Chemical Composition and Antioxidant Activity of Extracts from the Fruit, Leaf, and Branchlet of *Cupressus arizonica* Greene

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Potent antioxidant activities of solvent extracts (96% aqueous ethanol) from the fruit, leaf, and branchlet without adherent leaf of *Cupressus arizonica* were evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and compared with butylated hydroxytoluene (BHT) and ascorbic acid (AA). Their chemical compositions were analyzed using gas chromatography-mass spectrometry (GC/MS). Branchlet extracts (BE) were the most active as an antioxidant agent at 93.3% at the concentration of 0.493 mg/mL, which was higher than the value of vitamin C (63.3%) at the same concentration. The major components identified in the BE were communic acid (43.7%), followed by agatholic acid (20%), and ferruginol (10.4%). The extract from fruit had good antioxidant activity (90.3%) at a concentration of 0.015 mg/mL. The major compounds identified in the fruit extracts (FE) were communic acid (46.8%), spirohexane-5-carboxylic acid, 1,1,2,2-tetramethyl-, methyl ester (27.4%), and ferruginol (6%). Leaf extracts (LE) were more active as an antioxidant agent at 80.3%, which was higher than the value of BHT (75.7%) at the concentration of 0.015 mg/mL. The major components identified in the LE were hexadecanoic acid (45.1%), 1H,5H-pyrrolo[1',2':3,4]imidazo[1,5-a]pyridine, octahydro- (9%), bicyclo [3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)- (8.1%).

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Keywords: Cupressus arizonica; Fruit, leaf, and branchlet extracts; Antioxidant activity; Chemical composition; GC-MS

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INTRODUCTION

Arizona cypress (*Cupressus arizonica* Greene) is an evergreen (coniferous) and is a medium-sized tree at 50 to 60 feet tall and 15 to 30 inches in diameter. It has blue-green foliage and scaly reddish brown bark, and is widely distributed throughout the Southwest of USA (Little and Skolmen 1989; USDA 2002; Emami *et al*. 2010; Swearingen and Bargeron 2016). This species is native in the United States and found in California, New Mexico, Arizona, Texas, and Mexico (Fralish and Franklin 2002). This beautiful tree was introduced into Iran in 1954 and cultivated in various parts of the country as an ornamental tree and for reforestation purposes (Sabeti 1966). Persian names of this tree are "Sarve Noqrei" (Sabeti 1976) and "Sarve Simin" (Zare 2001).

Many researchers have yet to consider the chemical composition of *Cupressus* spp. from different parts of the world. Some of the species that have been

phytochemically analyzed include *C. arizonica* (Chéraif *et al.* 2007), *Cupressus lusitanica* (Kuiate *et al.* 2006), *Cupressus cashmeriana*, *Cupressus chengiana*, *Cupressus funebris*, *Cupressus duclouxiana*, *Cupressus guadalopensis*, *Cupressus macnabiana*, *Cupressus dupreziana* (Pierre-Leandri *et al.* 2003; Ramdani *et al.* 2011), and *Cupressus macrocarpa* (Zhang *et al.* 2012).

Mothana *et al.* (2009) demonstrated that *Cupressus sempervirens* L. leaves extract and essential oil have remarkable radical scavenging activity. Quercetin, rutin, caffeic acid, and p-coumaric acid have been isolated from *C. sempervirens* leaves (Ibrahim *et al.* 2007), but the bark extract of *Cupressus lusitanica* Mill. (Mexican white cedar) has shown high cytotoxicity on MCF-7 (estrogen receptor positive breast carcinoma) cells (Mbaveng *et al.* 2011).

C. arizonica Greene is an aromatic evergreen coniferous plant with great importance in urban horticulture and in the pharmaceutical and fragrance industries (Hassanpouraghdam 2011).

The results of the studies of Emami *et al.* (2013) showed that all methanol extracts of leaves and fruits of the six different species of Iranian common conifers: *Cupressus arizonica*, *Pinus halepensis*, *Pinus nigra*, *Pinus brutia* var. *elderica*, *Pinus wallichiana*, and *Cedrus deodara*, possessed antioxidant activity. Methanol extractions of *Pinus* spp. leaves and fruits showed the highest antioxidant activity (quite higher than α-tocopherol). Among the extracts examined, the leaves of *P. halpensis* methanol extract showed the lowest activity.

From the gas chromatography-mass spectrometry (GC-MS) analysis conducted by Abdulkhani *et al.* (2020) on the hydrophilic extractives of *C. arizonica* wood knots, different amounts of bioactive moieties were found, including matairesinol (MAT), curcumin, dienestrol, arctigenin (ARC), and sescoisolariciresinol (SEC). Additionally, the bioactivity of the hydrophilic extracts of *C. arizonica* was determined and compared with the raw hydrophilic extractives of *C. sempervirens* and *Picea excelsa*. The results of their studies revealed that *P. excelsa* with a total capacity of 318.8 $mg.mL^{-1}$ showed the highest level of phenolics, followed by unpurified *C. arizonica* (257.5 $mg.mL^{-1}$) and the solvent purified extract of *C. arizonica* (190.1 $mg.mL^{-1}$). They also found that the most powerful radical scavenging activity was a raw ethanolic extract from *P. excelsa* wood knot with 66.67%, followed by BHA and potassium acetate purified *C. arizonica* with 57.96 and 56.37%, respectively.

To the best of the authors' knowledge, the chemical composition of the fruit, leaves, and branchlet of the Arizona cypress (*Cupressus arizonica*) extracts have yet to be reported. However, the chemical composition of this part has been reported. Furthermore, this novel approach investigated the antioxidant activity of its extracts, as well as compared them with butylated hydroxytoluene (BHT) and ascorbic acid (AA). This paper is the first report on antioxidant activity of fruit extracts (FE), leaf extracts (LE), and branchlet extracts (BE) of *C. arizonica*.

