

Chemical composition and antibacterial activity of *Zygophyllum qatarense* Hadidi leaf extract

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ABSTRACT

Background and aims: In present study, chemical composition and antibacterial activity of *Zygophyllum Qatarense* Hadidi (ZQ) leaf extract were investigated. This study is the first report of evaluation and identification constituent of ZQ leaf that grown in Iran.

Methods: Phenolic compounds of methanolic extract of ZQ leaf were identified by High Performance Liquid Chromatography-DAD instrument (HPLC-DAD) and antibacterial activities of this plant against *Klebsiella pneumonia* and *Staphylococcus epidermidis* were evaluated by disk diffusion method, MIC and MBC methods

Results: The results showed that this plant contains chlorogenic acid, sinapic acid, caffeic acid, catachin, trans-ferulic acid and rosmarinic acid. The caffeic acid, catachin and rosmarinic acid are significant compound. The results showed methanolic extracts of ZQ leaf have a good potential against *K. pneumonia* and *S. epidermidis*.

Conclusion: ZQ has a wide range of biological properties and can be used in different industries such as pharmaceutical and food production.

Keywords: *Zygophyllum Qatarense* Hadidi (ZQ), Antibacterial activity, phenolic compounds, HPLC-DAD, Persian Gulf

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INTRODUCTION

The genus *Zygophyllum* L. is the largest genus of *Zygophyllaceae*. They comprise about 100 species known from the central Asia, South Africa and Australia.¹ *Zygophyllum Qatarense* Hadidi is a species from the *Zygophyllaceae* family and a plant native to Hormozgan province in Iran.² Hormozgan province is located southeast of Iran. The southern parts of this province are surrounded by warm waters of the Persian Gulf and Oman Sea that make a proper environment for growing of these plants.

The physiological characterization of this plant comprises an ascending accomplished with many branches. They are also highly succulent shrub gets with the height of 75 cm.³ ZQ is a drought resistant plant that grows in saline and non-saline habitats.^{4,5} Abbas has shown that change in soil salinity is reflected in the content of ZQ ash.³ ZQ is using for wound healing, earache, intestinal pain and also it has anti-inflammatory properties.^{2,6} Aqueous, butanol and ethanolic extracts of ZQ shows antimicrobial activity against both gram-negative bacteria *E. coli* and *P. aeruginosa*, two gram-positive *B.cereus* and *S.aureus*, and also two fungal species *C. albicans* and *A. flavus*⁶ Barzegar et al. have showed that methanolic extract of ZQ leaf can be used for synthesis of silver nanoparticles as an efficient green reagent without the need for chemical compounds. They have also showed that methanolic extract of ZQ leaf has antifungal activity against *Aspergillus niger* and *Penicillium digitatum*. They believed that the antifungal and antioxidant activities of methanolic extract can be related to poly hydroxyl organic compounds that possess plant extract.⁷ Phytochemical tests of ZQ were positive for alkaloids, sterols and coumarin.⁸ Phenolic compounds are secondary plant metabolites. They are almost present in all plants⁹ structurally,

phenolic compounds comprise an aromatic ring, they have bearing one or more hydroxyl substituent and they are also containing a wide range of aromatic cycles from simple phenolic molecules to highly polymerized compounds.¹⁰ Besides, Phenolic compounds have shown a series of wide range of biological properties, such as anticancer, antiviral, anti-inflammatory activities, antioxidant activity, anti-allergenic, anti-atherogenic and anti-thrombotic.^{10,11} Anti-microbial¹² Anti-proliferative, vasodilatory effects¹³ and effective cardiovascular disease.¹⁴ So, due to the properties that mentioned above, ease of availability of this plant and our interest in developing and identifying local medicinal plants of Iran we were encouraged to identify phenolic compound of methanolic extract of ZQ leaf by High performance liquid chromatography-DAD instrument (HPLC-DAD) and also we evaluated the antibacterial activity of ZQ leaf extract against *Klebsiella pneumonia* and *Staphylococcus epidermidis* by disk diffusion, MIC and MBC methods. To the best our knowledge this is the first report of evaluation and identification constituent of ZQ leaf that grown in Iran.

