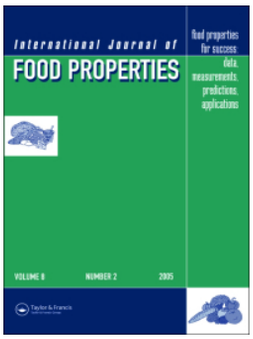

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ORIGINAL ARTICLE

Phytochemical variation and biological activities of *Zosima absinthifolia* during various stages of growth

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ABSTRACT

The knowledge of the medicinal plants' organs containing a great number of bioactive compounds is of high importance. Thus, the need to study phytochemical compositions and biological activities of different organs of *Zosima absinthifolia* is highly demanding rather than any time before in the past. The essential oil was analyzed using GC and GC/MS. Total phenol and flavonoid content, and phenolic compound analysis were evaluated via spectrophotometry, and HPLC methods, respectively. Antioxidant, antibacterial, and cytotoxic activities were assayed by DPPH, disk diffusion, and MTT methods, separately. The results illustrated the maximum yield of essential oil was achieved at the late-mature/ripe seeds (0.88%), while the leaves had the lowest yield (0.31%). The essential oil analysis showed that octyl acetate and 1-octanol were the main compounds in the early development, mid-mature, and late-mature seeds. The highest total phenolic and flavonoid content were achieved in the acetone extracts of different seeds. Moreover, only caffeic acid and salicylic acid were detected in the ethyl acetate, acetone, and methanolic extracts, and the acetone extract of all aerial parts of the plant showed the maximum content. The biological results revealed that the highest antioxidant and cytotoxic properties were obtained in the acetone extracts of flowers and the early development stage of seeds. Acetone extract of all parts of the plant was highly active against *Bacillus subtilis, B. pumilus*, and *Staphylococcus aureus* bacteria. Finally, it can be inferred that the different organs and extraction solvents could influence the phytochemical compounds and biological properties of *Z. absinthisfolia*.

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Introduction

The Zosima genus, belonging to the Apiaceae family in Iran, consists of six species. *Zosima absinthifolia* is a perennial herb and the well-known species of the genus that is native to some certain areas in Iran. This plant is widely distributed from the Middle East to Turkey, Iran, and Afghanistan. In some regions, the aerial parts of *Z. absinthifolia*, especially fruits, are used for different purposes such as food spice, edible after cooked and folk medicine.[1]

Z. absinthifolia has been utilized as a medicinal plant since ancient times in Iran, Turkey, and Pakistan. The essential oils and extracts of this plant are of many biological activities such as anti-inflammatory, anti-microbial (antibacterial and antifungal), and cytotoxic activity.[2,3] It has been shown that the essential oil of *Z. absinthifolia* fruits has a high antibacterial effect against some bacteria

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such as B. pumilus and Bacillus subtilis.[4] Karakaya et al. investigated the biological properties of essential oils and extracts from different parts of *Z. absinthifolia* and concluded that the flowers and fruit extracts have high antioxidant and anticholinesterase properties.[5] Bahadir et al. indicated that the n-hexane extract obtained from the aerial part of *Z. absinthifolia* had anti-inflammatory properties in rats.[6] In another study, the methanol extract of *Z. absinthifolia* fruits significantly showed high free radical scavenging, antibacterial activity, and cytotoxic properties.[2] Also, in studies on the essential oil compositions of *Z. absinthifolia* fruits, the major components of the essential oil were octyl acetate, octyl octanoate, octyl hexanoate, and 1-octanol.[3,4] Hence, different organs of medicinal plants may potentially be an excellent source of biological activity due to the attendance of phytochemical compounds.[7] Ecological conditions, phonological, harvest time, and genetic difference are important factors which could bring pressure to bear on the chemical compositions and biological properties of different organs of medicinal plants.[8–10] There are little reports on the phytochemical and biological activity of different parts of *Z. absinthisfolia*. Therefore, the present study aimed to investigate the phytoconstituents present in different parts of *Z. absinthisfolia*, and also, to estimate their antioxidant, antibacterial, and cytotoxicity activities.

Materials and methods

Plant materials

The aerial parts of *Zosima absinthifolia* were collected at different phenological stages (vegetative (leaves), full flowering (flowers)) and different stages of seeds (early development, mid-maturation, and late-mature/ripe) from its wild habitat in Tabriz, East Azerbaijan province, Iran, in the period between June and August of 2018. The GPS location details were the longitude of 46°18′E and latitude of 38°04′N, an altitude of 1360 m above sea level. Voucher specimen after identifying (ASMUH-98020) was deposited at the herbarium of Azarbaijan Shahid Madani University.