EXPERIMENTAL

Materials

C. arizonica tree fruits, leaves, and branchlets were collected in April 2022 from the yard of the college of Agriculture and Natural Resources at Karaj Islamic Azad University located on the south-west of Karaj city, Iran. Voucher specimen (No. 1425) of

the plant was deposited in the Herbarium of vascular plant (SOM) of the College of Agriculture and Natural Resources at Islamic Azad University, Karaj Branch, Karaj, Iran. The plant species were authenticated by Prof. Dr. Sayed Khosrow Hossein Ashrafi.

Preparation of Extracts

The innovation in this study is to consider parts of the plant that have not been tapped before for antioxidant activity. The tree parts of Arizona cypress, including fruits, leaves, and branchlets with an approximate moisture content 89.93, 94.17, and 47.06%, were separately cut into small pieces, and chopped (with 5 to 10, 5 to 15, and 5 to 20 mm particles size, respectively). In the first step, a little cotton was compressed and placed at the bottom of the 250-mL separatory funnel at the beginning of the outlet valve. In the second step, approximately 50, 50, and 40 g from pieces of fruits, leaves, and branchlets were poured into a separatory funnel, and then 200, 300, and 200 mL of 96% aqueous ethanol was added to each one. The mixture was macerated in the closed separatory funnel for 48 h. The outlet of the separatory funnel was opened, and the liquid was allowed to drip slowly, as specified in the method by Sabzikar *et al*. (2020). The liquid was clarified by filtration and finally concentrated to dryness, in a Petri plate at a laboratory temperature (25 ± 5 °C) under a laminar hood to avoid chemical alteration in the bioactive compounds with loss of their properties. The extracts were accumulated and dried over anhydrous sodium sulfate and then stored at 4 ºC until further analysis (Sabzikar *et al*. 2020). The extracts' weights from fruit, leaf, and branchlet components were 0.20, 0.25, and 0.15 g, respectively. Each extract was prepared in the concentrations of 0.985, 0.493, 0.246, 0.123, 0.062, 0.031, and 0.015 mg/mL by diluting the extract in 70% MeOH.

Free Radical Scavenging Activity by DPPH Assay

The free radical scavenging activities of the hydroethanolic extracts of fruit, leaf, and branchlet of *C. arizonica* were conducted using the 1,1-dipheny-2-picrylhydrazyl (DPPH) assay as described in literature (Kim *et al.* 2002), because the DPPH is a stable radical, which has been used to evaluate the total antioxidant activity of plant and microbial extracts (Halliwell 1997).

Briefly, a stock solution *via* dissolving 3.94 mg of DPPH powder in 100 mL of 70% methanol (the stock solution of 0.1 mM DPPH with concentration of 0.0394 mg/mL) served as oxidant and was prepared just before use and stored at 4 °C in the dark to minimize degradation. The control samples were prepared with the same volume of solution, without test compounds and the referenced standards (negative control) (Pillai *et al.* 2019; Alam *et al.* 2021).

To prepare the working solution from the hydroethanolic extract, 9.85 mg (dry powder) sample of each hydroethanolic extract was dissolved in 10 mL of 70% methanol (v/v) (concentration 0.985 mg/mL), separately. Serial dilutions were made from the stock solution of 0.1 mM DPPH and the working solution of tested extract to obtain concentrations of 0.985, 0.493, 0.246, 0.123, 0.062, 0.031, and 0.015 mg/mL. The schematic of preparation process of working solution are shown in Fig. 1 (Hosseinihashemi and Aghajani 2017).

Two stock solutions of ascorbic acid (AA) and butylated hydroxytoluene (BHT) with the same concentration (0.985 mg/mL) were prepared. These served as the reference standards (positive control). Pure methanol (Sigma-Aldrich, Darmstadt, Germany) was

used to make the control sample. The UV scanning spectrophotometer device (JENWAY 6320D, Standford, UK) was first calibrated and adjusted with 70% methanol.

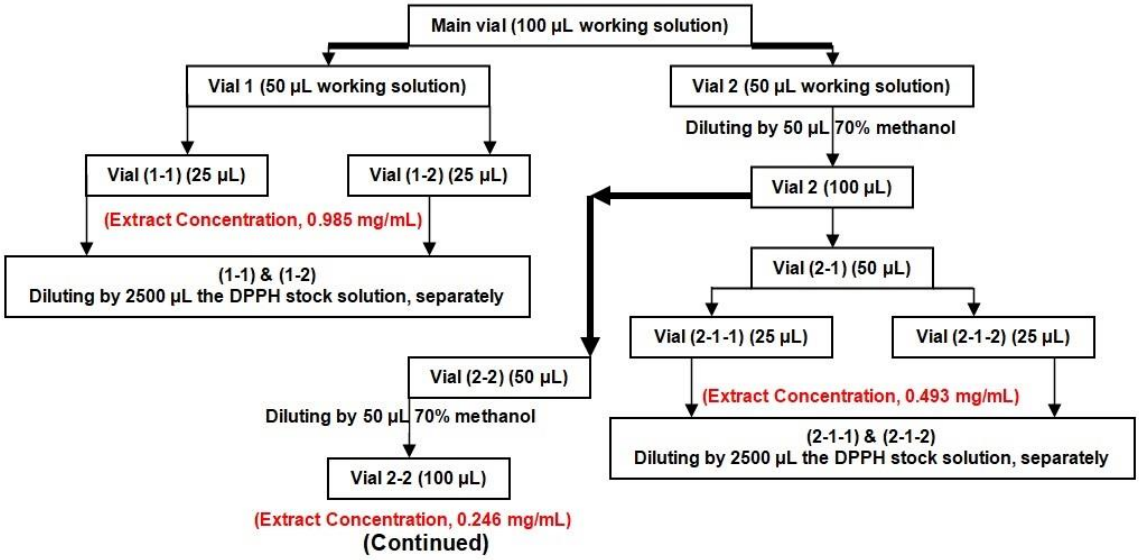

Fig. 1. Preparation process of working solutions

The reaction mixture was mixed for 10 s and left to stand at room temperature in a dark place for 30 min. The absorbance was measured at 517 nm, using a UV scanning spectrophotometer. The experiment was performed in triplicates, and the average absorbance was recorded for both extract and concentration, separately. The DPPH free radical scavenging activity (%) was calculated using Eq. 1,

$$\text{Inhibition (\%)} = 100 \times (Ac - As)/Ac \quad (1)$$

where the percentage inhibition value was calculated from the absorbance of the negative control, *Ac*, and of the sample, *As*.

