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Original Article

Chemical Constituents, Antioxidant and Antibacterial Properties of three Species (*Zeravschania membranacea*, *Zeravschania aucheri*, and *Aegopodium tribracteolatum*) of Apiaceae Family

Running title: Phytochemistry of some species of Apiaceae

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ABSTRACT

This research investigated some biological properties of three Apiaceae species (*Zeravschania membranacea*, *Zeravschania aucheri*, and *Aegopodium tribracteolatum*) collected from Kurdistan, Iran, commonly used in the local diets. Methanol Extracts were evaluated for chemical profile, antioxidant activity (DPPH and FRAP), antimicrobial tests included disc diffusion and microdilution method, against bacterial and fungal cells. *Aegopodium tribracteolatum* displayed the highest antioxidant properties (91.53 mg Trolox equivalent [TE]/g extract) with an IC_{50} of 294.6 ± 0.21 µg.mL^{-1} in the DPPH assay and ferric reducing power (105.87 mg Ascorbic acid [AA]/g extract), attributed to its high phenolic (79.81± 0.35 mg of gallic acid [GA]/gram extract) and flavonoid content (112.723 ± 8.32 mg of Quercetin [QE]/gram extract). GC-MS analysis revealed notable compounds such as benzocaine, indole, linolenic acid, quinic acid, xanthosine, and phytol. *Z. aucheri* and *Z. membranacea* chemical profiles were reported for the first time. Antimicrobial tests showed, *A. tribracteolatum* and *Z. aucheri* inhibited *Candida albicans* even at low concentrations (0.025 mg.ml^{-1}). All extracts notably inhibited *Bacillus cereus* and *Staphylococcus aureus* growth. But only *A. tribracteolatum* showed an inhibition zone (13± 0.11mm) for *Escherichia coli* at 0.5 mg.ml^{-1}. Minimum inhibitory concentration and minimum bactericidal concentration tests revealed *A. tribracteolatum* inhibited *C. albicans* (MIC= 12.5, MBC= 25 µg.ml^{-1}), and both *A. tribracteolatum* and *Z. membranacea* inhibited *B. cereus* (MIC= 25 µg.ml-1 and 50 µg.ml^{-1}, respectively). This study confirms that methanol extracts from these species are rich in natural antioxidants, suggesting potential uses in the food and pharmaceutical industries.

Keywords: Antifungal effect, Apiaceae, 2,2-Diphenyl-1-picrylhydrazyl, Ferric reducing antioxidant power, Gas Chromatography Mass Spectrometry

INTRODUCTION

Millions of individuals worldwide turn to medicinal plants, harnessing their healing properties. This practice extends beyond those who lack easy access to conventional allopathic healthcare systems, encompassing consumers in both developing and developed nations alike [1]. The Apiaceae family is one of the largest plant families, with approximately 438 genera and 3780 species, mostly dispersed in the Northern Hemisphere. In Iran, this family is represented by 121 genera and 322 species, out of which 118 species are exclusive to Iran. Geographically, these species are primarily distributed in regions of Azerbaijan, North Khorasan, the Kurdistan area, Elburz, and Zagros (including Northern, Southern, and Eastern Zagros), as well as the central basin of Iran. Their presence diminishes as one moves further south in Iran [2]. Ethnobotanical records attest to the use of many members of the Apiaceae family for relieving various human ailments. In traditional African and Asian cultures, different parts of these plants have been used to treat stomach disorders, nausea, appendicitis, digestion issues, hypertension, cardiovascular diseases, constipation, and even mosquito bites [3].

Resilient microorganisms, including both fungi and bacteria, pose a challenge in the medical field. Fungi such as *Candida*, *Aspergillus*, and *Cryptococcus neoformans* often develop resistance to azoles, as noted in various studies [4-6]. Similarly, certain strains of *S. aureus* have demonstrated resistance to methicillin, indicating an ongoing challenge in bacterial infections [7]. The impact of these resistant microorganisms extends beyond

clinical settings. Bacterial strains and the harmful substances produced during their metabolic processes can lead to foodborne illnesses, often resulting in gastrointestinal or even neurological symptoms [8]. Given these challenges, there is a growing interest in exploring new naturally occurring bioactive compounds as potential solutions.

Zeravschania membranacea (Boiss.) Pimenov, an Iranian native species. This perennial herb, with stems measuring 50 to 80 cm, thrives naturally in Iran's rocky northwest slopes at altitudes of 2500 to 3500 meters. Predominantly found in Iran's northwest, this plant is valued for its unique scent and plays essential roles in both food and traditional medicine. Furthermore, it contributes significantly to soil preservation and serves as vital summer grazing for livestock in semi-arid regions. Regrettably, indiscriminate harvesting in Kurdistan's Kosalan region has severely diminished its population, raising concerns about its imminent extinction [9].

Zeravschania aucheri (Boiss.) Pimenov, a member of the Peucedaneae tribe within the Apioideae subfamily, thrives as a perennial herb in the rocky mountainous terrain of northern and northwestern Iran. In traditional medicine, this plant is utilized by infusion or boiling to address kidney issues, expel worms, and alleviate stomach discomfort. Its infusion is also recognized for its potential in managing epilepsy. Notably, there is currently a lack of available documentation regarding the essential oil's or extract chemical composition, oil yield, antibacterial properties, and antioxidant activities across various *Z. aucheri* populations. While prior research has explored the essential oil composition of related species such as *Z. pastinacifolia* and *Z. membranacea*, as well as other members of the *Peucedanum* group [9-15].

