Determination of Active Biomarkers and the Antioxidant and Antibacterial Potential of Standardized *Zygophyllum spp* Extract

Mostafa Alamholo [1]

Abstract

This study aimed to identify the chemical composition, and investigation of antioxidant and antibacterial activity of *Zygophyllum fabago*, *Zygophyllum eurypterum*, *Zygophyllum propinquum* and *Zygophyllum megacarpum* extracts against human pathogenic bacterial. The samples were collected from West Azarbaijan province and analyzed in the Bu Ali Sina University, Iran biotechnology department. The antibacterial activity by agar well diffusion assay, minimum bactericidal concentrations (MBCs) and minimum inhibitory concentrations (MICs) by the serial dilution method and free radical activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were measured. Next, the phenolic and flavonoid contents were calculated by Folin-Ciocalteu and Aluminum Chloride methods, respectively, and the presence of the phytochemical compounds including alkaloids, saponins, and tannins were tested. In addition, chemical compositions analysis was done using a GCMS. The major components including as ar-curcumene (17.18%), methyl ester (21.53%), caryophyllène (17.07%), and carvacrol (23.71%) were dominant in *Z. fabago*, *Z. eurypterum*, *Z. propinquum and Z. megacarpum*, respectively. The highest sensitivity was observed on *S. epidermidis* with MIC of 3.125% on flower extract of *Z. fabago*. The most potent radical scavenging activity belonged to the flower extract of *Z.megacarpum*. The highest phenolic and flavonoid contents were obtained in *Z. fabago* root extract as 301.04 mgGA/DWg and 8.04 mgQ/DWg, respectively, and carvacrol was determined as the dominant compound. Based on the findings, *Zygophyllum spp* can be suggested for producing natural drugs and antimicrobial agents.

Keywords: Antibacterial, chemical composition, pathogenic bacteria, *Zygophyllum spp*

Significance | Identification of active compounds in medicinal plants to control resistant bacteria

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Introduction

Zygophyllum spp belongs to *Zygophyllaceae* and contains 25 genera and 240 species and is a perennial herbaceous. Moreover, its region is related to southwestern and central parts of Asia, south of Europe and north of Africa (Nickavar et al., 2005). Due to appearance of adverse effects and incompatibility of the synthetic compounds on human nature, scientists have been focused on herbal plants (Tigrine et al., 2011). The antimicrobial compounds level in the flowering and maturity stages is higher than others, and secondary metabolites are produced continuously or in response to elicitores in plants (Olthof et al., 2001). The important and dominant compounds with antimicrobial and antioxidant properties including carvacrol, caryophyllene, β-caryophyllene, thymol and camphor have been reported from *Zygophyllum spp*.

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Carvacrol is a best compound in genus *Zygophyllum* and is a natural monocyclic monoterpenoid with antioxidant, antifungal, anticancer, antibacterial, as well as theirs anti-inflammatory, antidiabetic, and neuroprotective properties have been reported (Davison &Wargo, 2001). Caryophyllene is a common constituent in the genus *Zygophyllum* and is a therapeutic target for treatment of diseases including as inflammation, atherosclerosis, and osteoporosis (Friedman, 2014) as well as β-caryophyllene useful for colitis, osteoarthritis, and diabetes (Raina et al., 2006). Moreover, another dominant compound in this genus is camphor witch has been used as a cold remedy for the relief of chest congestion (Chowdhury et al., 2008). In addition, the antibacterial, antifungal, antioxidant and anticancer properties of thymol and eugenol also have been reported (Salman et al., 2012).

Biological studies on Zygophyllum species have indicated various bioactive potentials including antioxidant, antidiabetic, antimicrobial, antitumor and anti-inflammatory effects. The antieczema, antispasmodic and hypoglycemia of *Zygophyllum gaetulum* has been reported (Bellakhdar et al., 1991). *Zygophyllum album* is used to treat rheumatism, asthma and skin cancer as well as Megdiche et al. (2013) evaluated the antioxidant activity and anti-proliferative capacity of the methanol, and ethyl acetate extract of Tunisian *Z. album* shoots. The antimicrobial, antioxidant and anti-inflammatory activity of *Zygophyllum fabago* has been reported (Zaidi &Crow, 2005; Yaripour et al., 2017). Based on Feng et al. (2009), the antibacterial and antioxidant properties as well as toxicity activity of *Zygophyllum simplex* has been reported. *Zygophyllum geslini* for treatment of diabetes, and *Zygophyllum decumbens* for treatment of rheumatism, fever and hypotension are used (Jaouhari et al., 2000).