The negative control contained reaction reagent except the extract or positive control substance. The values are presented as the means of triplicate analyses.

Analysis of Extracts

Gas chromatography-mass spectrometry (GC/MS) analysis of the *C. arizonica* fruit, leaf, and branchlet extracts was performed. Next, 100 µL of each extract was dehydrated by sodium sulfate salt and was dissolved with 100 µL of methanol, separately and run on a GC Agilent 7890A and MS Agilent 5975C mass spectrometer detector (Agilent Technologies, Palo Alto, CA, USA) equipped with a HP-5MS cross-linked capillary column (30 m long and 0.25 mm internal diameter, 0.25 µm film thickness). Helium was used as the carrier gas with a flow rate of 1 mL/min.

The GC/MS operation conditions were as follows: injector temperature of 260 °C; transfer line of 270 °C; oven temperature program of 60 °C for 4 min, 3 °C/min to 100 °C for 2 min, then 4 °C/min to 250 °C for 5 min; and carrier gas He at 1 mL/min. The intrinsic energy that hits the sample in the MS system was 70 eV. The split ratio of the sample was 50:1 with a split flow of 1 mL/min. Individual components were identified using mass spectra with data from literature, two mass spectrometric libraries (Wiley 275 L (http://www.palisade.com), 1998 and NIST-05 (http://www.nist.gov)), mass database

matching, and by comparing the retention times and mass spectra of constituents with published data (Julian and König 1988; Adams 1995, 2001).

Statistical Analysis

The results are given in mean values with their standard deviations. Statistical analysis was performed using the SPSS program, version 24.0 (International Business Machines (IBM) Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was conducted to determine the significance of differences between analytical results at $p < 0.05$ significance level.

RESULTS AND DISCUSSION

Antioxidant Activity (AOA)

The antioxidant activity of the 96% aqueous ethanol extracts from fruit, leaf, and branchlet of *Cupressus arizonica* at seven different concentrations of 0.15, 0.031, 0.062, 0.123, 0.246, 0.493, and 0.985 mg/mL were evaluated in the current study by using DPPH method and compared with BHT and vitamin C as the reference standards (Table 1 and Fig. 2). Statistically, there were highly significant differences among the treatments (fruit, leaf, branchlet extracts, BHT, vitamin C, and their concentrations (Table A1).

Table 1. Mean ± SD of Antioxidant Activity (%) as Affected by Concentration of Fruit, Leaf, and Branchlet of *C. arizonica* Extracts Compared with BHT and Vitamin C

Treatment	Concentration (mg/mL)							Duncan of Treatment
	0.015	0.031	0.062	0.123	0.246	0.493	0.985	
AOA (%) (FE)	90.27 ± 1.05	84.75 ± 0.19	80.03 ± 0.74	80.03 ± 1.04	75.51 ± 0.64	73.56 ± 1.46	73.32 ± 0.35	79.64^{b} ± 5.96
AOA (%) (LE)	80.34 ± 1.49	77.89 ± 0.24	76.75 ± 0.42	76.24 ± 2.34	72.52 ± 1.17	73.78 ± 1.00	70.94 ± 0.37	75.50^{a} ± 3.26
AOA (%) (BE)	74.12 ± 0.60	72.98 ± 5.74	84.41 ± 1.23	81.63 ± 4.02	80.92 ± 4.38	93.34 ± 0.08	93.33 ± 1.01	82.96^{c} ± 8.20
AOA (%) (BHT)	75.74 ± 2.65	66.45 ± 3.46	75.68 ± 0.73	76.81 ± 0.05	77.58 ± 2.20	74.92 ± 4.72	76.03 ± 3.49	74.74^{a} ± 4.31
AOA (%) (Vitamin C)	87.25 ± 6.70	68.01 ± 5.74	82.59 ± 2.26	80.49 ± 0.86	75.88 ± 0.31	63.25 ± 2.39	63.68 ± 1.59	74.44^{a} ± 9.58
Duncan of Concentration	76.83^{b} ± 9.39	73.06^{a} ± 8.68	78.55^{bcd} ± 3.60	79.04^{cd} ± 2.86	77.82^{bc} ± 4.22	76.72^{b} ± 9.20	80.17^{d} ± 9.06	

Different letters in final column and final row indicate a statistical difference ($p < 0.05$) among the treatment groups; FE: fruit extract; LE: Leaf extract; BE: Branchlet extract

As shown in Table 1 and Fig. 2, the BE exhibited high antioxidant activity overall. The lowest antioxidant activity, 70.9%, was observed in leaf extract at the concentration of 0.985 mg/mL, which was lower than for BHT (76.03%) at the same concentration. The highest activity came from branchlet extract (93.3%) at 0.985 and 0.493 mg/mL, which was also higher than the value of vitamin C (63.2 and 63.7%) at the same concentration. The same trend was observed with the reference, BHT. As the concentration of the extracts was increased, the antioxidant activity of the fruit and leaf extracts decreased, but the antioxidant activity of the branchlet extracts increased.

Additionally, among the assayed extracts, fruit and branchlet showed high antioxidant activity at 90.3 and 93.3%, respectively, when the concentration was 0.015 and 0.493 mg/mL, respectively.