Aegopodium tribracteolatum Schmalh., found in the Kosalan mountains of Kurdistan- Iran, has recently been acknowledged as its accepted name. This species carries three different synonyms: *Pimpinella anthriscoides*, *Pimpinella cervariifolia*, and *Pseudopimpinella anthriscoides*. The *Pimpinella* genus comprises approximately 150 species scattered across the northern hemisphere. *Pimpinella anisum* L., commonly known as anise, utilizes its fruit for its expectorant, antispasmodic, carminative, diuretic, and broncho-dilator properties, particularly in managing chronic bronchitis. Notable constituents of *Pimpinella anisum* L. essential oil include trans-anetole, estragole, γ-hymachalen, para-anisaldehyde, and methyl cavicol. Turkey's *Pimpinella* species have yielded phenylpropanoids, 4- (prop-2-enyl) phenyl angelate, 4-(3-methyloxiranyl) phenyl 2-methylbutyrate, bisabolene-type sesquiterpenoids, and trinorsesquiterpenes in their essential oils [16].

Surprisingly, there is a dearth of comprehensive studies on *Aegopodium tribracteolatum* or *Pimpinella anthriscoides* that evaluate and compare their antioxidant properties with other members of the Apiaceae family. Given the significant medicinal potential indicated by reports on other species of the Pimpinella genus, we were motivated to study this species in the Kosalan region. Our literature search has revealed a conspicuous absence of scientific publications detailing the phytochemistry of *Z. aucheri*. Hence, this study aims to compare the in vitro antioxidant activity, phenolic, and flavonoid contents among three selected Apiaceae species. The scientific insights derived from this research will establish a foundational dataset for the selection of Apiaceae species containing bioactive compounds with potential health benefits.

MATERIAL AND METHODS

Plant Material

The species studied in this research were collected in the spring of 2020 from the Kosalan region of Kurdistan (Fig. 1). Table 1 shows the information about the species.

To collect plants in the form of complete samples for identification, the protected area of Kosalan was visited several times. *Z. aucheri* were collected in late March, early April, and late June. *Z. membranacea* and *A. tribracteolatum* were collected in late April, early May, and late July. However, the flowering period of these species varies from June to late July. After identifying the species, a herbarium specimen was prepared and kept in the herbarium of Kurdistan University, Faculty of Science.

Preparation of the Extract Samples

The upper portions of the plant, having undergone air-drying, were converted into a fine powder utilizing a mechanical grinder. Subsequently, this powdered material was utilized for solvent extraction. To be precise, 25 grams of the powdered plant material underwent extraction with 250 ml methanol, a rotary evaporator equipped with a vacuum pump in room temperature for 45 minute. The obtained extract was condensed until it achieved a

state of desiccation through the application of reduced pressure, ultimately producing a desiccated raw methanol extract.

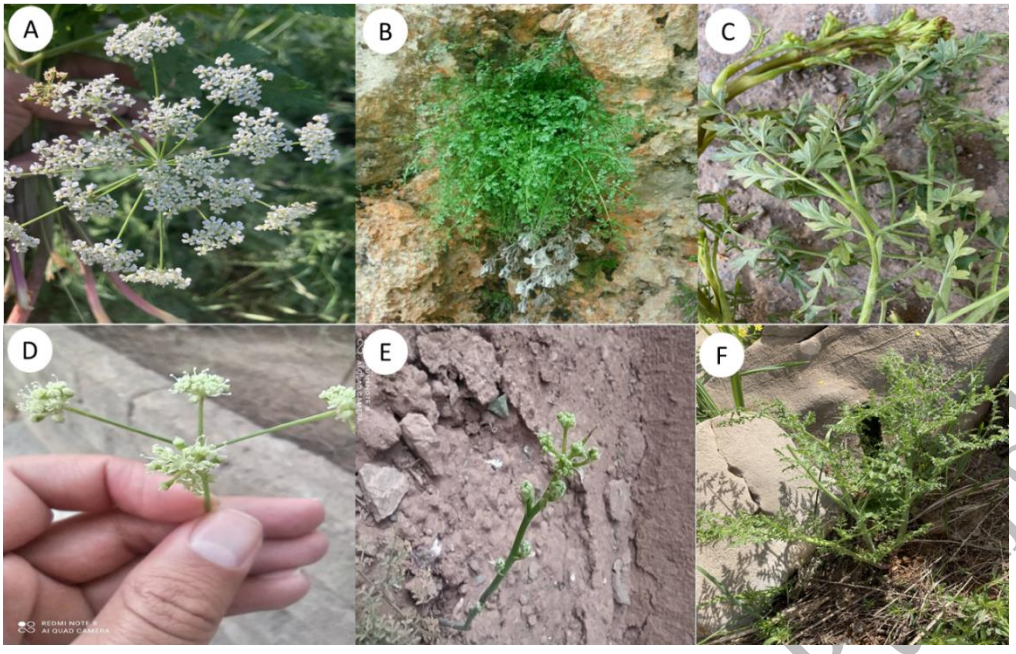


Fig. 1 Studied species in their habitat in Kosalan Mountains of Avroman. A: *A. tribracteolatum*; B, C: *Z. aucheri*; D-F*: Z. membranacea*.

Table 1 Specimen information of studied taxa

Family	Order	Taxon	Location	Herbarium Specimen
Apiaceae	Apiales	*Zeravschania membranacea*	35˚14’09”N 46˚22’49”E	UOK-145
		Aegopodium tribracteolatum	35˚12’51”N 46˚27’18”E	UOK-140
		Zeravschania aucheri	35˚13’23”N 46˚26’21”E	UOK-146

Quantification of total phenolic content (TPC)

The total phenolic content (TPC) of the aerial plant extracts was determined using the Folin-Ciocalteu reagent method [17].To achieve this, 0.5 mL of a methanol solution of each extract (at a concentration of 1.0 mg.mL^{-1}) was introduced into test tubes containing 0.5 mL of Folin-Ciocalteu reagent, followed by the addition of 2.0 mL of sodium carbonate solution (7% W/V). Subsequently, the tubes were vigorously agitated. The resulting mixture was then incubated at 45°C for 60 minutes with intermittent shaking. The absorbance was measured at 765 nm utilizing an Elico SL 164 UV-Vis spectrophotometer. A calibration curve was generated using gallic acid as the standard, spanning concentrations from 0 to 0.8 mg.mL^{-1}. The findings were expressed in milligrams of gallic acid equivalents per gram (mg GAE/g) of dried extract.