Extraction of essential oil

The plant (50 g) was subjected to hydro-distilled with 500 mL of distilled water for 3 hours using a Clevenger apparatus. The obtained essential oils were dehydrated using Na_2SO_4 and then stored in a sealed dark glass (−20°C in a freezer) before the next analyses.

GC-MS analysis

The essential oil analysis was done on an Agilent Technologies GCMS instrument equipped with an HP-5 fused silica capillary column (30 m × 0.32 mm (ID), 0.25 micron (FT)). The injector temperature was adjusted at 240°C, and the oven temperature program was set at 60°C (3 min) to 210°C with a ramp-up of 3°C/min, then increased to 240°C (20°C/min), finally, held for 8.5 min. The EI-MS operating parameters were set as follows: ionization voltage, 70 eV; ion source temperature, 200°C. Wiley and NIST 11.0 mass-spectral libraries, Kovats Indices (KI), and previous literature were used for the qualitative identification of essential oil constituents. The compound percent was calculated by the electronic integration of FID peak areas without the use of correction factors.

Preparation of the extracts

Five different solvents including hexane, dichloromethane, ethyl acetate, acetone, and methanol were hired for the extraction of bioactive compounds from different parts of *Z. absinthisfolia*. Approximately, 30 mL of the solvents was added to 2 g of samples and then put into an ultrasonic bath (frequency, 100 kHz; power intensity, 160 W; temperature, 35°C) for 30 min. Next, the extract

was filtered, evaporated, and then stored in an amber glass vial (–20°C in a freezer) for further analysis.

Total phenolic content

The total phenolic content was quantified by the Folin–Ciocalteau colorimetric method.[11] Briefly, Folin–Ciocalteau reagent (250 µL, 10%, v/v) was mixed with the extract (50 µL, 1 mg/mL). The obtained solution was shaken for 5 min, and then 250 µL of 7% carbonate sodium (7%, v/v) was added. Finally, after 2 hours, the absorbance of the sample was read at 765 nm.

Total flavonoid content

The total flavonoid content was quantified according to Hazrati et al. method.[10] Briefly, $AlCl_3$ solution (100 µL, 2% w/v) was added to the extract (200 µL, 1 mg/mL), and shaken for 10 min. Then, the absorbance of the sample was read at 430 nm.

DPPH free radical scavenging activity

The measurement of DPPH radical scavenging activity of the *Z. absinthisfolia* extracts was carried out according to the method proposed by Mollaei et al. with some modifications.[11] Briefly, the DPPH solution (100 µL, 1 mM) was added to 100 µL of the extract at different concentrations (25–500 µg/mL). The absorbance of the obtained solution was read at 517 nm after incubating at 25°C for 30 min. Finally, the radical scaven ging activity (%) was calculated by Equation (1):

$$\text{Radical scavenging activity}(\%) = \left(\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{control}}\right) \times 100 \quad (1)$$

Antibacterial activities

The extracts of *Z. absinthisfolia* were tested individually against a range of seven bacteria, including *Bacillus pumilus* PTCC1274, *Bacillus subtilis* ATCC 465, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* PTCC1015 (Persian Type Culture Collection number), *Klebsiella pneumoniae* ATCC10031, *Escherichia coli* ATCC25922 (American Type Culture Collection number), and *Staphylococcus epidermidis* ATCC12228. All bacterial strains were achieved from the Pasteur Institute of Iran (PII). Bacterial strains were cultured in Mueller Hinton Agar plates, and then the antimicrobial activity of extracts was studied using the disk diffusion method with the determination of inhibition zones. Also, the MIC values were determined by the broth microdilution assay.

Cytotoxic activities

The cytotoxicity activity of the *Z. absinthisfolia* acetone extract was studied according to MTT assay.[12] Briefly, the human breast adenocarcinoma (MCF-7) cell lines were cultured and then the 100 µL of cell solution at the density of 1×10^4 cells/mL was plated in 96-well plates. Then, the culture media was replaced with fresh media (100 µL) and treated with different concentrations of the acetone extract. After 72 h, MTT solution (20 µL) was added to each well and then incubated for 4 h. Lastly, the absorption of MTT was read at 570 nm. The cell viability (%) is calculated according to Equation (2):

$$\text{Cell viability}(\%) = \left(OD_{\text{sample}}/OD_{\text{control}}\right) \times 100 \quad (2)$$

Extraction of phenolic compounds

The extraction of phenolic compounds was done conforming with the method employed by Hazrati et al. with some modifications.[10] Briefly, 10 mL of 80% ethanol was added to 1.0 g of the dried powdered plant and then was vigorously shaken. After centrifuging at the speed of 6000 × g for 5 min, the supernatant was collected, evaporated, and then stored in an amber glass vial (–20°C in a freezer) for further analysis using HPLC.