On the one hand, the results of the study on the antioxidant activity and chemical composition of hydroethanolic extracts of different parts of *C. arizonica* indicated that *C. arizonica* is a rich source of phenolic compounds, with a high antioxidant capacity which may provide protection against scavenge free radicals.

On the other hand, looking at the detail of the data in Table 1 and Fig. 2, it is apparent that among the different parts of the plant, the fruit and branchlet extracts exhibited good antioxidant activity compared with the leaf extracts from *C. arizonica*, coinciding with high levels of terpenes and phenolic compounds in these parts.

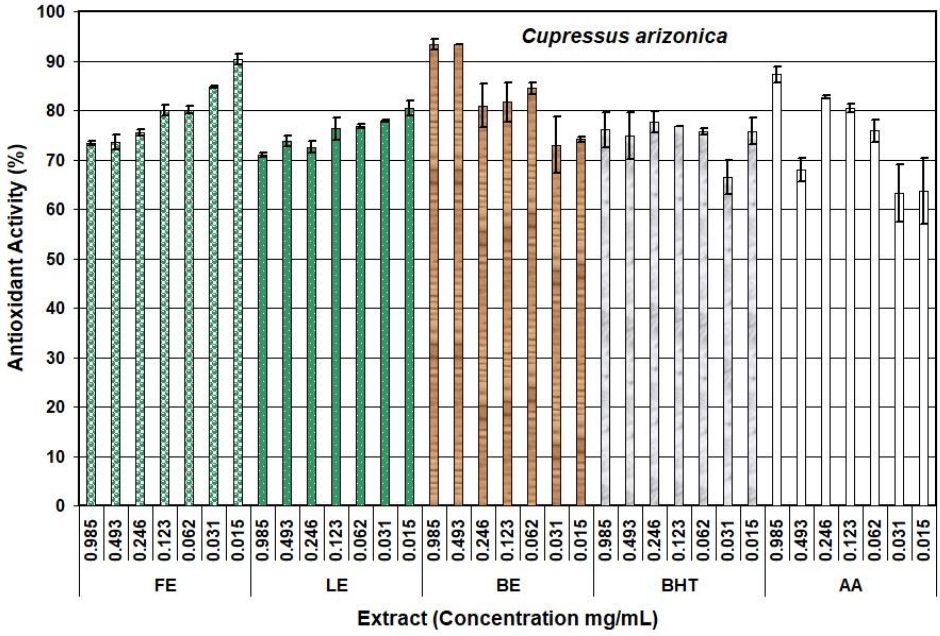

Fig. 2. The antioxidant activity of extracts from the fruit (FE), leaf (LE), and branchlet (BE) of *C. arizonica*

According to the findings of Mikucka *et al.* (2022) and in accordance with the current research findings in Table 2, the content of total polyphenols and phenolic acids strongly correlated with antioxidant activity, indicating that these compounds provide a substantial contribution to the bioactive properties of the extracts.

Phenolic compounds are widely found in plants and play an important role in antioxidant activity and scavenging free radicals through their hydroxyl groups (Cosme *et al.* 2020; Ebrahimnezhad *et al.* 2022), where deviates depending on their molecular structure and characteristics (Qian *et al.* 2020; Falah *et al.* 2021). In addition to phenolic compounds, other structures such as terpenes could develop antioxidant capacity in plant organs both solitarily or synergistically (Álvarez-Martínez *et al.* 2021).

The GC/MS analysis study revealed that the tested extracts contained an abundant amount of components that can contribute to their effective antioxidant potential in DPPH assay. The chemical components identified that are classified in Table 2 confirm these statements, so that some of these components from classes such as labdane di-terpenes, abietane di-terpenes, phenolic di-terpenes, oxygenated cembrene di-terpenes, oxygenated abietane di-terpenes, phenolic acids, resin acids, monoterpene hydrocarbons,

oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, phenolic compounds, volatile phenolic compounds, tocopherols (Ebrahimnezhad *et al.* 2022), lignans (Abdulkhani *et al.* 2020), triterpenoids, and coumarins inhibited free radical scavenging activity *in-vitro* bioassay.

Table 2. Classification of the Identified Chemical Components of the *C. arizonica* Fruit, Leaf, and Branchlet Hydroethanolic Extracts by GC/MS Data Analysis

Components	Fruit (%)	Leaf (%)	Branchlet (%)	Components	Fruit (%)	Leaf (%)	Branchlet (%)
D, LD, AD, PD, DA OCD, OAD, OD	57.83	1.99	63.22	S, CS	0.33	-	0.61
FAc	0.53	45.10	4.61	PAHs	-	0.39	-
Es, AEs, FEs, FAEs	27.35	3.86	0.33	Et, Vet	0.55	-	0.30
PAc, RAc	-	1.66	20.01	P	0.04	0.50	-
A	3.45	9.32	0.29	Alka	-	0.44	-
AM, MH, OM, SH, OS, ES	3.46	8.60	1.71	Ald (K)	-	0.28	-
Alc, AAlc, FAlc	0.22	3.95	-	L	0.18	0.18	0.08
PC, VC, VPC	0.07	1.76	2.83	CP	-	0.15	-
LOC	0.07	-	-	Alke	-	-	0.12
St	0.12	0.17	2.11	TT	0.11	-	-
V	0.06	1.40	-	Co	-	-	0.03
Re	1.24	-	-	O	2.91	15.76	2.87