Quantification of the total flavonoid content (TFC)

An initial examination for flavonoids was conducted employing the lead acetate, ferric chloride, and ammonium hydroxide reagent, which yielded affirmative results. Consequently, the total flavonoid content was determined. The quantification of total flavonoids was carried out following the methodology outlined in Chang-Yang's procedure [18]. A 0.5 mL aliquot of the analyte solution, possessing a concentration of 1 mg/mL, was mixed well with 0.1 mL of aluminum chloride (at a concentration of 10%), 0.1 mL of potassium acetate exhibiting a molarity of 1 mol/L, and 2.8 mL of distilled water. This assemblage of reagents underwent a thorough mixing process to ensure uniformity within the solution. Subsequently, the mixed solution was allowed a quiescent standing period of 30 minutes at ambient temperature to facilitate the requisite reactions. Post the elapse of the stipulated time, the supernatant was subjected to spectrophotometric analysis, wherein the absorbance was assiduously recorded

at a wavelength of 415 nm. A yellow hue in the mixture signaled flavonoids' presence. A standard calibration curve was plotted using quercetin, and findings were articulated as quercetin equivalents (mg QE/g) per gram of desiccated extract.

In vitro Antioxidant Activity Analysis

Ferric-reducing power (FRAP) assay

The extract's reducing capacity was measured according to the method by Yen and Chen (1995) [19]. Concisely, aliquots of 1.0 mL of sample solutions at varying concentrations were amalgamated with 2.5 mL of phosphate buffer (0.2 mol.L^{-1}, pH 6.6) and 2.5 mL of 1% (W/V) $K_3Fe(CN)_6$. Subsequent to a 20-minute incubation period at 50ºC, 2.5 mL of 10% Trichloroacetic acid was introduced to the mixture. Centrifugation at 12,000 r.min^{-1} for 10 minutes was conducted as necessary. From the resultant supernatant, an aliquot of 2.5 mL was extracted and combined with 2.5 mL of distilled water and 0.5 mL of 0.1% (W/V) $FeCl_3$. Absorbance readings were taken at 700 nm utilizing an Elico SL 164 UV–Vis spectrophotometer [20]. The blank sample contains all the reagents except the plant extract. Ascorbic acid (concentrations ranging from 25 to 400 µg.mL^{-1}) served as the positive control for gauging reducing power, with each test executed thrice and the results subsequently averaged.

DPPH radical scavenging activity assay

To assess the sample's DPPH radical scavenging activity, a 1 mL solution of 0.1 mmol. L^{-1} DPPH in methanol was mixed with 3.0 mL of extract at various concentrations (62.5, 125, 250, 500, 1000 µg/ml). The mixture was incubated in darkness at room temperature for 15 minutes. Post-incubation, absorbance was measured at 517 nm using a spectrophotometer [21]. Scavenging activity, represented as inhibition percentage ($I_{\%}$), was calculated with the equation: $I_{\%} = [(OD_{control} - OD_{sample}) / OD_{control}] \times 100$ $OD_{sample.}$ Here OD_{sample} is the extract-containing solution's absorbance, while $OD_{control}$ is the control solution's absorbance. A higher $I_{\%}$ indicates stronger antioxidant activity by the extract, evidenced by effective DPPH radical neutralization.

Antibacterial and Antifungal Activity Assay

Disk Diffusion Method

At first, five tested microbes including, four strains of bacterial pathogens, Gram-positive *Staphylococcus aureus*, *Bacillus cereus*, and Gram-negative *Escherichia coli* and *pseudomonas aeruginosa* and one strain of fungal pathogen, *Candida albicans* each with a concentration of 108 cfu.ml^{-1}, were inoculated on Muller-Hinton agar plates by using sterile swab sticks. Subsequently, six-mm paper discs were impregnated with 25µl of various extract stocks, from each 100% and 200% stock concentration, placed aseptically on the agar surface. Chloramphenicol discs (30µg) were utilized as positive control. 100% DMSO was employed as the negative control. After 24 hours of incubation at 37°C, zones of inhibition on the plates were determined using a ruler [22].

Dilution method to obtain MIC and MBC

Following the agar disc diffusion, MIC and MBC test were conducted on the compounds that exhibited antibacterial activity. The determination of minimal growth inhibitory concentration and minimum bactericidal concentration was carried out using the dilution method with tryptone soy broth (TSB) culture medium. A volume of 90µl of liquid tryptone soy culture medium, containing stocks of a specific concentration, was combined with 50µl of a suspension of the microbe culture. This mixture was then subjected to serial dilution from the first well. The half-McFarland standard was employed to generate a turbidity level comparable to a bacterial suspension with a concentration of 0.5×10^8 cfu/ml. The tubes were thereafter placed in an incubator set at a temperature of 35 °C for a duration of 24 hours. The final microtube, in which bacterial growth and turbidity were absent, contained extracts and was reported as the minimum inhibitory concentration (MIC) tube. To determine the MBC, the well preceding the one showing no visible growth (MIC) was chosen and sub-cultured on a fresh Tryptone Soy Agar plate. Absence of growth after incubation indicated the MBC [23].