ZQ leaf with herbarium code 201-MP-IRAN (Medicinal and Pesticide Plants "IRAN" Herbarium) was Collected and dried from Hormozgan province, Iran. High Performance Liquid Chromatography instrument (HPLC/DAD Analysis), Agilent Technologies 1200 series, was applied for analysis. Gallic acid, Catechin, Caffeic acid, Rutin, Vanillin, *p*-coumaric acid, Trans-ferulic acid, Sinapic acid, Hesperidin, Ellagic acid, Rosmarinic acid, Quercetin, Hesperetin, Eugenol, Carvacrol were purchased as standard from Sigma-Aldrich chemical company and methanol, DMSO, nutrient agar (NA) and nutrient broth (NB) from Merck company. *K. pneumonia* and *S.*

epidermidis were prepared from Shiraz branch, Islamic Azad University.

The extraction of *Zygophyllum Qatarense Hadidi* leaf

ZQ was extracted by maceration method. ZQ leaves (10 gr) and methanol (100 mL, 80%) were mixed in 250 ml beaker. The mixture was shaken for three days. Then after, the methanolic extract was formed, filtered and used without any further purification.

HPLC columns and solvent systems

The Column of HPLC instrument that used this research was Zorbax eclipse C18 (4.6 × 150 mm, 5µm particle Agilent) and the temperature column was at 30 °C and also the flow rate was 1.0 ml/min. The detector of HPLC instrument was DAD and the chromatograms of phenolic compounds were recorded at 320 and 280 nm. The mobile phase was a buffer containing methanol and formic acid 1% in different ratio and time.

Standardization of microbial solution

K. pneumonia and *S. epidermidis* were used for making suspension after specialization and culture in nutrient agar medium. In this experiment, 0.5 McFarland standard solution used for standardization of bacteria suspension of. A 0.5 McFarland standard is 1.5 × 10⁸ CFU/ml. suspensions of bacteria after making in nutrient broth medium were adjusted with 0.5 McFarland standard solutions.

The antibacterial activity by disk diffusion method

Methanolic extract of ZQ leaf and controls (Ampicillin and Erythromycin were as control) were made in 512, 256, 128, 64 µg/ml concentrations with Dimethyl sulfoxide (DMSO) solvent. After that, the disks contain four concentration of treatment was placed in NA medium that contains *K. pneumonia* and *S. epidermidis*. Finally, samples were placed to incubator for 24 hours and at 37 °C. The size of the disks was

5mm. In research was carried out in three replications. Analysis of variance of traits according to factorial method in a totally random design were analysis by using SAS Software and the average of treatments were compared by using Duncan's test in Level 5%.

The minimum inhibition concentration (MIC)

The minimum inhibition concentration of ZQ leaf was evaluated using MIC method. First of all, 95µl of NB solution was added to cells of micro plate rows (Micro plates has 96 cells in 8 rows); then, 100µl of each treatment was added to the first cell of each row. After mixing the contents of the first cells, 100 µl was removed and added to the next cell and so till ninth cells of every row. Finally, 5µl of bacteria suspension was added to all cells of micro plate except tenth cells. The tenth cells of each row were as a control.

The minimum bacterial concentration (MBC)

The minimum bacterial concentration of ZQ leaf was evaluated using MBC method. In this method, all contents of cells from MIC method were cultured in NA medium and after that were placed inside the incubator during 24 hours at 37 °C. The lowest concentration of treatments that 99.9 % of fungal have not growth was considered as minimum *bactericidal* concentration (MBC). [15]

RESULTS

HPLC-DAD analysis

Obtained results of HPLC-DAD chromatogram of the phenolic standards and methanolic extract (Fig 1-4) showed in Table 1. HPLC-DAD chromatogram of methanolic extract of ZQ leaf was compared with chromatogram of the phenolic standards. This comparison showed that methanolic extract of *Zygophyllum*

Qatarense Hadidi leaf contains Sinapic acid (16.46 min), Caffeic acid (11.36 min), Catachin (8.86 min), Trans-ferulic acid (16.27 min) and Rosmarinic acid(19.24 min) at 280 nm and Chlorogenic acid (11.34 min) at 320 nm. (Table 1) The highest quantity of phenolic compounds of this plant

respectively were Rosmarinic acid (86.7681mg/lit), Sinapic acid (65.84166 mg/lit), Catachin (59.36007mg/lit), Caffeic acid (46.77162 mg/ml), Trans-ferulic acid (41.54233 mg/lit), Chlorogenic acid (21.57132 mg/ml) (Table 1).