D: Diterpenes; LD: Labdane diterpenes; AD: Abietane diterpenes; PD: Phenolic diterpenes; DA: Diterpene aldehydes; OCD: Oxygenated cembrene diterpenes; OAD: Oxygenated Abietane diterpenes; OD: Oxygenated diterpenes; FAc: Fatty acids; Es: Esters; AEs: Acid esters; FEs: Fatty esters; FAEs: Fatty acid esters; PAc: Phenolic acids; RAc: Resin acids; A: Alkaloids; AM: Aromatic monoterpenes; MH: Monoterpene hydrocarbons; OM: Oxygenated monoterpenes; SH: Sesquiterpene hydrocarbons; OS: Oxygenated sesquiterpenes; ES: Elemene sesquiterpenes; Alc: Alcohols; AAlc: Aromatic alcohols; FAlc: Fatty alcohols; PC: Phenolic compounds; VC: Volatile compounds; VPC: Volatile phenolic compounds; LOC: Low molecular oxygenated compounds; St: Steroids; V: Vitamins; Re: Retinoids; S: Sugars; CS: Carboccylic sugars; PAHs: Polyaromatic hydrocarbons; Et: Ethers; VEt: Vinyl ethers; P: Peptides; Alka: Alkanes; Ald (K): Aldehydes (Ketones); L: Lignans; CP: Cyclic polyols; Alke: Alkenes; TT: Triterpenoides; Co: Coumarins; O: Others

The antioxidant activity of hydroethanolic extracts of branchlet of *C. arizonica* was higher than that of fruit, leaf, BHT, and AA, which can be caused by the amount of the some mentioned antioxidant components (Table 2).

Extracts Analysis

It is clear that the extracts of the plant varied according to the plant location, variety, and position. The yields of extracts isolated from *C. arizonica* ranged from 0.44 to 0.79% depending on the part of the plant analyzed. The greatest yields were in branchlets and leaves (0.79 and 0.53%, respectively) and the lowest extract was in the fruits (0.44%). The extracts obtained from fresh fruit, leaf, and branchlet without adherent leaf of *C. arizonica* growing in Iran, were analyzed by GC/MS. A total of 55, 60, and 53 compounds were identified accounting for 98.52, 95.51, and 99.12% of the total extracts in fruit, leaf, and branchlet, respectively.

Phytochemical analysis of the fruit, leaf, and branchlet hydroethanolic extracts of *C. arizonica* showed the different amounts of bioactive moieties as summarized in Table

2. The preliminary phytochemical analysis of *C. arizonica* wood knots extract showed different amounts of bioactive moieties including matairesinol (MAT), curcumin, dienestrol, arctigenin (ARC), and sescoisolariciresinol (SEC) were found (Abdulkhani *et al.* 2020).

Chemical composition of C. arizonica fruit, leaf, and branchlet extracts

Figure 3 indicates the main chemical constituents in the fruit, leaf, and branchlet extracts.

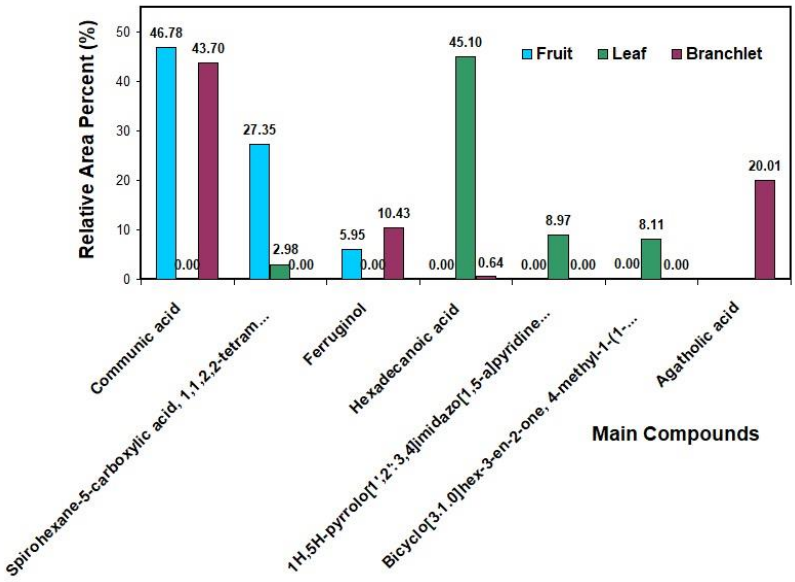

Fig. 3. The main chemical constituents identified in the hydroethanolic extracts from fruit, leaf, and branchlet of *C. arizonica*

The chemical components identified in the hydroethanolic extracts from fruit, leaf, and branchlet of *C. arizonica* are presented in Tables 3, 4, and 5. The GC-MS profiling revealed the fruit, leaf, and branchlet hydroethanolic extracts of *C. arizonica* contained 55, 60, and 53 bioactive components, respectively. Fruit, leaf, and branchlet extracts were more active, active, and most active, respectively, and also possessed good antioxidant activity.

Other identified compounds shown in Table 3 with moderate percentages included 8-amino-2,5-dimethyl-6-methoxyquinoline (2.98%), sugiol (2.89%), totarol (1.66%), bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl- (1.36%), and β-retinoic acid (1.24%). A few other compounds with lower percentages were identified in the fruit extracts, including bicycloelemene, 1,10-dicyanodecane, gamma-elemene, (1R*,2R*,3S)-3-isopropenyl-2-vinylcyclohex-1-yl vinyl ether, benzenamine, 3-chloro-N-(2-pyridinylmethylene)-, di-epi-.alpha.-cedrene-(I), and mom inositol.

The other identified compounds shown in Table 4 with moderate percentages included 1-(adamantyl-1)butanol-1 (2.05%), benzeneethanol, .beta.-ethenyl- (1.75%), phyllacladan-16.alpha.-ol (1.45%), vitamin E (1.40%), pimaric acid (1.27), and totarol (1.09%). There were a few other compounds with lower percentages identified in the leaf extracts, including 1S,CIS-calamenene, aromadendrene VI, di-epi-.alpha.-cedrene-(I), phytol, catechol, 5-hydroxy-1,3,4-trimethoxy-7-methyl-6-propargynaphthalene, and .alpha.-cedrol. The other identified compounds shown in Table 5 with moderate

percentages included isopimaral (2.93%), phenol, 4-ethyl-2-methoxy- (2.68%), sugiol (2.46%), 9,12-octadecadienoic acid (Z,Z)- (2.18%), (23S)-ethylcholest-5-en-3.beta.-ol (1.98%), totarol (1.44%), and 9-octadecenoic acid, (E)- (1.29%). There were a few other compounds with lower percentages identified in the branchlet extracts that included dispiro[2.1.2.1] octane, 1,1,6,6-tetramethyl-, cembrene, labda-8(17),13Z-dien-15-ol, carvacrol, hexadecanoic acid, mome inositol, and bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-.