GC Analysis

Samples were extracted with toluene and analyzed using Agilent's 7890B GC System/597A MSD. Chromatographic separation utilized an HP-5 ms column (30m x 0.25mm x 0.25µm), with temperatures set at 280°C for the injection port and auxiliary. The temperature program started at 5°C (held for 2 min), increased at

7°C/min to 26°C, and held for 8 min. The mass spectrometer, operating in electron ionization mode at 70 eV, covered a range of 550 AMU with ionization sources at 230 and 150°C. Helium (99.959% pure) was used as the carrier gas at 34 psi and 1 ml/min. Compound identification involved database comparison (NIST, Wiley), inhibition indices, and fragmentation patterns.

Statistical Analysis

The dataset, comprising triplicate measurements, underwent statistical scrutiny through analysis of variance (ANOVA), with results presented as mean values ± standard error of the mean (SEM), based on a sample size (n) of 3. A two-way ANOVA, supplemented by Tukey's multiple comparison tests (data not shown), was utilized for data analysis, executed via SPSS software version 16.0 (SPSS Inc., USA). A P-value < 0.05 was the criterion for statistical significance.

RESULTS

In vitro Antioxidant Activity

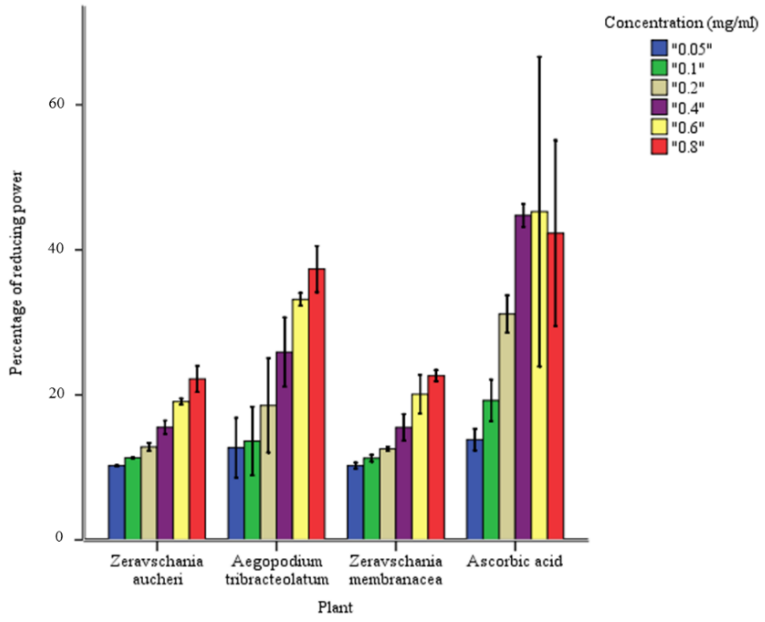
In the assessment of reducing power capacity, the extracts exhibited the ability to convert the Fe^{3+}/ferricyanide complex into its ferrous form, leading to the formation of Perl's Prussian blue, which was measured at 700 nm. As depicted in Figure 2A, it becomes clear that the reduction percentage increased with higher concentrations of the extract across all species. In terms of the highest concentration, the order of reducing power percentage was as follows: ascorbic acid> *A. tribracteolatum*> *Z. membranacea*> *Z. aucheri*. However, when subject to multiple comparison analysis, no significant differences were observed in the reduction power percentage between *Z. membranacea* and *Z. aucheri* at lower concentrations. Figure 2B displays dose-response curves, comparing the reducing powers of the extracts from the studied species to ascorbic acid, with *A. tribracteolatum* showing the highest ferric reducing power (105.87 mg AA/g extract) (Table 2).

DPPH, a stable free radical, reacts with antioxidants by accepting an electron, leading to decolorization. The antioxidant's efficacy in scavenging DPPH is directly linked to its electron donation capacity. Plant extracts showed concentration-dependent DPPH scavenging, with *A. tribracteolatum*'s methanol extract displaying the lowest IC_{50} value (294.6 ± 0.21 µg.mL^{-1}) (Fig. 3A, B and Table 2). *A. tribracteolatum* exhibited the highest scavenging capacity with 91.53 mg TE/g extract. Trolox, the positive control, showed an IC_{50} value of (249.21 ± 0.03) µg.mL^{-1}. Other species failed to inhibit 50% of the radicals even at higher concentrations (1000 µg.mL^{-1}). The exceptional DPPH scavenging of *A. tribracteolatum* extracts is due to their polyphenolic compounds' hydrogen-donating ability. These compounds are known for their antioxidant properties, countering radicals like DPPH by donating hydrogen atoms. This process quenches the radical's reactivity, reducing color and indicating the extract's antioxidant potential.