Scientists and microbiologists are always trying to find compounds with antioxidant activity to reduce the effects of free radicals on the human body. Natural antioxidants have been replaced with synthetic antioxidants due to their toxic effects (Kumaran & Karunakaran, 2006). Moreover, 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a type of stable organic radical and the potent of biological reagents to scavenge the DPPH radical can be suggested as antioxidant reagent (Deng et al., 2011). The secondary metabolites including flavonoid, monoterpene, triterpenoid, sesquiterpenoid, anthocyanin, flavonoid, and isoflavonoid compounds have been reported from *Zygophyllum spp* (Ahmed et al., 2015). The anticancer and antiradical properties of secondary metabolites including flavonoid and phenol which reduce the risk of cardiovascular disease in human has reported (Nickavar et al., 2005). Dichloromethane extract of *Z. album* showed the highest antioxidant activity (IC50= 57 µg/ml) and anticancer capacity against human lung carcinoma (A-549), colon adenocarcinoma (DLD-1) (Ksouri et al., 2013), and hepatocellular carcinoma (HepG2) (El-Attar et al., 2019) cells (IC50 = 37, 48, and 27.74 µg/ml, respectively).

This research aimed to identify the bioactive compounds, antioxidant and antibacterial properties of *Zygophyllum fabago, Zygophyllum eurypterum, Zygophyllum propinquum* and *Zygophyllum megacarpum* methanol extracts against some human infectious bacteria under in vitro conditions.

Materials and Methods

The culture media including Mueller-Hinton Agar (MHA) Nutrient Broth (NB), and the antioxidant materials such as DPPH, Quercetin (Q) and Gallic acid (GA) from Merck Co. (Germany) as well as gentamycin and clindamycin antibiotics from Paten Tab Co. (Iran) were provided. The tested organs including root and flower of *Z fabago, Z. eurypterum, Z. propinquum* and *Z. megacarpum* were collected from West Azerbaijan province, Iran. Moreover, a volume of 300 mL of the methanol was mixed with 30 g of the powder and were shaken. Finally, the obtained extracts were centrifuged at 10000 rpm for 6 min and transferred to an oven at 37˚C as well as the crude extract was stored at -24°C (Fuselli et al., 2008).

Bacterial suspension

The sensitivity and resistance of gram-positive bacteria including *Enterococcus faecalis* (PTCC-1195), *Staphylococcus epidermidis* (ATCC1054), *Arcanobacterium haemolyticum* (ATCC3389) and *Staphylococcus saprophyticus* (ATCC7791), and the gram-negative bacteria including *Proteus mirabilis (PTCC-1287,) Neisseria meningitides (PTCC-4578), Acinetobacter baumannii (PTCC-4413)*, and *Klebsiella pneumoniae* (PTCC-1129) were tested against extracts. However, a loop of cultured bacterial colony obtained on the MHA medium was transferred to the NB medium and incubated. Next, the bacterial suspension equivalent 0.5 McFarland standard as 1.5×10^8 CFU was prepared.

Antibacterial test

The antibacterial activity of the root and flower methanol extracts of *Z. fabago, Z. eurypterum, Z. propinquum* and *Z. megacarpum* by agar well diffusion assay as100 and 200 mg mL^{-1} from the crude extract was studied. In addition, a bacterial suspension (1.5×10^8 CFU) as 250 mL was spread on MHA medium. Then, for incubation, a volume of 100 µL of each extract was transferred into wells with 5 diameters (Yang et al., 2018). Moreover, methanol as negative control and clindamycin and gentamycin antibiotics were selected as positive control. Finally, the collected data were analyzed by SAS 9.3 software with three replications based on millimeter (mm).

Minimum Inhibitory and Minimum Bactericidal Concentration (MIC and MBC)

The serial dilution assay of methanol extract was used to measure MIC and MBC. Accordingly, the dilution series including 100, 50,

25, 12.5, 6.25, and 3.125 mg mL-1 were used for MIC test. Next, a volume of 200 µL of the extract of 200 mg mL^{-1} mixed with 185 µL of the NB and then, 200 µL from the first tube was transferred to the second tube, followed. For incubation, the bacterial suspension as 15 µL was added to all tested tubes. The lowest dilution was detected as MIC with lack of bacterial growth. Finally, the tubes with lack of bacterial growth were selected to measure MBC (Shojaemehr et al., 2020).