Analysis of phenolic compounds

The analysis of phenolic compounds was performed using an HPLC (Knauer, Berlin, Germany) system equipped with a 20 µl loop, a diode-array detector, and an ODS column (250 mm × 0.46 mm, 5 µm). The reverse-phase separation was done with gradient elution solvent A and B, namely methanol-TFA (99.9:0.1, v/v) and water-TFA (99.9:0.1, v/v), respectively. Gradient conditions were: 20% A, in 0 min; 30% A, in 10 min; 60% A, in 30 min; 80% A, in 40 min; 100% A, in 45 min; 20% A, in 52 min; isocratic, 6 min. The flow rate of the mobile phase was adjusted at 1 mL/min and the wavelength was set to 254, 275, and 320 nm. Study on the quantification method of the studied phenolic compounds was accomplished via the external standard method.

Statistical analysis

Data were analyzed by the SAS 9.2 using a one-way analysis of variance (ANOVA) of a completely randomized design, and the mean comparisons were determined by Tukey's test ($p < .05$).

Result and discussion

Essential oil content in different parts

The essential oil yields (%, w/w) were studied in different organs of *Z. absinthisfolia* and the results showed that the yields of the leaves, flowers, early development of seeds, mid-mature seeds, and late-mature/ripe seeds were 0.31%, 0.42%, 0.46%, 0.35%, and 0.88% (w/w), respectively. Karakaya et al. studied the essential oils of root, flower, and fruit of *Z. absinthisfolia*, and concluded that the yield of the root essential oil was low compared to others and the best yield result was obtained for fruit.[5] Başer et al. indicated that the essential oil yield of the air-dried fruits of *Z. absinthisfolia* was 0.9%.[4] In another research on the essential oil from the dried fruits of *Z. absinthisfolia*, the essential oil yield was 0.05% (w/w).[13] The variations in essential oil content of different parts could be related to physiological differentiation occurred during the phenological cycle. According to the "source-sink transportation hypothesis," the photosynthesis and metabolites are diverted to secondary metabolism pathways in flowers and seeds, and an increase in the essential oil amount in the ripe seeds could construe to significant biological and ecological repercussions.[14] These results suggest that *Z. absinthisfolia* leaves may posit a metabolic effect on growth and photosynthesis, resulting in lower essential oil yield.

Chemical compounds of essential oils

Table 1 indicates the chemical compositions of various aerial organs of *Z. absinthisfolia* (Table 1). As shown in Table 1, different compositions were recognized with respect to the leaves, flowers, early development, mid-mature, and late-mature/ripe seeds, which represented 96.64%, 96.34%, 97.14%, 97.91%, and 95.64% of the total identified compounds, respectively. The main compounds were Germacrene-D (13.14%), α-pinene (7.08%), β-pinene (5.32%), 3-Methylnonane (6.07%), β-Caryophyllene (7.84%), and Octyl-acetate (7.52%) in the leaves; Camphor (35.24%), Octyl acetate (10.55%), Caryophyllene oxide (5.87%), and β-Caryophyllene (5.47%) in the

Table 1. Chemical composition of the essential oils of *Z. absinthifolia* at different phenological stages.