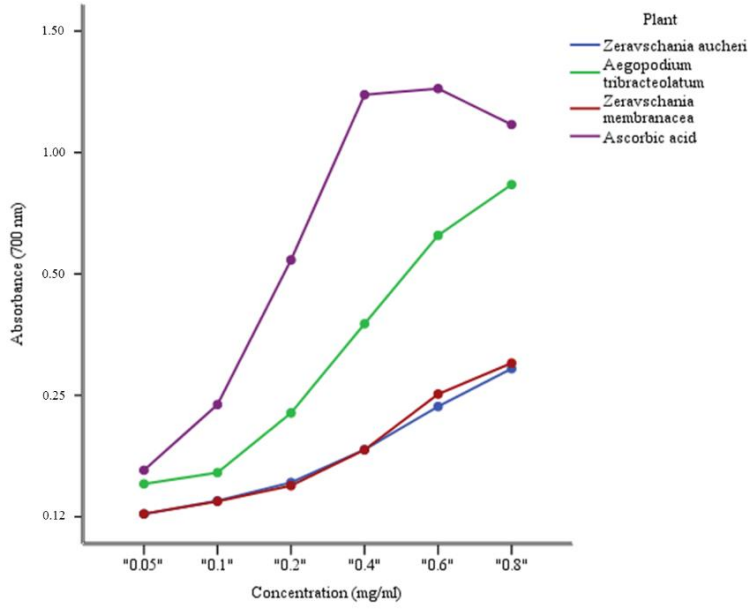
Total Phenolic and Flavonoid Content

To ascertain the total phenolic content, the Folin-Ciocalteu reagent was applied to methanol extracts of the plants after appropriate dilution. A calibration curve was constructed using gallic acid as the reference standard, with the equation Y=0.0057x+0.3694 and a coefficient of determination (R^2) of 0.9817 (as illustrated in Fig. 4A). The results revealed that the total phenolic content varied among the different species but did not exhibit statistically significant differences (P-value = 0.05) (as summarized in Table 2). Notably, *A. tribracteolatum* demonstrated the highest phenolic content, measuring approximately (79.81±0.35) mg GAE/g of extract.

The assessment of total flavonoid content was carried out using calibration curves constructed with quercetin as the reference standard. The equation for the calibration curve was Y=0.0011x+0.0748, and the coefficient of determination (R^2) was 0.9649, as illustrated in Figure 4B. The results revealed significant variations in the total flavonoid content among the studied plants (P-value = 0.05), as summarized in Table 2. Notably, *A. tribracteolatum* exhibited the highest flavonoid content, measuring approximately (112.723 ± 8.32) mg QE/g of extract.



A)

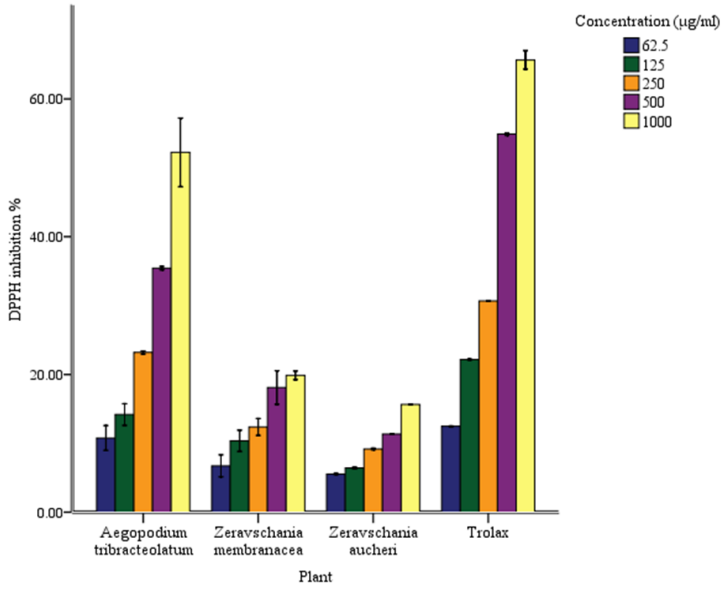


B)

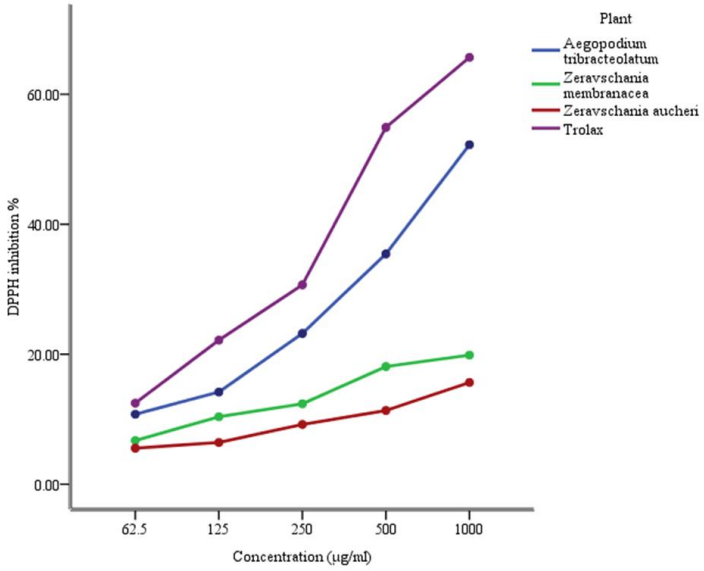
Fig. 2 Reducing power ability of different extracts of studied species at different concentrations, (mean ± SEM, n = 3). A and B: Comparison of reducing power in different concentrations of studied plants in comparison with ascorbic acid.

Table 2 Total phenolic and flavonoid contents and antioxidant activities of the methanol extracts of studied species (mean ± SEM). [a] Standard deviation. The data display the mean ± SD of three independent experiments ($p < 0.05$). QE: Quercetin, GAE: Gallic acid, AA: Ascorbic acid, TE: Trolox equivalent

Taxon	Total flavonoid content mg QE/g of dry) (Material ± SD[a]	Total phenolic content mg GAE/g of dry) (Material ± SD[a]	IC_{50} (µg.mL−1) DPPH radical	mg AA/g extract	mg TE/g extract
Z. membranacea	35.05 ± 8.59	65.65 ± 1.61	1096 ± 0.07	93.43	78.52
A. tribracteolatum	112.723 ± 8.32	79.81 ± 0.35	294.6 ± 0.21	105.87	91.53
Z. aucheri	52.02 ± 3.77	67.75 ± 1.78	1250 ± 0.03	90.16	56.78