Antioxidant activity by DPPH

To investigate the antioxidant activity, different concentrations including as 0.2, 0.4, 0.6, 0.8 and 1 mg mL^{-1} were prepared from the root and flower methanol extract of *Z. fabago, Z. eurypterum, Z. propinquum* and *Z.megacarpum*. Moreover, the used standard as ascorbic acid and the samples absorption at 517 nm using a spectrophotometer as well as the free radical scavenging activity (RSA) (%) was calculated as follow (Stojicevic et al., 2008):

RSA (%) =100 (1 - (As - Ab)/Ac As: Sample Ab: Blank (methanol 99%) Ac: Control

Determination of flavonoid and phenolic contents

The flavonoid and phenolic contents were calculated with Aluminum Chloride and Folin–Ciocalteu methods, respectively. Accordingly, the sample absorption for measure the flavonoid content at 415 nm as mgQ/gDW as well as, for calculate the phenolic content at 765 nm as mgGA/gDW through spectrophotometer were done (Choi et al., 2002).

Confirming the presence of secondary metabolites

To determine the exist of alkaloid, a volume of 5 mL HCl (1.0%) mixed with 0.5 g of the methanol extract and then for 5 minutes kept in a warm water and was crossed by the filter paper. Finally, the exist of alkaloid was detected as the turbidity or sediment. Moreover, to determine the exist of tannin, a volume of 5 mL distilled water mixed with 0.5 g of the methanol extract and then crossed by a filter paper and a few drops of FeCl3chloride (10%) were added. Next, the exist of tannin was detected as black-green color. To determine the exist of saponin, 0.25 g of the methanol extract mixed with a volume of 20 mL of distilled water and then crossed through a filter paper and finally, the exist of saponin detected as stable foam on the paper (Uko et al., 2001).

Gas Chromatograph Mass Spectrometry (GCMS)

The chemical compositions of *Z. fabago, Z. eurypterum, Z. propinquum* and *Z.megacarpum* methanol extracts were analyzed by GCMS (Urmia University, Iran). The GCMS analysis was performed by an Agilent 6890N coupled to Agilent S973 mass detector as well as initial temperature of 275 °C kept for 2 min. Moreover, the temperature was rose to 120 °C and then, injection port temperature was determined as 350°C and the helium flow rate at 0.9 $mLmin^{-1}$. Next, a volume of sample as 1 µl was injected through split/splitless mode.

Statistical analysis

For data analysis a completely randomized design was used. Then, the average comparisons done by the Duncan test at ($p<0.05$) by SAS 9.3 software with three replications.

Results

Antibacterial activity

The agar well diffusion method was used to determine the antibacterial properties. To detect the inhibitory zone was used of *Z. fabago, Z. eurypterum, Z. propinquum and Z.megacarpum* methanol extract to infectious bacteria which are represented in Table 1. Totally, the inhibition zone rised by increasing extract concentration, as well as the most susceptibility was exhibited on gram-positive than gram-negative bacteria for tested extracts.

The most sensitivity was demonstrated on *S. epidermidis* against flower methanol extract of *Z. fabago* as 22.8±0.66 mm as well as *N.meningitides* showed resistant. Accordingly, flower methanol extract exhibited better inhibitory effect compared to root extract. The most susceptibility was measured on *E. faecalis* against root methanol extract of *Z. eurypterum* as 21.5±00 mm as well as *N. meningitides* and *P. mirabilis* showed resistant. Additionally, methanol extract of *Z. eurypterum* showed an inhibitory effect on all gram-positive bacteria. The flower and root extracts of *Z. propinquum* demonstrated the inhibitory effect against all tested bacteria. In addition, the most susceptibility was obtained on *S. saprophyticus* against flower extract as18.5±0.88 mm. Furthermore, the root extract of *Z. megacarpum* showed the strong antibacterial effect as 20±0.66 mm on *A. haemolyticum* as well as all gram negative bacteria showed resistant against *Z. megacarpum* extracts.

Determination of MIC and MBC

As shown in Table 2, MIC and MBC of *Z. fabago* flower extract against *S. epidermidis* of 3.125% and 6.25%, as well as MIC and MBC of *Z. eurypterum* root extract against *E. faecalisas* of 6.25% were demonstrated, respectively. MIC and MBC of *Z. propinquum* flower extract against *E. faecalisas* of 6.25% and 12.5%, as well as MIC and MBC of *Z. megacarpum* root extract against *A. haemolyticum* of 6.25% were observed, respectively. Next, MBC didn't show on the flower extract of *Z. megacarpum* against all tested bacteria. Totally, *K.pneumoniae* demontrated resistant against tested extracts.