No.	Compound name	RI	Different organs of plant*				
			1	2	3	4	5
1	α-pinene	922	7.08	2.23	1.38	0.11	0
2	Camphene	934	2.99	1.66	0.92	0.9	0.1
3	Sabinene	958	3.43	1.26	0.31	0.2	0.1
4	β-pinene	970	5.32	2.12	0.31	0.21	0
5	3-Methylnonane	976	6.07	2.65	0	0	0
6	Octanal	999	0	0	1.27	0.8	0
7	gama-4-Carene	1001	1.69	0.71	0	0	0
8	*p*-Cymene	1016	0.99	0.37	0	0	0
9	limonene	1023	3.28	0.7	0	0	0
10	Cineole	1026	0	0	0.36	0	0
11	Trans- β-ocimene	1047	4.82	1.06	0.28	0	0
12	Linalool	1054	0	0.19	0.17	0	0
13	1-Octanol	1060	2.16	3.12	7.62	14.22	17.06
14	β-Myrcene	1083	2.64	1.21	0	0	0
15	1-hexyl-3-methyl Cyclopentane	1097	2.92	1.15	0	0	0
16	3-Methylundecane	1109	4.71	1.11	0.1	0.18	0.1
17	Camphor	1133	0	35.24	0	0	0
18	endo-Borneol	1157	0	2.76	0	0	0
19	Octanoic acid	1166	1.69	0	3.47	0	0
20	(+)-α-Terpineol	1181	0	0.41	0.28	0	0
21	Decen-1-ol (4Z)	1261	1.89	0	0.34	0	0
22	Bornyl acetate	1273	1.54	0.62	1.06	1.36	0.53
23	Citronellyl acetate	1347	0	0	0.34	0	0
24	Hexyl hexanoate	1378	0.52	0	0	0.7	0.78
25	Geranyl acetate	1379	0	0	0.31	0	0
26	Octyl butanoate	1380	0	0	0.27	0	0
27	β-Bourbonene	1386	2.95	0.69	0.45	0	0
28	Decenyl acetate	1397	0.59	0	5.02	4.4	0.31
29	gamma-elemene	1430	2.49	1.15	0.95	0.43	0
30	β-Caryophyllene	1439	7.84	5.47	2.57	0.77	0
31	Octyl acetate	1470	7.52	10.55	59.72	66.82	69.69
32	N-octyl 2-methyl butyrate	1472	0.41	0.92	0.4	0.68	0.71
33	Germacrene-d	1482	13.42	4.14	1.59	0.38	0
34	β-Selinene	1479	1.73	1.37	0	0	0
35	(+) -Spathulenol	1577	2.46	2.68	2.63	1.05	0
36	Caryophyllene oxide	1574	1.10	5.87	1.85	0.5	0
37	3-Methylene-bicyclo[3.2.1]oct-6-en-8-ol	1607	0.41	0.9	0	0	0
38	β-Eudesmol	1632	0.71	1.84	0	0	0
39	Isoaromadendrene epoxide	1638	0	0	0	0	0
40	α-Bisabolol oxide B	1655	0	1.45	0	0	0
41	Octadecanal	1358	0.1	0	2.12	4.06	6.09
42	α-Bisabolol	1673	0.85	0.57	0.38	0	0
43	Octanoic acid	1677	0.17	0.17	0.15	0.14	0.17
44	Farnesyl acetate	1841	0.15	0	0.52	0	0
45	Hexadecanoic acid	1943	0.15	0.00	0.52	0.00	0.00
	Hydrocarbon		74.37	29.05	8.86	3.18	0.3
	Alcohol		7.86	11.22	11.04	15.27	17.06
	Ether		1.72	41.05	2.59	0.5	0
	Aldehyde		0	0	1.27	0.8	0
	Ketone		0	2.76	0	0	0
	Acid		1.94	0	6.11	4.06	6.09
	Ester		10.75	12.26	67.27	74.1	72.19
	Total		96.64	96.34	97.14	97.91	95.64

*1: Leaves; 2: Flowers; 3: Early development seeds; 4: Mid-maturation seeds; 5: Late-mature/ripe seeds.

flowers; Octyl-acetate (59.72%), 1-Octanol (7.62%), and Decenyl acetate (5.02%), in the early development seeds. Also, in the mid-mature seeds essential oils, Octyl acetate, and 1-Octanol were the major components, with 66.82%, and 14.22%, respectively, while in the late-mature/ripe seeds essential oil, these components were correspondingly 69.69% and 17.06% (Table 1).

According to the results, the Octyl acetate and 1-Octanol percentages increased during the ripening seeds compared to the other parts, while there was a decline with regard to α-Pinene, Camphene, Sabinene, β-pinene, 3-Methylnonane, Trans-β-ocimene, 3-Methylundecane, β-Caryophyllene, and Germacrene-D. Also, based on the results, Camphor (35.24%), endo-Borneol (2.76%), and β-Bisabolol oxide B (1.45%) observed only in the flower organ. However, in comparison to other organs, a-pinene, β-pinene, 3-Methylnonane, Trans-β-ocimene, and Germacrene-D were of the highest amounts in the leaves (7.08%, 5.32%, 6.07%, 4.82%, and 13.14%, respectively) (Table 1).

Furthermore, according to functional groups, the essential oil compounds have been classified into seven groups including hydrocarbon, alcohol, ether, aldehyde, ketone, acid, and ester (Table 1). Concerning essential oils of the leaves, hydrocarbons were the major group, while, the percentage of this group decreased during the maturation of seeds. These results proposed that hydrocarbons contained more compounds that were volatile in the early stages of plant growth. Moreover, the essential oil of the mid-mature and late-mature/ripe seeds had a significantly higher percentage of alcohol, acid, and ester. Başer et al. reported Octyl acetate and Octyl hexanoate like esters as the dominant compositions of the *Z. absinthisfolia* essential oil.[13] Karakaya et al. indicated ester such as Octyl acetate was the main composition in the seed essential oil of *Z. absinthisfolia*, and it also was significantly different within the flowers essential oil.[5] The above-mentioned findings were in concert with the results of our study. Thus, it can be concluded that the essential oil constituents could be dissimilar within each plant organ. Other relevant studies have investigated the changes in essential oil constituents in different organs of the Apiaceae family such as *Heracleum persicum* and *Ferulago angulata*.[10,15]