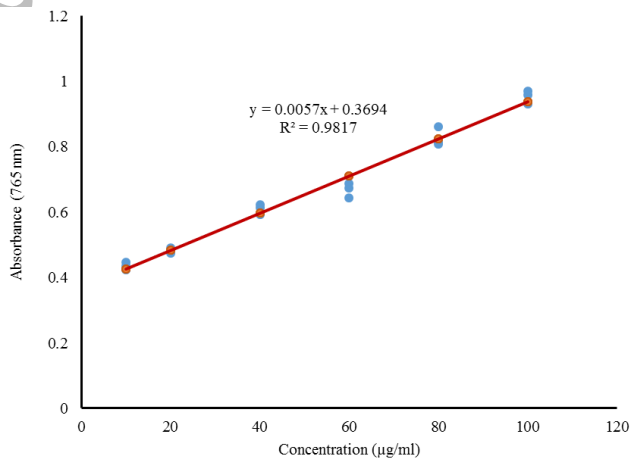


A)

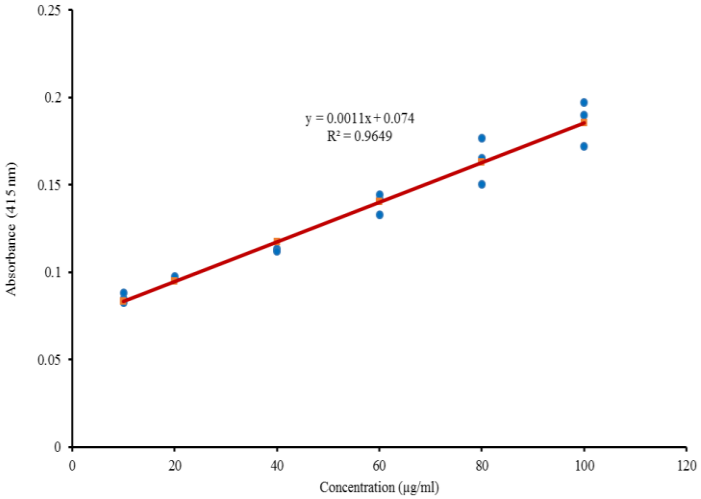


B)

Fig. 3 The inhibition percent of studied species extracts on DPPH radicals, (mean ± SEM, n = 3). A and B: Comparison of radical inhibition percent in different concentrations of studied plants in comparison with trolax.



A)



B)

Fig. 4 A: Calibration curve of Gallic acid to determine the amount of phenol in the studied species. B: Calibration curve of Quercetin to determine the amount of flavonoids in the studied species.

Table 3 Major Chemical compositions and percentage of the identified components from studied species RT: Retention time

No	Compound	*Z. membranacea* %	RT (min)	*Z. aucheri* %	RT (min)	*A. tribracteolatum* %	RT (min)
1	Benzocaine	17.83	22.04	-	-	-	-
2	Indoline	15.28	21.14	-	-	-	-
	α-linolenic acid	12.17	27.37	32.59	27.24	33.07	27.18
3	Linalyl acetate	8.71	13.05	-	-	-	-
4	Propanamide	8.04	21. 7	-	-	-	-
5	Quinic acid	7.85	20.93	-	-	-	-
6	Palmitic acid	5.88	24.95	21.45	24.96	16.25	24.87
7	Xanthosine	2.62	17.94	-	-	-	-
8	tetrahydrobenzofuran-2(4H)-one	2.25	22.32	-	-	-	-
9	Quinic acid	-	-	7.70	24.63	26.56	20.54
10	1,2,3,4-Tetrahydronaphthalene-1-carboxylic acid, 2-amino-, methyl ester	-	-	-	-	5.71	28.02
11	Carbonic acid, 2-ethylhexyl octadecyl ester	-	-	-	-	4.56	20.86
12	5-Phenyl-3-isoxazolol	-	-	-	-	4.07	17.37
13	Phytol	-	-	26.83	3.60	3.99	26.82
14	Neophytadiene		-			2.00	22.99
15	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	-	-	4.73	10.93	-	-
16	1,1-Dimethoxycyclohexane	-	-	7.68	4.41	-	-
17	Hydratropaldehyde dimethyl acetal	-	-	14.22	2.59	-	-
18	2-Butanone, 4,4-dimethoxy	-	-	1.95	14.36	-	-

Table 4 The diameter of the inhibition zone of antibiotic and plant extracts (mm), by disk diffusion method.

Pathogen strain	Plant species/Antibiotic	Concentration (mg/ml)	The mean of inhibition zone (mm) ± SD	Chloramphenicol 30 mg/Disc
S. aureus	*Z. membranacea*	0.025	13± 0.06	-
		0.5	14± 0.21	
	A. tribracteolatum	0.025	10± 0.32	
		0.5	11± 0.11	
	Z. aucheri	0.025	8± 0.07	
		0.5	11± 0.04	
B. cereus	*Z. membranacea*	0.025	10± 0.01	19± 0.02
		0.5	12± 0.02	
	A. tribracteolatum	0.025	12± 0.23	
		0.5	15± 0.05	
	Z. aucheri	0.025	9± 0.14	
		0.5	12± 0.03	
E. coli	*Z. membranacea*	0.025	-	18± 0.02
		0.5	-	
	A. tribracteolatum	0.025	10± 0.37	
		0.5	13± 0.11	
	Z. aucheri	0.025	-	
		0.5	-	
P. aerogenosa	*Z. membranacea*	0.025	-	-
		0.5	-	
	A. tribracteolatum	0.025	-	
		0.5	-	
	Z. aucheri	0.025	-	
		0.5	-	
C. albicans	*Z. membranacea*	0.025	-	-
		0.5	-	
	A. tribracteolatum	0.025	15± 0.07	
		0.5	18± 0.31	
	Z. aucheri	0.025	-	
		0.5	-	