Investigation of antiradical activity through DPPH

Inhibition percentage of DPPH in different concentrations of tested extracts is represented in Table 3. Accordingly, the increase in the concentration of the tested extracts showed a direct relationship with the inhibition rate of free radicals. The most and lowest potent radical scavenging activity was observed in flower extract of *Z. megacarpum* and root extract of *Z. fabago,* respectively. Moreover, ascorbic acid was used as control as well as a significant difference was observed between the IC50 values of

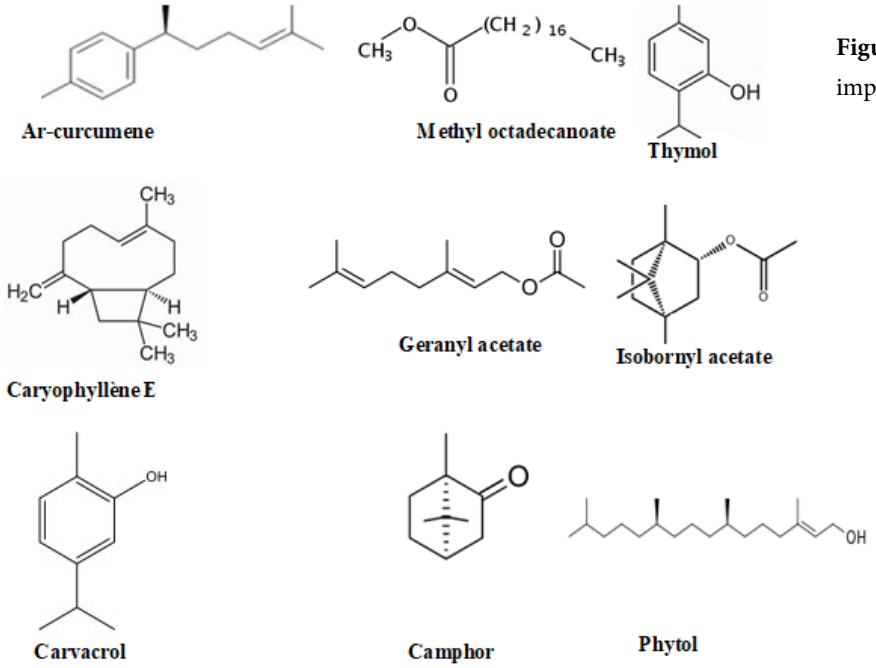

Figure 1. The chemical structures of some important bioactive compounds

Table 1. Inhibitory zone diameters (mm) of *Z. fabago, Z. eurypterum, Z. propinquum* and *Z. megacarpum* extracts against human infectious bacteria

Species	Organ	Con	*E. faecalis*	*S. epidermidis*	*A. haemolyticum*	*S. saprophyticus*	*P. mirabilis*	*N. meningitides*	*A. baumannii*	*K. pneumoniae*
Z. fabago	Root	100	$10.3\pm.33^{g}$	11 ± 0.88^{fg}	10.5 ± 1.2^{g}	-	9 ± 0.88^{gh}	-	-	-
		200	12 ± 0.66^{f}	14.5 ± 0.88^{e}	10 ± 00^{g}	-	10 ± 0.33^{g}	-	-	-
	Flower	100	11 ± 0.33^{fg}	17 ± 0.22^{cd}	13.4 ± 0.33^{ef}	12 ± 00^{f}	9 ± 00^{gh}	-	12 ± 0.33^{f}	11.2 ± 0.33^{fg}
		200	13.2 ± 0.6^{ef}	22.8 ± 0.66^{a}	15 ± 00^{de}	12.5 ± 0.66^{f}	12 ± 0.88^{f}	-	14 ± 0.88^{e}	13 ± 00^{ef}
Z. eurypterum	Root	100	16 ± 00^{d}	$12.3\pm.33^{f}$	$14\pm.0.33^{e}$	$10\pm.0.66^{g}$	-	-	$12\pm.0.66^{f}$	$12\pm.0.22^{f}$
		200	21.5 ± 00^{ab}	15 ± 0.88^{de}	$16\pm.00^{d}$	$11\pm.0.33^{fg}$	-	-	$12.5\pm.0.66^{f}$	$12.9\pm.0.88^{f}$
	Flower	100	14 ± 0.33^{e}	$12\pm.00^{f}$	$11\pm.0.33^{fg}$	11 ± 0.33^{fg}	-	-	$12\pm.0.33^{f}$	-
		200	15.6 ± 0.8^{de}	$15.2\pm.0.66^{de}$	$11\pm.0.88^{fg}$	12.5 ± 0.88^{f}	-	-	$13.5\pm.0.2^{ef}$	-
Z. propinquum	Root	100	8 ± 0.33^{h}	9 ± 0.55^{gh}	8.5 ± 033^{h}	11 ± 0.66^{fg}	7.5 ± 0.33^{hi}	8 ± 0.55^{h}	7.2 ± 66^{hi}	$8\pm.0.33^{h}$
		200	9 ± 00^{gh}	11 ± 0.88^{fg}	12 ± 0.88^{f}	14 ± 0.2^{e}	9±00gh	10.2 ± 0.88^{g}	10 ± 0.88^{g}	$8.5\pm.0.66^{h}$
	Flower	100	9.2 ± 0.33^{gh}	11 ± 00^{fg}	12 ± 0.88^{f}	13.5 ± 0.66^{ef}	8.2 ± 0.66^{h}	11 ± 00^{fg}	9 ± 0.88^{gh}	$9\pm.0.55^{gh}$
		200	10.5 ± 0.6^{g}	11.2 ± 0.33^{fg}	14 ± 0.55^{e}	18.5 ± 0.88^{c}	9.5 ± 0.6^{gh}	11.2 ± 0.66^{fg}	10.6 ± 0.66^{g}	$10.8\pm.1.2^{g}$
Z.megacarpum	Root	100	12 ± 0.33^{f}	13 ± 0.88^{ef}	15.6 ± 0.55^{de}	10 ± 0.2^{g}	11 ± 0.33^{fg}	10.8 ± 00^{g}	9 ± 0.33^{gh}	9.5 ± 0.88^{gh}
		200	12.6 ± 0.66^{f}	15 ± 0.33^{de}	20 ± 0.66^{b}	11 ± 0.66^{fg}	12.5 ± 0.6^{f}	12 ± 0.66^{f}	10.2 ± 0.88^{g}	12 ± 0.33^{f}
	Flower	100	11 ± 0.88^{fg}	12.8 ± 0.66^{f}	14 ± 0.33^{e}	-	-	-	-	-
		200	12.3 ± 00^{f}	$13.3\pm0.1.2^{ef}$	14 ± 0.88^{e}	-	-	-	-	-
Clindamycin as control			16 ± 0.88^{d}	16 ± 0.33^{d}	19 ± 0.33^{bc}	15 ± 0.88^{de}	18.5 ± 0.57^{c}	23 ± 0.57^{a}	19 ± 0.33^{b}	18 ± 1^{c}
Gentamycin as control			19 ± 0.66^{bc}	21.1 ± 0.88^{ab}	22.4 ± 0.33^{a}	22 ± 0.55^{a}	23 ± 0.33^{a}	19.7 ± 0.88^{bc}	22 ± 0.66^{a}	23.1 ± 0.33^{a}