Extract yield of different parts

Figure 1 shows the yield of different parts of *Z. absinthisfolia* (leaves, flowers, early development, mil-mature, and late-mature/ripe seeds) extracted by different solvents. The extracted yield of different parts was highly of sharp difference. Among all parts of the plant, the extract yield was recorded highest in the acetone and lowest in n-hexane extract of *Z. absinthisfolia*. In needles, the yield was

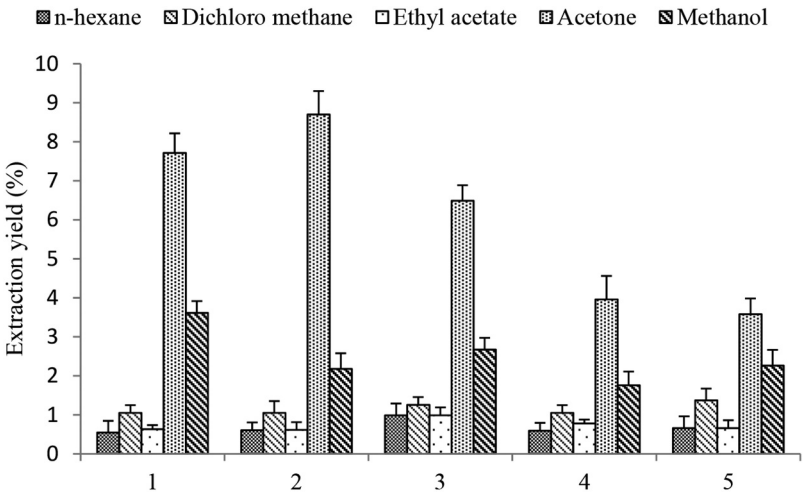

Figure 1. Yield of the extracts obtained from the different parts of *Z. absinthisfolia*. 1: Leaves; 2: Flowers; 3: Early development seeds; 4: Mid-maturation seeds; and 5: late-mature/ripe seeds.