Table 5 Different levels of MIC and MBC of plant extracts on studied bacterial strains

Microbial cells	Plant extracts	MIC (µg / ml)	MBC (µg /ml)
B. cereus	*Z. membranacea*	50	100
	A. tribracteolatum	25	50
	Z. aucheri	-	-
C. albicans	*Z. membranacea*	-	-
	A. tribracteolatum	12.5	25
	Z. aucheri	-	-

Gas Chromatography- mass Spectrometry (GC-MS) Analysis

The characterization of plant extract combinations was conducted through GC-MS studies, enabling the precise identification of various organic compounds within the methanolic extracts of the three studied plants. The results of these analyses are presented in Table 3, revealing the presence of several phytochemicals with potential medicinal significance. Notably, Benzocaine, Indole, Propanamide, and Linalyl acetate were exclusively detected in *Z. membranacea*. Octadecatrienoic acid was identified in all three plants, but its concentration was notably higher in *Z. aucheri*. Similarly, Quinic acid and Hexadecanoic acid were found in all three plants, with relatively higher proportions in *Z. aucheri* and *A. tribracteolatum*, respectively. These findings offer valuable insights into

the chemical composition of the studied plant extracts, shedding light on the presence of potentially valuable phytochemicals that may contribute to their medicinal properties.

Anti-microbial Tests Assessment

The influence of two concentrations (0.025 mg/ml and 0.50 mg/ml) of plant extracts on the susceptibility and resistance of common bacterial and fungal strains, including *S. aureus, B. cereus, E. coli, P. aeruginosa* and *C. albicans*, was scrutinized. The diameters of inhibition zones against each strain are presented in Table 4. These measurements, in conjunction with those for the inhibition zone in the antibiotic control, underwent thorough analysis.

The data in (Table 4) underscore that the inhibitory effect was not universally observed for all three examined extracts across all tested pathogens. Notably, *A. tribracteolatum* and *Z. aucheri* exhibited a discernible inhibitory effect on *C. albicans*, whereas *Z. membranacea* demonstrated no discernible impact on it. All extract had an inhibitory effect on both gram positive bacteria (*S. aureus* and *B. cereus*) among them *Z. membranacea* show more effect on *S. aureus* at lower concentration (13± 0.06 mm). None of the plant extract were able to inhibited gram negative strains (*P. aeruginosa* and *E. coli*) except for *A. tribracteolatum* which could produce an inhibition zone for *E. coli* at higher concentration (13± 0.01 mm).

The MIC and MBC of the extracts were meticulously assessed to ascertain the lowest concentration that inhibits microbial growth and the minimum concentration that leads to bacterial cell death, respectively. These results are documented in (Table 5). No result was found for *S. aureus, E. coli* and *P. aeruginosa* and strikingly, the findings revealed that none of the extracts exhibited inhibitory effects on *C. albicans* except for *A. tribracteolatum* (MIC 12.5, MBC 25 $\mu g.ml^{-1}$). This species demonstrated inhibitory activity against *B. cereus* (MIC 25 $\mu g.ml^{-1}$) as well. *Z. membranacea* inhibit *B. cereus* strain at higher concentration (MIC 50 $\mu g.ml^{-1}$). While *Z. aucheri* extract showed no discernible effect on this bacterial strain.

DISCAUSSION

The present study aimed to evaluate the antioxidant properties of three plant species: *Z. membranacea, A. tribracteolatum*, and *Z. aucheri*. These species were part of the daily diet of the local people in the Avroman region of Kurdistan province during the spring season. The data analysis revealed that the anti-radical properties of the extracts, similar to ascorbic acid, were concentration-dependent. As the concentration of each plant extract increased, so did the free radical inhibitory activity. This increase in anti-radical properties was attributed to the presence of phenolic and flavonoid compounds in the extracts. Higher concentrations of these compounds led to an increased presence of hydroxyl agents in the reaction, thereby enhancing the possibility of hydrogen transfer to free radicals and consequently increasing the inhibitory ability of the extract.

The chemical profile of the leaf extract of *Z. aucheri* was reported for the first time and revealed the detection of 38 different chemical compounds. The most abundant constituents in the extract were n-Hexadecanoic acid (Palmitic acid), which is a common saturated fatty acid found in animals, plants, and microorganisms, and 9, 12, 15-Octadecatrienoic acid (linolenic acid).

In the case of *Z. membranacea* extract, among the 33 different compounds detected, Benzocaine and Indole were found to be the most abundant. However, it is interesting to note that in a previous study by Pirbalouti et al. (2013), which reported constituents of the essential oils from various populations of *Z. membranacea*, they identified different compounds including cis-β-ocimene, sabinene, trans-β-ocimene, α-pinene, γ-terpinene, α-terpinolene, and β-pinene. The present study and Ghasemi et al. (2013) study both detected β-ocimene (0.29%) in the extract, but there were no traces of Benzocaine and Indole in the latter study.

These findings highlight the uniqueness and diversity of the chemical compositions of *Z. aucheri* and *Z. membranacea* extracts, which could have potential implications for their medicinal properties. Further research is needed to explore the therapeutic potential of these compounds and understand the differences in their chemical profiles among different populations and species of these plants.

Among the studied species, *A. tribracteolatum* exhibited the highest free radical scavenging properties due to its rich flavonoid content. Specifically, it contained 112.723 ± 8.32 mg of quercetin per gram of extract, and its total phenol content was 79.81±0.35 mg of gallic acid per gram of extract, making it the highest among the tested species. Interestingly, the research on the biological activity and phytochemistry of *A. tribracteolatum* was not

found, but previous studies referred to it as "*Pimpinella anthriscoides*." Molecular studies have confirmed that the Southwest Asian *Pimpinella anthriscoides* (Boiss.) F. Ghahrem., Khajepiri & Mozaff has been transferred to the genus *Aegopodium* as *A. tribracteolatum* Schmalh. [24].