Table 2. MIC and MBC (mg mL^{-1}) of *Z. fabago, Z. eurypterum, Z. propinquum* and *Z.megacarpum* extracts against human infectious bacteria

Species	Organ		*E. faecalis*	*S. epidermidis*	*A. haemolyticum*	*S. saprophyticus*	*P. mirabilis*	*N. meningitides*	*A. baumannii*	*K. pneumoniae*
Z. fabago	Root	MIC	50	6.25	50	100	100	-	-	-
		MBC	100	12.5	50	-	-	-	-	-
	Flower	MIC	25	3.125	50	100	100	100	100	-
		MBC	50	6.25	100	100	100	-	100	-
Z. eurypterum	Root	MIC	6.25	50	50	-	100	-	100	-
		MBC	6.25	50	100	-	-	-	100	-
	Flower	MIC	25	100	100	-	-	100	100	-
		MBC	50	-	-	-	-	100	-	-
Z. propinquum	Root	MIC	50	50	100	50	100	-	-	-
		MBC	50	100	100	50	100	-	-	-
	Flower	MIC	6.25	100	50	12.5	50	-	100	-
		MBC	12.5	100	-	12.5	100	-	100	-
Z.megacarpum	Root	MIC	50	50	6.25	100	-	100	100	-
		MBC	100	100	6.25	100	-	100	100	-
	Flower	MIC	100	100	50	-	-	100	-	-
		MBC	-	-	-	-	-	-	-	-

Table 3. The inhibition percentage of DPPH and IC50 values of *Z. fabago, Z. eurypterum, Z. propinquum* and *Z. megacarpum* methanol extracts

Species	Organ	Inhibition percentage of DPPH (mg mL^{-1})					IC50
		0.2	0.4	0.6	0.8	1	
Z. fabago	Root	86.25	89.36	92.39	94.12	97.27	0.3508^{a}
	Flower	89.87	92.78	94.08	97.17	98.01	0.2581^{b}
Z. eurypterum	Root	91.25	92.39	94.58	94.89	96.61	0.1907^{c}
	Flower	90.25	91.27	94.27	95.98	96.01	0.1809^{c}
Z. propinquum	Root	89.12	90.83	92.57	94.09	95.20	0.1503^{d}
	Flower	90.35	91.25	93.38	94.08	95.81	0.1604^{d}
Z.megacarpum	Root	87.24	89.55	92.18	95.68	99.02	0.2905^{b}
	Flower	91.99	93.25	94.50	95.02	97.04	0.1080^{e}
Ascorbic acid		91.3	92.41	96.58	98.47	99.13	0.1091^{e}