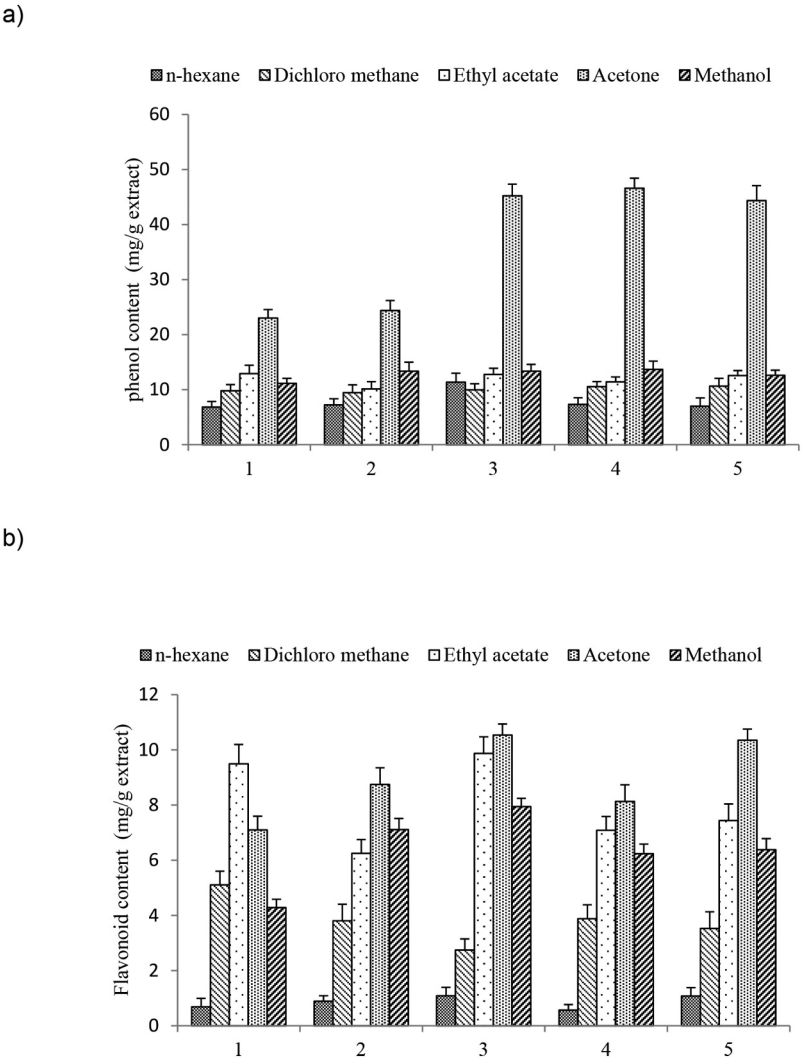

Figure 2. Phenol and flavonoid contents of the extracts obtained from the different parts of *Z. absinthisfolia*. 1: Leaves; 2: Flowers; 3: Early development seeds; 4: Mid-maturation seeds; and 5: late-mature/ripe seeds.

estimated higher in the acetone extracts in comparison to the other extracts and it was recorded lowest in hexane, chloroform, and ethyl acetate. Higher extract yield with polar solvents revealed the presence of more polar compounds in *Z. absinthisfolia*. It is probably the case that low extract yield in hexane, chloroform, and ethyl acetate in comparison to acetone and methanol was due to the poor dielectric constant.

Total phenolic and flavonoid content

The total phenol and flavonoid content depends on the plant parts of *Z. absinthisfolia* and extract solvents. According to Figure 2a, the phenol and flavonoid contents in the acetone extracts of *Z. absinthisfolia* seeds were more than the other parts and solvent extracts. A study on the flavonoid content indicated that ethyl acetate and acetone as extraction solvents resulted in maximum flavonoid content followed by dichloromethane, methanol, and n-hexane, respectively. Once the acetone was used

as an extraction solvent, it resulted in a maximum extraction of flavonoids from early development and late-mature seeds of *Z. absinthisfolia* (10.53 and 10.35 mg QE/g of extract, respectively) (Figure 2b).

Phenolic compound analysis

In the present study, different solvents and parts of *Z. absinthisfolia* were examined for their individual phenolic compounds. For this purpose, 16 phenolic compounds, i.e. gallic acid, catechin, epicatechin, vanillic acid, ferulic acid, para-coumaric acid, meta-coumaric acid, kaempferol, caffeic acid, rosmarinic acid, rutin, cinnamic acid, salicylic acid, chlorogenic acid, hesperidin, and apigenin were used and then analyzed hiring the RP-HPLC method. Among the studied phenolic compounds, the dominant phenolic compounds were caffeic acid and salicylic acid. Both were detected in the ethyl acetate, acetone, and methanolic extracts, while these compounds were not observed in the n-hexane and dichloromethane extracts of all parts of the plant.[16] Also, the acetone extract of all plant parts indicated the maximum caffeic acid and salicylic acid content (32.86 mg/g extract), followed by ethyl acetate (10.95 mg/g extract), and methanolic extracts (6.51 mg/g extract). Moreover, the acetone late-mature seed extract had the highest amount of these compounds (9.85 mg/g extract), followed by mid-mature (9.24 mg/g extract) and early development seeds (8.65 mg/g extract).

As shown in Table 2, salicylic acid was the major phenolic compound in the extracts of early development, mid and late-mature seeds of 6.36, 7.54, and 7.94 mg/g extract, respectively (Table 2). Salicylic acid is a natural product that has many biological activities including antioxidant, anti-aging, anti-cancer, anti-rheumatic, and anti-allergy.[17] Salicylic acid is widely utilized in the cosmetic and food industries owing to its high biological activities and low toxicity.[18] It has been observed within all organs of the plant, with the highest amount related to the ripe seed organs (7.94 mg/g of extract). Moreover, the minimum amount was associated with the ethyl acetate and methanolic extracts of the leaves with values of 0.10 and 0.33 mg/g extract, respectively. Still, salicylic acid was not detected in ethyl acetate and methanolic extracts of the flower organs.

Caffeic acid, another identified phenolic compound, has been reported to have considerable amounts of biological properties such as anti-cancer, anti-rheumatic, and anti-allergy properties.[19] This compound was found in the ethyl acetate, acetone, and methanolic extracts of all parts of *Z. absinthisfolia*, with the highest amount in the acetone extract of flowers and early development seeds (2.28 and 2.29 mg/g of extract), respectively (Table 2). Hence, the quantity of phenolic compounds was variant at disparate extracts and parts of *Z. absinthisfolia*, with the highest and lowest values of 9.85 and 0.31 mg/g each for the acetone extract of late-mature seeds and the ethyl acetate extract of leaves.