Table 3. Chemical Composition of Fruit Hydroethanolic Extracts of *C. arizonica*

Compound	RT[a] (min)	RAP[b] (%)	CC[c]	Compound	RT[a] (min)	RAP[b] (%)	CC
α-Thujene	5.14	0.16	MH	9,12-Octadecadienoic acid (Z,Z)-	29.06	0.19	FAc
Sabinene	6.14	0.13	MH	(Z)6,(Z)9-Pentadecadien-1-ol	29.15	0.17	FAlc
dl-3,4-Dehydroproline	7.21	0.04	P	Octadecanoic acid	29.45	0.05	FAc
γ-Terpinene	8.09	0.04	MH	Benzenamine, 3-chloro-N-(2-pyridinylmethylene)-	30.22	0.47	A
β-Citronellol	12.11	0.10	OM	(1R*,2R*,3S)-3-Isopropenyl-2-vinylcyclohex-1-yl vinyl ether	30.38	0.51	Et
4-Vinylguaiacol	14.14	0.07	VPC	Labda-8(17),13Z-dien-15-ol	30.63	0.28	LD
α-Cubebene	14.91	0.08	SH	Torulosol	31.16	0.11	LD
Trans-caryophyllene	16.46	0.13	SH	Bicyclo[4.3.0]nonane, 7-methylene-2,4,4-trimethyl-2-vinyl-	31.35	1.36	O
Myrtensaeure	16.89	0.07	LOC	Totarol	31.68	1.66	PD
α-Humulene	17.17	0.22	SH	8-Amino-2,5-dimethyl-6-methoxyquinoline	31.92	2.98	A
α-Amorphene	17.38	0.05	SH	Ferruginol	32.04	5.95	AD
β-Selinene	17.85	0.04	SH	Communic acid	32.47	46.78	LD
α-Selinene	18.03	0.03	SH	Methyl communate	33.25	0.05	O
δ-Cadinene	18.56	0.07	SH	β-Retinoic acid	33.81	1.24	R
Nerolidol	19.31	0.11	OS	Bicycloelemene	34.20	0.84	ES
α-Cedrol	20.17	0.06	OS	Podocarpa-8,11,13-trien-3-one, 14-isopropyl-13-methoxy-	34.47	0.28	O
Di-epi-α-cedrene-(I)	20.70	0.38	MH	Spirohexane-5-carboxylic acid, 1,1,2,2-tetramethyl-, methyl ester	35.74	27.35	Es
α-Cadinol	21.14	0.13	OS	gamma-Elemene	35.96	0.54	SH
4,6-Di-O-methyl-α-d-galactose	24.30	0.04	S	Sugiol	36.13	2.89	AD
Propyl isopropyl ether	24.47	0.04	Et	(+)-Aromadendrene	36.51	0.31	SH
Mome inositol	25.65	0.29	S	Shyobunol	40.96	0.04	OS
5-Nonanol	26.23	0.05	Alc	1,10-Dicyanodecane	41.21	0.89	O
Hexadecanoic acid	26.37	0.29	FAc	Matairesinol	41.51	0.18	L
Ent-pimara-8(14),15-diene	26.53	0.05	D	D-alpha-Tocopherol	41.64	0.06	V
13-Epimanoyl oxide	26.98	0.11	TT	Dihydrotachysterol	43.46	0.06	St
Cembrene	27.27	0.05	OCD	1-Cyclohexyl-4-(1'-decahydrona	44.29	0.15	O
9H-1,8-	28.38	0.18	O	(23S)-ethylcholest-5-en-	44.41	0.06	St

[1]Propen[1]yl[3]ylidene-7H-benzocycloheptene, p				3.beta.-ol			
Phytol	28.70	0.06	OAD	-	-	-	-

[a] RT means retention time; [b] RAP means relative area percent; [c] CC: Compounds class