Note. The different letters shown significantly different via Duncan test at $p<0.05$

Table 4. The phenol and flavonoid contents of *Z. fabago, Z. eurypterum, Z. propinquum* and *Z. megacarpum* extracts

Species	*Z. fabago*		*Z. eurypterum*		*Z. propinquum*		*Z.megacarpum*	
Organ	Root	Flower	Root	Flower	Root	Flower	Root	Flower
Phenol (mgGA/DWg)	301.04 ± 0.33^{a}	280.12 ± 0.22^{a}	268.07 ± 0.66^{a}	198.12 ± 0.88^{b}	201.08 ± 0.55^{b}	145.25 ± 0.33^{c}	108.09 ± 0.66^{d}	98.07 ± 0.57^{d}
Flavonoid (mgQ/DWg)	8.04 ± 0.57^{a}	7.58 ± 1.2^{a}	7.19 ± 0.88^{b}	6.81 ± 0.33^{b}	3.07 ± 0.57^{d}	3.98 ± 0.66^{d}	4.17 ± 0.88^{c}	5.89 ± 0.66^{c}

Note. The different letters shown significantly different via Duncan test at $p<0.05$

Table 5. The presence of alkaloids, saponins, and tannins in *Z. fabago, Z. eurypterum, Z. propinquum* and *Z. megacarpum* extracts.

Species	*Z. fabago*		*Z. eurypterum*		*Z. propinquum*		*Z.megacarpum*	
	Root	Flower	Root	Flower	Root	Flower	Root	Flower
Alkaloid	+	+	+	+	+	+	+	+
Saponin	-	-	+	+	+	+	-	-
Tannin	+	+	-	-	+	+	+	+

Note: Presence +, Absence –

Table 6. Identified compounds of methanol extracts of *Z. fabago, Z. eurypterum, Z. propinquum* and *Z.megacarpum* by GCMS.

Z. fabago	Compound content (%)	*Z. eurypterum*	Compound content (%)	*Z. propinquum*	Compound content (%)	*Z.megacarpum*	Compound content (%)
Decene	1.35	Glucopyranosyl ester	0.58	α-Terpineol	2.37	Nonanal	0.22
Lavandulol	2.1	Quinovic acid-3-O-β-D-quinovopyranoside	1.35	Isoquercetin	1.11	Decene	4.99
(Z)-α-Damascone	1.08	Atricarpan	1.06	Androsin	2.36	Carvacrol	23.71
(Z)-β-Damascone	0.38	Atriplicosaponin	2.36	Isorhamnetin-3, 7-diglucoside	2.01	Aminocaproic acid	1.27
Liguloxide	1.58	Ursolic acid	2.89	Hyacinthine	0.35	Thymol	7.17
Diethyl phatalate	5.13	Zygophylloside S	3.87	Cocolactone	13.22	Eugenol	
α-Cadinol	1.02	β –sitosterol	2.08	Delta decalactone	10.23	2,6-Di(tert-butyl) phenol	9.06
Geranyl valerate	7.05	Rutin	8.15	Isobornyl acetate	14.28	Camphor	8.19
β-Bisabolenol	1.48	Kaempferol	3.07	α-Copaene	1.44	Isobornéol	5.88
ar-Curcumene	17.18	Isorhamnetin	2.81	Caryophyllène	17.07	Bornyl acetate	5.22
Bicyclogermacrene	0.28	Eicosane	1.37	β-Amorphène	1.02	(Z) Farnescene	0.28
Caryophyllene oxide	2.03	Hinesol	1.99	Decanone	0.89	α-(Z) Santalol	1.88
Hexadecanoic acid	14.38	Atractylenolactam	5.06	Bornyl acetate	4.88	n-Pentadecanol	2.33
Neophytadiene	0.78	β-eudesmol	0.97	Geranyl acetate	15.77	Linalool	4.49
Pentacosane	1.14	Pubinernoid A	4.5	Z-Lanceol acetate	0.74	β-Damascenone	1.57
β-Ionone	1.56	Octadecane	3.15	(E,Z)-Geranyl linalool	1.77	α-Terpineol	1.83
Phytol	9.07	σ-deca lactone	1.88	Eicosane	0.32	Eicosane	1.72
Oleanolic acid	12.05	Decene	2.44	(Z)-α-Damascone	2.17	(Z)-α-Damascone	2.88
Isorhamnetin	2.01	Methyl ester	21.53	β-Damascenone	0.99	Diethyl phatalate	6.35
Pomolic acid 3-O-α-L-arabinoside	13.05	Linalool	7.77	Camphor	4.05	Geranyl valerate	5.17
β –sitosterol	0.25	Delta octadecanoate	16.51				
Safranal	1.47						