Table 2. Content of Caffeic acid (CaA), and Salicylic acid (SA) (mg/g dried extract) at different solvent and parts of *Z. absinthifolia.*

	n-Hexane		Dichloromethane		Ethyl acetate		Acetone		Methanol	
	CaA	SA	CaA	SA	CaA	SA	CaA	SA	CaA	SA
Leaves	nd	nd	nd	nd	0.21	0.10	1.56	0.64	0.54	0.33
Flowers	nd	nd	nd	nd	0.19	nd	2.28	0.64	nd	nd
Early development of seeds	nd	nd	nd	nd	1.06	2.14	2.29	6.36	0.81	1.19
Mid-maturation of seeds	nd	nd	nd	nd	1.12	2.48	1.70	7.54	1.12	1.14
Late-mature/ripe of seeds	nd	nd	nd	nd	1.34	2.31	1.91	7.94	0.49	0.89
				Total	10.95		32.86		6.51	

CaA: Caffeic acid; SA: Salicylic acid; nt: not detect. All experiments were done in triplicate.

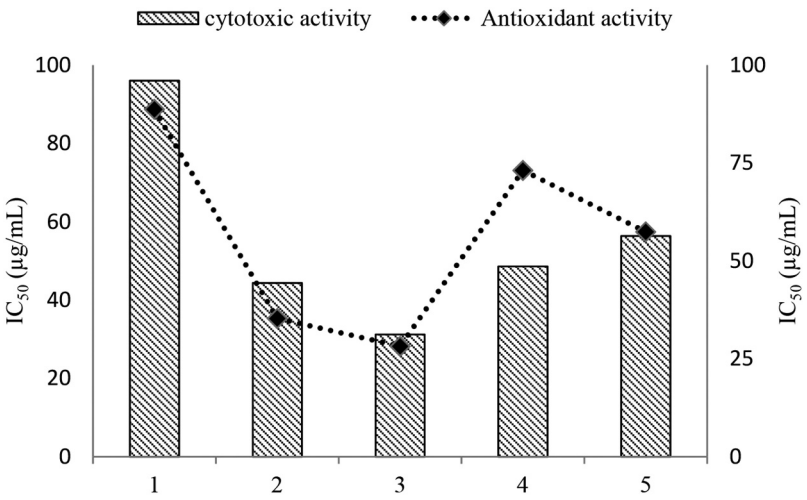

Figure 3. Antioxidant and cytotoxic activities of the acetone extract obtained from the different parts of *Z. absinthisfolia*. 1: Leaves; 2: Flowers; 3: Early development seeds; 4: Mid-maturation seeds; and 5: late-mature/ripe seeds.

Biological activity of acetone extract

According to the results, it could be concluded that the acetone extract had the highest extract yield, total phenol, and flavonoid content, as well as the maximum caffeic acid and salicylic acid amounts. To that end, it appears logical to investigate the biological activity of the acetone extract.

Antioxidant activity

The results of DPPH scavenging for activity among different parts of *Z. absinthisfolia* are shown in Figure 3. In general, the amount of IC_{50} among different parts was from 28.2 ± 0.1 to 88.7 ± 0.2 µg/mL. In comparison with the various parts of *Z. absinthisfolia*, the acetone extract in the early development seeds showed the highest activity in the scavenging process using DPPH assay. Meanwhile, the acetone extract of flowers and early development seeds had the highest antioxidant activity (35.3 ± 0.3 and 28.2 ± 0.1 µg/mL, respectively) among the extracts. Thus, it can be concluded that acetone has been the finest solvent for the extraction of natural antioxidant compounds from *Z. absinthisfolia* early development seeds. Our results are in line with Martins et al.,[20] which reported that acetone can be considered as a useful solvent for the extraction of phenolic compounds with high antioxidant activity.

Cytotoxicity activity

The cytotoxic activity of the *Z. absinthifolia* seeds was studied and the results indicated that the methanolic extract of *Z. absinthifolia* seeds significantly exhibited cytotoxic activities against the Hela cell line.[2] However, the cytotoxic study of the different parts of *Z. absinthisfolia* has not investigated. So, the current study was the first concerning the cytotoxic activity of the acetone extract of different parts of *Z. absinthisfolia*. The cytotoxicity results exposed that almost all organs of the plant have cytotoxic properties against MCF-7 cell lines (Figure 3). Moreover, the extracts of flowers and early development seeds indicated the lowest amount of IC_{50} (41.39 and 31.22 µg/mL, respectively). As a result, the acetone extracts of flowers and early development seeds could potentially be considered as an anti-proliferative agent, which this fact may be due to the presence of high antioxidant compounds.[21,22] García-Pérez et al. reported the cytotoxic activity of the different extracts of

Table 3. *In vitro* Antimicrobial activities of the acetone extract obtained from the different parts of *Z. absinthisfolia* (Disk diffusion method) against various microorganisms.