Table 4. Chemical Composition of Leaf Hydroethanolic Extracts of *C. arizonica*

Compound	RT[a] (min)	RAP[b] (%)	CC[c]	Compound	RT[a] (min)	RAP[b] (%)	CC
δ-3-Carene	5.14	0.20	MH	P-Toluenesulfinamide	21.38	0.21	O
trans-2-Penten-1-al	6.25	0.30	VC	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	21.52	0.39	PAHs
m-Cymene	7.29	0.17	AM	14-Norcadin-5-en-4-one isomer B	21.80	2.02	O
α-p-Dimethylstyrene	8.84	0.46	MH	Cyclopentanepropanoic acid, 1-acetyl-2,2-dimethyl-, methyl ester	22.70	0.32	AEs
Benzenemethanol	10.09	0.15	AAlc	Pluchidiol	23.64	0.14	O
4,5-epoxy-1-isopropyl-4-methyl-1-cyclohexene	10.18	0.17	VC	Hexadecanoic acid	26.36	45.10	FAc
Bicyclo[3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)-	10.88	8.11	O	2-Hydroxy-12-methoxy-19-norpodocarpa-4,8,11,13-tetraen-3-one	28.38	0.34	O
5-Aminobenzimidazole	11.51	0.50	P	Phytol	28.70	0.90	OAD
Catechol	11.90	0.75	PC	Ethyl linoleolate	29.13	0.20	FAEs
Catecholborane	12.09	0.15	PC	6,6,10-Trimethyl-1-phenylthiospiro(3.6)dec-1-ene	29.94	0.14	O
Thymol	13.63	0.14	OM	Phyllacladan-16.alpha.-ol	30.24	1.45	O
4-Vinylguaiacol	14.13	0.15	VPC	Aromadendrene	30.37	0.16	SH
α-Terpinolene	14.87	0.20	MH	12-(Cyanomethyl)indolo[1,2-c]quinazoline	31.16	0.21	A
Benzeneacetonitrile, 4-F	16.63	0.19	O	Totarol	31.67	1.09	PD
Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	17.01	1.77	O	5-Hydroxy-1,3,4-trimethoxy-7-methyl-6-propargynaphthalene	31.91	0.62	O
(+)-Epi-bicyclosesquiphellandrene	17.37	2.63	SH	4,4-dimethyltricyclo[6.3.2.0(2,5)]trideca-8-ene-1-ol	32.00	0.26	O
Germacrene D	17.72	0.14	SH	Pimaric acid	32.32	1.27	RAc
Aromadendrene VI	18.08	0.99	SH	Heneicosane	34.06	0.18	Alka
1S,cis-Calamenene	18.57	1.00	SH	Cotinine	34.23	0.14	A
Benzoic acid, 3-hydroxy-	18.73	0.16	PAc	Di-(2-ethylhexyl)phthalate	34.73	0.36	AEs
Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-	18.81	0.24	PC	Spirohexane-5-carboxylic acid, 1,1,2,2-tetramethyl-, methyl ester	35.04	2.98	AEs
α-Calacorene	18.99	0.58	SH	Benzeneethanol, .beta.-ethenyl-	35.46	1.75	AAlc
Vanillic acid	19.66	0.23	PA	1-(Adamantyl-1)butanol-1	35.86	2.05	Alc
2-(cyclohepen-1-yl)cycloheptone oxime	19.77	0.28	Ald (K)	2-Hydroxy-12-methoxy-19-norpodocarpa-4,8,11,13-tetraen-3-one	36.13	0.18	O
α-Cedrol	20.18	0.58	OS	Octadecane	36.55	0.26	Alka
(5R)-5-Ethyl-2-	20.41	0.17	O	1H,5H-	41.22	8.97	A

methylteneetyrahydrpyran				Pyrrolo[1',2':3,4]imidazo[1,5-a]pyridine, octahydro-			
4-Oxo-dihydro-.beta.-irol	20.60	0.16	O	Matairesinol	41.46	0.18	L
Di-epi-α-cedrene-(I)	20.70	0.96	MH	Vitamin E	41.63	1.40	V
(1R,3R,4R,5R)-(-)-Quinic acid	21.03	0.15	CP	Ergost-5-en-3.beta.-ol	42.95	0.17	St
α-Cadinol	21.14	0.39	OS	-	-	-	-

[a] RT means retention time; [b] RAP means relative area percent; [c] CC: Compounds class

Table 5. Characterized Chemical Composition of Branchlet Hydroethanolic Extracts of *C. arizonica*

Compound	RT[a] (min)	RAP[b] (%)	CC[c]	Compound	RT[a] (min)	RAP[b] (%)	CC
Limonene	5.15	0.11	MH	3-Methyl-1-phenyl-2-azafluorene	31.18	0.12	A
Sabinene	6.14	0.04	MH	Dispiro[2.1.2.1]octane, 1,1,6,6-tetramethyl-	31.32	0.96	O
Mequinol	8.93	0.06	PC	Totarol	31.68	1.44	PD
Catechol	11.94	0.05	PC	Ferruginol	32.04	10.43	AD
Carvacrol	13.84	0.66	OM	Communic acid	33.06	43.70	LD
4-Vinylguaiacol	14.14	0.04	VPC	Aciphyllyl alcohol	33.27	0.05	OM
Perilla alcohol	16.93	0.23	OM	Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-	33.75	0.50	O
α-Muurolene	18.08	0.04	SH	(4S,5R)-5-Hydroxycaryophyll-8(13)-ene-4,12-epoxide	34.04	0.17	OS
l-β-Bisabolene	18.24	0.06	SH	.gamma.-Elemene	34.16	0.22	SH
Nerolidol	19.31	0.09	OS	Podocarpa-8,11,13-trien-3-one, 14-isopropyl-13-methoxy-	34.46	0.25	O
t-Muurolol	21.12	0.04	OM	Di-(2-ethylhexyl)phthalate	34.78	0.20	AEs
(+-)-Dihydro-8-methoxy-4,6-dimethylcoumarin	21.78	0.03	Co	Agatholic acid	35.59	20.01	RA
4,7-Dioxa-tricyclo [7.2.1.0(3,8)]dodec-2-ene	22.69	0.19	O	(3S,4R,5R,6R)-4,5-Bis(hydroxymethyl)-3,6-dimethylcyclohexene	35.80	0.34	O
Tetradecanoic acid	22.98	0.05	FAc	Sugiol	36.05	2.46	AD
dl-cis-2,5-Dimethoxymethylcyclohexane	24.29	0.07	O	Thunbergol	36.40	0.14	OD
Propyl isopropyl ether	24.48	0.30	Et	Dihydroaflatoxine B1	36.64	0.37	O
Hexadecanoic acid	26.41	0.64	FAc	14-.beta.-H-pregna	37.46	0.13	St
Mome inositol	26.71	0.61	S	(-)-Anhydrosecoi	39.24	0.05	O
Cembrene	27.27	1.15	OCD	.alpha.trans-sesquicyclogeraniol	40.96	0.05	O
Phytol	28.71	0.10	OAD	1-Pentadecene	41.19	0.12	Alke
9,12-Octadecadienoic acid (Z,Z)-	29.17	2.18	FAc	Phenol, 4-ethyl-2-methoxy-	41.57	2.68	PC
9-Octadecenoic acid, (E)-	29.26	1.29	FAc	7-Oxabicyclo[4.1.0]heptane, 3-penten-2-one deriv.	42.22	0.09	O
Linoleic acid ethyl ester	29.44	0.13	FAEs	Deoxyisopodophyllotoxin	44.22	0.08	L