Table 7. Antimicrobial, antioxidant, anticancer, anti-inflammatory and IC50 properties of the main compounds obtained by GC/MS of *Zygophyllum* species

	Main Active Compound	Antioxidant Activity	Anti-inflammatory Activity	Anti-cancer Activity	Antimicrobial Activity	IC50	Reference
Z. fabago	ar-Curcumene, and Hexadecanoic acid	Strong	Moderate	-	Strong	Strong	Yaripour et al., 2017; Orhan et al., 2004
	Oleanolic acid	-	Moderate	Moderate	Moderate	-	Castellano et al., 2022
	Pomolic acid	Strong	Moderate	Moderate	Low	Strong	Chan et al., 2023; Schinella et al., 2020
Z. eurypterum	Linalool	-	-	Strong	Strong	-	An et al., 2021
	Methyl ester	Low	-	-	Strong	Low	Sati et al., 2016
	Rutin	Strong	Strong	Strong		Strong	Ganeshpurkar, & Saluja 2017
Z. propinquum	Cocolactone	Strong	-	-	Strong	Strong	Kchaou et al., 2016
	Isobornyl acetate	Strong			Strong		Kumar et al., 2010; Sıcaka & Eliuzb 2019
	Caryophyllène	Strong	-	-	Moderate	Strong	Rehman et al., 2022, Salifou et al., 2020
Z.megacarpum	Geranyl acetate	Moderate	-	Strong	Moderate	Moderate	Ahmad et al., 2023; Celuppi et al., 2023
	Carvacrol	Strong	Strong	Strong	Strong	Moderate	Davison & Wargo, 2001; Sharifi-Rad et al., 2018
	Thymol	Strong	Moderate	Strong	Strong	Moderate	Salman et al., 2012
	Camphor	Moderate	Moderate	Strong	Strong	Moderate	Carvalho et al., 2019; Singh et al., 2023

tested extracts.

Measurement of flavonoid and phenolic contents

The flavonoid and phenolic contents of root and flower methanol extracts of *Z. fabago, Z. eurypterum, Z. propinquum* and *Z.megacarpum* are represented in Table 4. The highest and lowest phenolic content on the root extract of *Z. fabago* and flower extract of *Z. megacarpum* were calculated as 301.04±0.33 and 98.07±0.57 mgGA/DWg, respectively. Next, the most and lowest flavonoid content on the root extract of *Z. fabago* and *Z. propinquum* were measured as 8.04 and 3.07mgQ/DWg, respectively.

Investigation of the presence of secondary metabolites

The presence and absence of alkaloid, saponin, and tannin were tested in root and flower methanol extracts of *Z. fabago, Z. eurypterum, Z. propinquum* and *Z.megacarpum*. Accordingly, the presence of alkaloid in all tested extracts, and saponin in *Z. eurypterum* and *Z. propinquum* extracts were observed. Moreover, the presence of tannin was not confirmed in *Z. eurypterum* extract (Table 5).

Identification of chemical compositions by GCMS

The chemical compositions of *Z. fabago, Z. eurypterum, Z. propinquum* and *Z.megacarpum* methanol extract are shown in Table 6. Twenty two (as 96.42%), twenty (as 97.04), twenty one (as 95.39%), and twenty (as 94.21%) compounds were measured in *Z. fabago, Z. propinquum, Z. eurypterum,* and *Z.megacarpum* extracts, respectively. The dominant compounds in the *Z. fabago* extract were ar-curcumene (17.18%), hexadecanoic acid (14.38%), and pomolic acid 3-O-α-L-arabinoside (13.05%). The major constituents in *Z. eurypterum* extract included methyl ester (21.53%), delta octadecanoate (16.51%), and rutin (8.15%). Moreover, the chemical components including caryophyllène (17.07%), geranyl acetate (15.77%), and isobornyl acetate (14.28%) in *Z. propinquum* extract were dominant constituents. The major compounds in *Z. megacarpum* methanol extract included carvacrol (23.71%), 2, 6-di (tert-butyl) phenol (9.06) and camphor (8.19%). The important compounds with medical properties including caryophyllène, carvacrol and camphor were identified by GC/MS in this research. The chemical structures of some important compounds from *Zygophyllum spp* has been shown in Figure1.