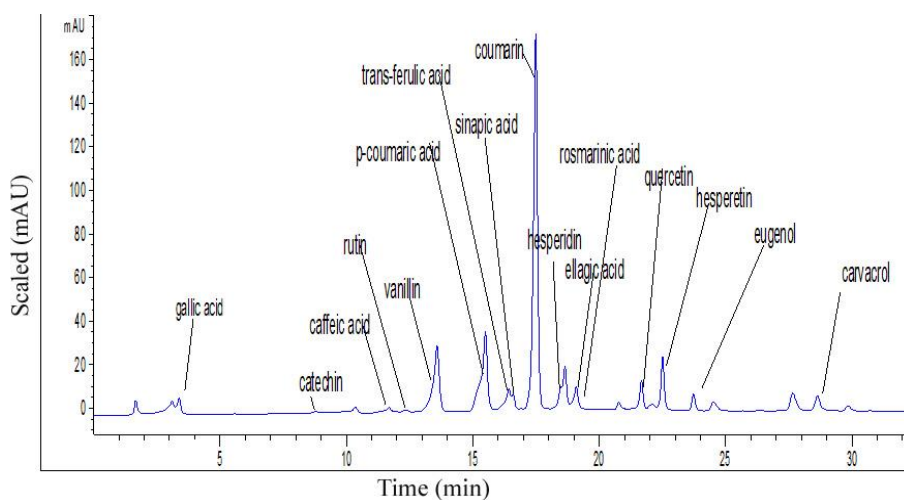
Table 1. Various phenolic compounds in methanolic extract of ZQ leaf measured by HPLC-DAD method.

Constituent of Phenolic compounds in <i>Zygodhylum Qatarense Hadidi</i>	Retention time (St) ^a (min)	Retention time (Ext) ^b (min)	amount of Phenolic compounds (mg/lit)
Rosmarinic acid	19.17	19.24	86.7681
Sinapic acid	16.54	16.46	65.84166
Catechin	8.59	8.86	59.36007
Caffeic acid	11.35	11.36	46.77162
Trans-ferulic acid	16.38	16.27	41.54233
Chlorogenic acid	10.35	10.54	21.57132
Rutin	12.42	ND ^c	ND
Quercetin	21.6	ND	ND
p-Coumaric acid	15.96	ND	ND
Carvacerol	28.48	ND	ND
Vanilin	13.54	ND	ND
Hesperedin	18.59	ND	ND
Ellagic acid	19.02	ND	ND
Eugenol	23.74	ND	ND
Hesperetin	22.45	ND	ND
Gallic acid	3.39	ND	ND

Table 2: The inhibition zone size of *Zygophyllum Qatarense Hadidi* leaf against *Klebsiella pneumoniae* and *Staphylococcus epidermidis* in millimeter and the results of MIC and MBC method in $\mu\text{g/ml}$

	Disk diffusion method				MIC	MFC
	(mm \pm SD)				($\mu\text{g/ml}$)	($\mu\text{g/ml}$)
<i>Klebsiella pneumoniae</i>						
	512 $\mu\text{g/ml}$	256 $\mu\text{g/ml}$	128 $\mu\text{g/ml}$	64 $\mu\text{g/ml}$		
Methanolic extract of <i>Zygophyllum Qatarense hadidi</i>	14.0 \pm 0.0	13.70 \pm 0.65	12.93 \pm 0.11	11.50 \pm 0.50	64	64
Ampicillin	14.0 \pm 0.0	13.90 \pm 0.79	12.50 \pm 0.5	13.0 \pm 1.0	64	128
<i>Staphylococcus epidermidis</i>						
Methanolic extract of <i>Zygophyllum Qatarense hadidi</i>	14.00 \pm 0.0	13.25 \pm 0.25	12.93 \pm 0.11	11.93 \pm 0.11	64	64
Erythromycin	14.50 \pm 0.5	14.25 \pm 0.25	13.25 \pm .25	13.0 \pm 0.0	4	16

Ampicillin and Erythromycin were as a control. DMSO did not show any antibacterial activity