	Inhibition zone diameters (mm)[a]						
Sample	*B. pumilus*	*B. subtilis*	*S. aureus*	*B. cereus*	*K. pneumonia*	*E. coli*	*S. epidermidis*
Leaves	15	17	18	11	12	12	10
Flowers	19	18	19	12	14	12	12
Early development of seeds	17	17	12	11	11	12	11
Mid-maturation of seeds	15	17	14	11	10	12	11
Late-mature/ripe of seeds	15	18	15	10	10	11	12
Ampicillin[b]	15	14	13	nt	nt	12	19

a: Zone of inhibition (in mm) includes diameter of the disc (6 mm), values as mg ml^{-1}, (–): Inactive, (7–13): moderately active, (> 14): highly active, nt: not tested, A quantity of 10 µl of EtOH without sample (negative control) was inactive. All experiments were done in triplicate. [b]Tested at 1 µg/disc.

Table 4. Minimum inhibitory concentrations (MIC [mg/ml]) of the acetone extract obtained from the different parts of *Z. absinthisfolia.*

	Microorganism						
Sample	*B. pumilus*	*B. subtilis*	*S. aureus*	*B. cereus*	*K. pneumonia*	*E. coli*	*S. epidermidis*
Leaves	15	7.5	7.5	>15	>15	>15	>15
Flowers	7.5	7.5	7.5	15	7.5	15	15
Early development of seed	7.5	7.5	15	15	>15	15	>15
Mid-maturation of seeds	15	7.5	7.5	15	>15	15	>15
Late-mature/ripe of seeds	15	7.5	7.5	>15	15	15	15
Ampicillin[b]	15	15	15	nt	nt	15	15

nt: not tested, All experiments were done in triplicate. [b]Tested at 1 µg/disc.

Poliomintha glabrescens against colon cancer cells HT-29, and thereafter, concluded that cytotoxicity activity could be linked to the existence of flavonoids such as luteolin and apigenin.[21] According to Nile et al., the high cytotoxicity of the *Origanum vulgare* extracts against MFC-7 was due to its high phenolic content.[22] The findings confirm that the acetone extract of *Z. absinthisfolia* flowers and early development seeds have cytotoxicity activities and are enriched with phenolic, flavonoid, and phenolic acid, which could be regarded as a promising source for the development of new drugs.

Antibacterial activity

The antibacterial activity was tested in opposition to two gram-negative and five gram-positive bacteria. The inhibition zone diameter and Minimum Inhibitory Concentration (MIC) values of *Z. absinthifolia* extracts are displayed in Tables 3 and 4. The results showed the extracts were of moderate-to-high inhibitory activity against the tested bacteria. In accordance with the results, the acetone extracts of all parts of the plant were highly active against *B. subtilis, B. pumilus*, and *S. aureus* bacteria, while, in the cause of other studied bacteria, the extracts were moderately active. The extract of flowers was more active against studied bacteria compared to other parts of the plant. There was and still is a body of ongoing research about the antibacterial effect of the essential oil and the extract obtained from the *Z. absinthisfolia* fruits.[2,3] Razavi et al. showed that the methanolic extract of the *Z. absinthisfolia* fruits was found to have a significant antibacterial effect against *B. cereus* and *S. epidermidis*.[2] In another study, the essential oil of the *Z. absinthisfolia* fruits revealed high antibacterial effects against *B. subtilis, B. pumilus*, and also modest-to-strong effects on different bacteria and fungi.[3] However, no research has been done on the antimicrobial activity of the different parts of *Z. absinthisfolia* in Iran and other countries thus far.

Conclusion

The results indicated there are remarkable differences in the chemical compositions of *Z. absinthisfolia* between the different organs of the plant. As a result, the kinds of organs and extraction solvents were the most important factors, which could affect the chemical compositions and biological properties of *Z. absinthisfolia*. Regarding the essential oils of the leaves, the majority of the compositions were hydrocarbons, while, the percentage of this group decreased during the development phase of seeds. In the acetone extracts of *Z. absinthisfolia* seed, the phenol and flavonoid content, as well as the caffeic acid and salicylic acid amounts, were more than the other parts and extracts. The acetone extracts of flowers and early development seeds showed the highest antioxidant, antibacterial, and cytotoxic activity among the extracts. Thus, it can be concluded that acetone was the best solvent for the extraction of bioactive compounds from the early development phase of seeds of *Z. absinthisfolia*. Finally, when *Z. absinthisfolia* is utilized as herbal medicine, the selection of early development seeds would be notably of considerable importance.

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