Discussion

Today, due to the high cost of treatment with chemical drugs and the side effects of some antibiotics, there is an urgent need to identify and introduce new and effective plants in the production of natural antibiotics with high bioaccumulation potential (Fusco et al., 2007). Plants are a major source of antimicrobial properties that have been used in the treatment of infectious diseases since ancient times. Biological studies on *Zygophyllum* species have indicated significant antioxidant, antidiabetic, antitumor, antimicrobial and anti-inflammatory activities (Barzegar et al., 2018). According to He et al. (2016), the bioactive compounds with antimicrobial and antioxidant including triterpenes, flavonoids, saponins, sterols, phenolic, essential oils and esters have been isolated from *Zygophyllum spp*.

In this research, the highest phenolic and flavonoid contents were measured as 301.04±0.33 mgGA/DWg, and 8.04±0.57 mgQ/DWg, respectively from the root methanol extract of *Z. fabago*. Moreover, the presence of alkaloid in all tested extracts was observed. In addition, carvacrol and cocolactone were the major constituents from methanol extract of Z. *megacarpum* and *Z. propinquum*, respectively. Kchaou et al. (2016) reported the highest phenolic and flavonoid contents of *Z. album* methanol extract as 403.4+8.0 mgGA/DWg and 120.2+0.2 mgQ/DWg, respectively, which was contrast with the present study. Similarity, the presence of alkaloid was confirmed as well as cocolactone and carvacrol were the main compounds of *Z. album* essential oil.

Moreover, ar-curcumene as 17.18% and geranyl valerate as 7.05% were identified from *Z. fabago* methanol extract. In addition, the presence of secondary metabolites including alkaloid and tannin as well as the absence of saponin were demonstrated. Yaripour et al. (2017) identified major constituents comprising phytol (62.1%) and ar-curcumene (20.5%) from leaf and flower essential oils of *Z. fabago*, which was approximately similar to the present results.

Chemical compositions including methyl ester and caryophyllene E as 21.53% and 17.07% were dominant compounds in *Z. eurypterum* and *Z. propinquum* methanol extract, respectively. According to El Abdouni Khayari (2017) results, caryophyllene E, and methyl ester as 19.18 %, and 35.9 % were reported as dominant compounds in leaf and fruit of *Z. gaetulum,* respectively, as well as the presence of saponins, and alkaloids were confirmed, which were similar to the present study. Cybulska et al. (2014) demonstrated the presence of alkaloids, sterols and coumarin from *Z. qatarense* methanol extract. Similarity, in our study the presence of alkaloid was confirmed from *Zygophyllum spp* methanol extracts.

In the present study, the most sensitivity was reported on *S. epidermidis* against flower methanol extract of *Z. fabago* as 22.8±0.66 mm. Kchaou et al. (2016) reported the highest sensitivity on *E. faecalis* as 18±1 mm against *Z. fabago* essential oil, which was less than our data. Antirheumatic, anthelminthic, antiasthmatic, and antiinflammatory properties have been reported from the shoot and flower extract of *Z. fabago* (Khan et al., 2014). The antibacterial properties of *Z. fabago* leaf alcoholic extract against infectious bacteria including *P. mirabilis, E. coli, K. pneumonia, S. aureus* and *S. typhi* have reported, accordingly, the highest susceptibility was observed on *S. aureus* (Kumaran & Karunakaran, 2006). Moreover, the antimicrobial activity of the

aqueous, butanol and ethanol extracts of *Z. qatarense* against some infectious bacteria including *E. coli, P. aeruginosa, B.cereus,* and *S.aureus* (Mahasneh, 2002) as well as antimicrobial effect of *Z. oxianum* extract against these pathogens have been proven (Jaouhari et al., 2000). Based on the findings, these differences could be related to different in the geographical regions and climate conditions, collected time, developmental stage and growth conditions of plant, and type of extract assays. In general, the antimicrobial and anticancer properties of herbal plants mainly depend on the compounds present in the plant and are directly related to each other (Ksouri et al., 2013). Accordingly, compounds found in the plants with antimicrobial activity have showed antioxidant properties (Sıcaka &Eliuzb, 2019), which was also observed in the results of this research (Table 7).

Conclusion

Overall, the most susceptibility shown on *S. epidermidis* against the flower methanol extract of *Z. fabago*. The results of this research showed better antiradical and antioxidant activities and were confirmed the antimicrobial components including carvacrol, ar curcumene and caryophyllène. Based on the findings, detected components of *Zygophyllum spp* may have the potent to be used as anti-pathogenic agent against antibiotic-resistant infective bacteria and also the produce of herbal drugs.

Author Contributions

M.A. conceptualized, performed the experiments and revised the article.

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Competing financial interests

The authors have no conflict of interest.

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