**Figure I:** HPLC-DAD chromatogram peaks of phenolic standards at 280 nm

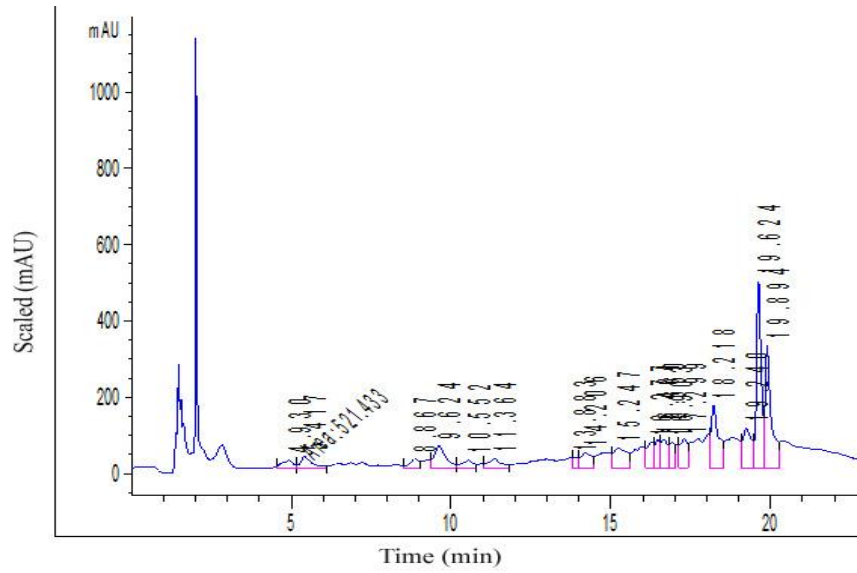


Figure II. HPLC-DAD chromatogram peaks of *Zygophyllum Qatarense Hadidi* leaf at 280 nm

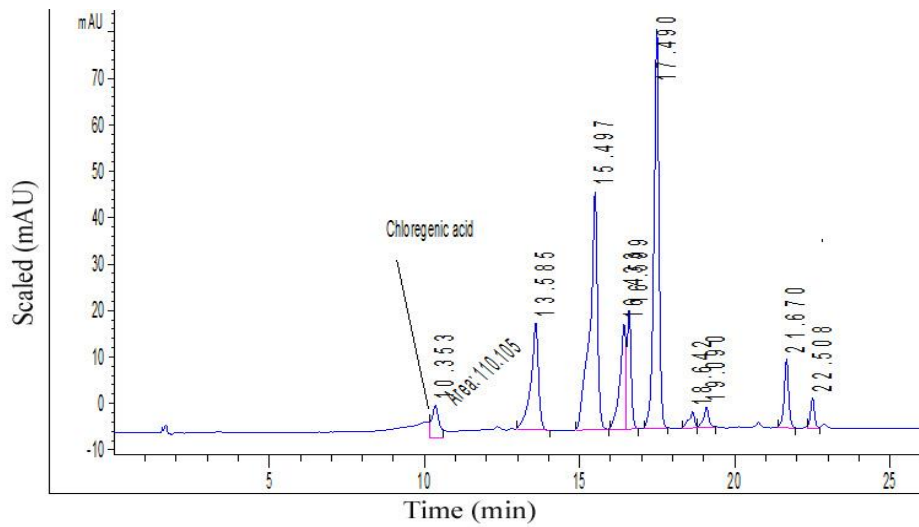


Figure III. HPLC-DAD chromatogram peak of phenolic standards in retention time for chlorogenic acid (10.35 min) at 320 nm

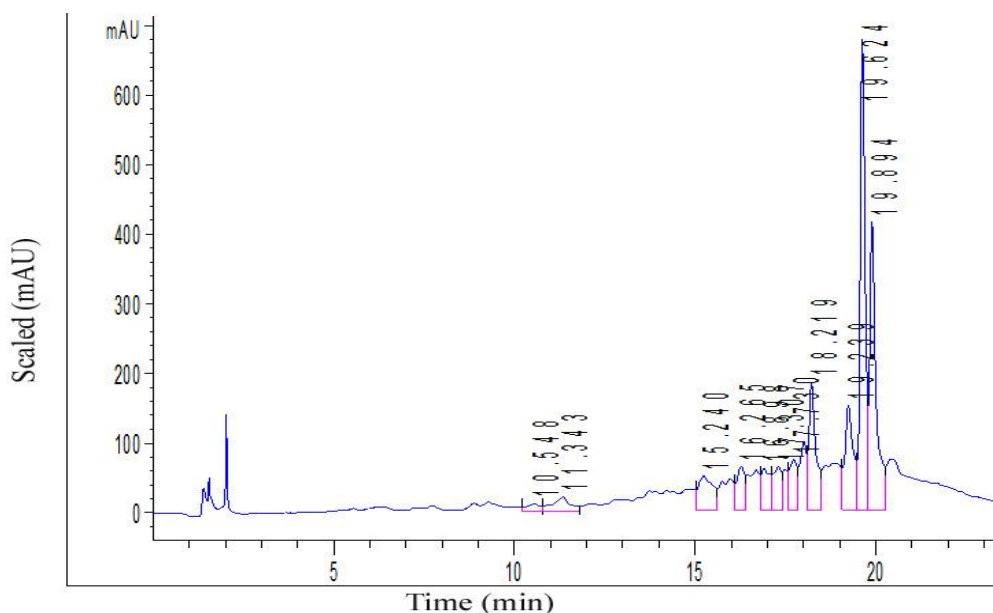


Figure IV: HPLC-DAD chromatogram peak of *Zygophyllum Qatarense Hadidi* leaf in retention time for chlorogenic acid (10.54 min) at 320 nm