In Zengin et al. (2020) investigation, *P. anthriscoides* (former name for *A. tribracteolatum*) exhibited the highest flavonoid content among the six studied species. Furthermore, the present study identified Quinic Acid as the most prominent component in the leaf extract of *A. tribracteolatum*. Quinic acid, a constituent of the tara tannins, is known for its astringent properties and serves as a versatile chiral starting material for pharmaceutical synthesis. Notably, it is a building block in the synthesis of Oseltamivir, used to treat influenza A and B. The presence of Quinic Acid in *P. anthriscoides* was confirmed in previous studies [25-28]. Additionally, *A. tribracteolatum* exhibited the second-highest percentage of linolenic acid (9, 12, 15-octadecatrienoic acid), which was isolated as an anti-inflammatory compound [28].

The ethanolic extract of *Pimpinella boisseiri* root showed the presence of chlorogenic acids, including 3, 4, 5, -Caffeoylquinic acid, 3, 4 Dicaffeoly Quinic Acid, and 3, 5-Feruloyl Quinic Acid [25]. *Pimpinella* species have been traditionally used to treat various ailments, exhibiting a wide range of therapeutic values, including carminative, expectorant, sedative, antidepressant, antiseptic, insecticidal, antiviral, antispasmodic, nematocidal, utagenic, analgesic, antifungal, antibacterial, diuretic, estrogenic, and antimalarial properties [29].

Moreover, *Pimpinella* species, including *A. tribracteolatum*, are known for their economic importance and have been used as condiments or vegetables, in addition to their traditional medicinal uses. The study's results suggest that *A. tribracteolatum*, also known as *P. anthriscoides*, could be a valuable medicinal plant for various industrial applications, similar to *P. anisum*.

The study undertook an assessment of the antimicrobial properties exhibited by plant extracts against a spectrum of common bacterial and fungal strains, including *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa*, and *C. albicans*. Disc diffusion assays were conducted using two concentrations (0.025 mg/ml and 0.50 mg/ml) of the extracts. The findings revealed that the extract from *A. tribracteolatum* notably hindered the growth of *C. albicans* and demonstrated inhibitory effects on both gram-positive bacteria, namely *S. aureus* and *B. cereus*. Noteworthy antifungal activities of certain Apiaceae species, such as *Deverra tortuosa* and *Anethum graveolens*, against *C. albicans* and *Bacillus subtilis* have also been reported [30]. Similarly, *Bunium persicum*, *Cuminum cyminum*, and *Carum copticum* exhibited antibacterial effects against *S. aureus*, *B. cereus*, and *E. coli* [31]. *Prangos peucedanifolia* displayed promising antibacterial potential against *B. cereus* (Minimum Inhibition Concentration MIC: 0.37 $mg.ml^{-1}$), *P. aeruginosa*, and *E. coli* (MIC: 0.27 $mg.ml^{-1}$) [32]. Moreover, the essential oil derived from various populations of *Z. membranacea* exhibited heightened antibacterial activity against *Proteus vulgaris* [9]. However, no antimicrobial effects of the extracts from both *A. tribracteolatum* and *Z. aucheri* were found in the literature. The antimicrobial outcomes of this study resonate with some, but not all, of the findings from other recent investigations. The variance in results suggests the need for further investigation into the antibacterial activity of the studied plant extracts against these pathogens under standardized conditions. This underscores the significance of considering diverse factors that may influence the effectiveness of plant extracts as antimicrobial agents.

CONCLUSION

The study underscores the significance of assessing the antioxidant properties of these plants, largely attributed to the presence of phenolic and flavonoid compounds. *A. tribracteolatum* emerges as particularly noteworthy due to its highest free radical scavenging properties, stemming from its abundant flavonoid content. This highlights its potential as a valuable medicinal plant for a variety of industrial applications. Moreover, the research offers fresh insights into the chemical compositions of *Z. aucheri* and *Z. membranacea* extracts, unveiling the presence of numerous phytochemicals with potential medicinal value. This diversity in chemical profiles accentuates the distinctiveness of each plant species. The observed correlation between antioxidant activity and phenolic content further underscores the pivotal role of phenolic compounds in determining the antioxidant potential of medicinal plants. In addition to evaluating the antimicrobial properties of the plant extracts against common bacterial and fungal strains, the study found that the extract from *A. tribracteolatum* exhibited significant inhibitory effects on *C. albicans*, *S. aureus*, and *B. cereus*. These findings suggest the considerable potential of *A. tribracteolatum* as a valuable antimicrobial agent.

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Conflict of Interests

The authors have not declared any conflict of interests.

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Figur captions:

Figure 1. Studied species in their habitat in Kosalan Mountains of Avroman. A: *A. tribracteolatum*; B, C: *Z. aucheri*; D-F*: Z. membranacea*.

Figure 2. Reducing power ability of different extracts of studied species at different concentrations, (mean ± SEM, n = 3). A and B: Comparison of reducing power in different concentrations of studied plants in comparison with ascorbic acid.

Figure 3. The inhibition percent of studied species extracts on DPPH radicals, (mean ± SEM, n = 3). A and B: Comparison of radical inhibition percent in different concentrations of studied plants in comparison with trolax.

Figure 4. A: Calibration curve of Gallic acid to determine the amount of phenol in the studied species. B: Calibration curve of Quercetin to determine the amount of flavonoids in the studied species.