectively. No antimicrobial effect was observed with *n*-butanol extracts on microorganisms. Concludingly, increasing the polarity of the solvents has led to a decrease in the extraction of effective antimicrobial substances. *Anabasis setifera* tested here was effective against all tested bacterial and fungal strains, although a mixture of two polar solvents extracted them. This conflict can be caused by the difference in the geographical area where the plants grow, plant collection season, extracting solvents, and the lack of use of the underground organs of the plant.

The evaluation of the antibacterial effects of the ethyl acetate extract and the isolates extracted from the roots of *C. imbricatum* (collected in November 2015 from Arar, Saudi Arabia) showed that both compounds and ethyl acetate extract possess remarkable antibacterial properties against three gram-positive (*S. epidermidis*, *S. aureus*, *M. luteus*) and three gram-negative (*E. coli*, *P. aeruginosa*, S. typhimurium) bacteria with MIC values ranging from 16 to 64 µg/ml (17). These results generally contradict the findings in this paper since different organs of the plant have been investigated using different solvents. However, it can be speculated that underground parts of *C. imbricatum* show a higher antibacterial effect than aerial organs, which is rational in desert perennial plants that keep their important ingredients underground. To the best of our knowledge, no reports exist on the antifungal effects of *C. imbricatum* in the literature. Besides, the current study confirmed the effectiveness of hydroethanolic extract of this plant on *A. fumigatus* and *F. oxysporum* for the first time.

The antioxidant activity of acetonic and methanolic extracts of the aerial parts of *Anabasis setifera* collected from Qum, Iran, was studied *via* the DPPH radical scavenging method (20). Percentage inhibitions of 28.62 and 40.58 were recorded with acetonic and methanolic extracts, respectively. Both percentage inhibitions were lower than the activity the authors recorded at a concentration of 62.5 µg/ml (49.55%). Although both plants were collected during October, the study's hydroethanolic extract has a higher antioxidant content.

Potent DPPH free radical scavenging property was reported for whole plant ethanolic and methanolic extracts of *Caroxylon imbricatum,* while ethyl acetate and acetone extracts manifested weak antioxidant properties (16). The two bioactive compounds of biphenylpropanoids and biphenylsalsonoid extracted from the root of *Caroxylon imbricatum* showed IC_{50} of 86.5 and 122.3 µg/ml (17), which are predictably lower than that of the hydroalcolholic extract of aerial parts of the plant in this study. These researchers used roots as sources of antioxidants and measured the radical scavenging effect of pure bioactive compounds, while here, the antioxidant activity of crud extract of aerial parts of the plant has been evaluated.

Conclusion

This study emphasized the diverse biological and pharmacological properties of two valuable plant species, *Anabasis setifera* and *Caroxylon imbricatum*. Their hydroethanolic extracts effectively inhibited several important bacterial and fungal pathogens. Extracts had particularly significant inhibitory activities against gram-negative *K. pneumoniae* and gram-positive *B. cereus*. *Anabasis setifera* inhibited the tested fungi at much lower concentrations than *Caroxylon imbricatum*. No remarkable antioxidant effects were observed from the extracts. The findings indicate that these extracts can efficiently prevent and treat bacterial and fungal infectious diseases.

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Ethical statement

Not applicable

Conflicts of interest

The authors declare that they have no competing interests.

Author contributions

Mehdi Dehghani Kazemi contributed to the plant sampling, identification, and manuscript preparation. Zahra Ebrahimnezhad collected the laboratory data. Hamid Beyzaei supervised the data collection and prepared the final version of the manuscript. All authors have approved the final version of the manuscript.

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