The antibacterial activity

The results of antibacterial activity by disk diffusion method showed that the inhabitation zone size of methanolic extract of ZQ against *K. pneumonia* in 512, 256, 128 and 64 $\mu\text{g/ml}$ concentrations were 14.0 ± 0.0 mm, 13.70 ± 0.65 , 12.93 ± 0.11 and 11.50 ± 0.50 mm, respectively and against *S. epidermidis* were 14.00 ± 0.0 mm, 13.25 ± 0.25 , 12.93 ± 0.11 mm and 11.93 ± 0.11 (Table 2). Also the results of antibacterial activity of methanolic extract of ZQ against *K. pneumonia* and *S. epidermidis* by MIC method was 64 $\mu\text{g/ml}$ and by MBC method was 64 $\mu\text{g/ml}$ (Table 2).

Discussion

Phytochemical investigation of the genus *Zygophyllum* shows that it's very rich in saponins and quinovic acids and also flavonoids and alkaloids were identified from this genus¹⁶ and based on studied *Zygophyllum album*, *Zygophyllum simplex*

and *Zygophyllum fabago* that are from the *Zygophyllum* genus contains flavonoids and phenolic acid.^{17,18,19} In present study was proved that ZQ are contains some phenolic acids like other species from the genus *Zygophyllum*. A large number of these phenolic compounds have been identified as strong antioxidant compounds.²⁰ The antioxidant activity of phenolic compounds is related to the position of hydroxyl groups and their number. The nature of substitutions on aromatic rings can be increased electron delocalization of formed radical intermediates and consequently stabilize free radical.^{10,20} The efficiency of rosmarinic acid and caffeic acid is higher than other phenolic compounds due to their unique chemical structures according to the above mentioned.²⁰ In plants, rosmarinic acid is supposed to act as a preformed constitutively accumulated defense compound and has a numerous interesting biological activities, such as antiviral,

antibacterial, anti-inflammatory and antioxidant.²¹ Recent studies have clearly showed that caffeic acid is an effective antioxidant among polyphenolic bio-resource compounds as an in vitro antioxidant assays.⁹ Besides, biochemical in vitro studies have also reported catechin, helps to prevent oxidation of plasma low-density lipoprotein (LDL).²² So phenolic compounds that reside in ZQ like certain species of *Zygophyllum* are very significant and can be used in wide range of pharmaceutical industries.

Also the results of antibacterial activity of ZQ against *K. pneumonia* and *S. epidermidis* by three method and comparison with Ampicillin and Erythromycin (were as control) showed that this plant has antibacterial activity against *K. pneumonia* and *S. epidermidis*. Based on studied many plants belong to the genus *Zygophyllum* have been shown various biological effects.²³ like *Z. album*.²⁴ *Z. simplex*¹⁸ and *Z. fabago*.¹⁶ In a study, Mahasneh investigated antibacterial and antifungal activity of ZQ against both gram-negative bacteria *E. coli* and *P. aeruginosa*, two gram-positive *B.cereus* and *S.aureus* also two fungal species *C. albicans* and *A. flavus*. Also Barzegar et al investigated antifungal activity of ZQ against *A. niger* and *P. digitatum*. Their result showed that ZQ extract has antimicrobial activity against some microbes.^{6,7} Generally our research and other reported this plant have good potential against different bacteria and fungi. These activities related to phenolic compounds present in ZQ that in this study identified by HPLC method.

CONCLUSIONS

The results showed that methanolic extracts of ZQ leaf contains Chlorogenic acid, Sinapic acid, Caffeic acid, Catachin, Trans-ferulic acid and Rosmarinic acid. The

Rosmarinic acid, Caffeic acid and Catachin are significant compounds in methanolic extracts of ZQ leaf. Also the results of antibacterial activity of ZQ against *K. pneumonia* and *S. epidermidis* by three method were shown this plant have good potential against *K. pneumonia* and *S. epidermidis*. Based on this research and other reported this plant has a wide range of physiological and biological properties, and can be used in different industries such as pharmaceutical and food production.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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