Octadecanoic acid	29.52	0.38	FAc	(23S)-ethylcholest-5-en-3.beta.-ol	44.42	1.98	St
Isopimaral	30.41	2.93	DA	3-(3,4-Dimethoxyphenyl)-propionic acid	46.30	0.07	FAc
Labda-8(17),13Z-dien-15-ol	30.64	0.83	LD	1,2-Benzisothiazole, 3-butoxy-	48.49	0.17	A
13-Epitorreferol	30.90	0.04	LD	-	-	-	-

[a] RT means retention time; [b] RAP means relative area percent; [c] CC: Compounds class

The monoterpene amounted to 0.81% in FE, 2.13% in LE, and 1.13% in BE, whereas sesquiterpenes accounted for 2.65% in FE, 6.47% in LE, and 0.58% in BE, with high amount of diterpenes 57.83% in FE and 63.22% in BE and with low amount 1.99% in LE.

In monoterpenes, monoterpene hydrocarbons were the major constituents, accounting 0.71 and 1.82%, respectively in fruit and leaf, and 0.15% in branchlet. The main monoterpene hydrocarbons were di-epi-α-cedrene-(I) 0.38% in fruits, 0.96% in leaves, and sabinene 0.04% in branchlet, respectively. In sesquiterpenes, sesquiterpene hydrocarbons were the major constituents 1.58% in fruits, 5.50% in leaves and 0.32% in branchlets.

In diterpenes, labdane diterpenoids were the major constituents, accounting 47.17 and 44.57%, respectively in fruit and branchlet extracts, but the compound was not identified in leaf extract. Furthermore, abietane-type diterpenes also were the major constituents, accounting 8.84 and 12.89%, respectively in fruit and branchlet extracts, but the compound was not found in leaf extract.

Abdulkhani *et al.* (2020) stated that in addition to phenolic compounds, other moieties in ethanolic extracts caused an increase in total radical scavenging activity. It has been reported that the antioxidant activity of phenolic compounds depends on the number and location of hydroxyl groups in the phenolic compounds (Sok *et al.* 2009). Matairesinol (MAT) was the defatted lignan in ethanolic fruit extracts of *C. arizonica* that have been previously reported by Abdulkhani *et al.* (2020) in wood knot as a predominant lignan. Dl-3,4-dehydroproline inhibits the growth of *Lactobacillus arabinosus*, *Streptococcus lactis*, *Pediococcus cerevisiae*, *Leuconostoc dextranicum*, and *Escherichia coli*, and the toxicities are competitively reversed by l-proline with inhibition indices of 3, 3, 3, 10, and 10, respectively (Smith *et al.* 1962).

Benzimidazole and some of its derivatives as 4-nitro and 5-nitro-benzimidazoles, 2-amino-, 4-amino-, and 5-aminobenzimidazoles have been tested on gastric acid secretion in Shay rats. Only 5-aminobenzimidazole decreased the gastric secretion process basally or stimulated by betazole (Trivulzio *et al.* 1988).

Communic acids are diterpenes with labdane skeletons found in many plant species, primarily conifers, predominating in the genus *Juniperus* (fam. Cupressaceae). These acids have been isolated from different parts of the plant (fruits, wood, bark, leaves, roots, *etc.*); they are primarily found in leaves, fruits, and bark as well as have different biological activities (antibacterial, antitumoral, hypolipidemic, relaxing smooth muscle, *etc.*) (Barrerol *et al.* 2012).

Ferruginol has antimicrobial activity (Li *et al.* 2008; Matsushita *et al.* 2006), antioxidant activity (Wang *et al.* 2002), gastroprotective and ulcer healing effects (Rodríguez *et al.* 2006), termite resistance effects (Kano *et al.* 2004), and growth inhibition activity against *Heterosigma akashiwo* (Saijo *et al.* 2013).

The results obtained from Wang *et al.* (2002) indicate that ferruginol possesses a significant inhibitory activity against the DPPH radical, followed by hinokiol, secoabietane dialdehyde, 6β-acetoxy-7α hydroxyroyleanone, and isopimarinol, with sugiol showing the least radical scavenging activity as well as ferruginol that has potential for use as a natural food preservative. The ferruginol compound value in the BE was twice that of the FE, but in the LE, this compound was not present at all. It seems that, for this reason the antioxidant activity of the BE was higher than the FE and the FE higher than the LE.

It has also been demonstrated that ferruginol is an effective antifungal compound of *Taiwania cryptomerioides* heartwood (Chang *et al.* 1999). Because both white-rot and brown-rot fungus release oxidase to cleave the cellulose or lignin of wood, it is plausible that ferruginol inhibits the growth of fungus by blocking the radical transition (Wang *et al.* 2002).

GC/MS analysis of *Stevia rebaudiana* extracts deal with *in vitro* antidiabetic potential has shown the major compounds of 1-hepta-triacotanol, duvatrienediol, dihydroxanthin, β-amyrin, lupenone, phytol, γ-sitosterol, agatholic acid, and fatty acids (Zaidan *et al.* 2019). Agatholic acid also was found in the wheat (1.46%), infected wheat by *Caloglyphus berlesei* (4.05%), maize (0.64%), infected maize by *Caloglyphus berlesei* (1.41%), and fishmeal (4.69%) extracts after three months stored (Gamal El-Din *et al.* 2019) as well as in the fruit benzene/acetone (10:1 *v/v*) extracts of *Forsythia suspensa* to yield of 9 mg (Kuo *et al.* 2014).

Diterpenoids totarol and sugiol were also detected as the major compounds in the fossil species *Taxodium dubium* (Otto *et al.* 1997). The major compound in resin extracts from *T. distichum* was totarol (36.75%), followed by limonene (19.24%), and α-pinene (16.06%), while resins of *T. ascendens* also contained higher levels of totarol (46.85%) and α-pinene (29.39%), but limonene reached only 5.42% (Špaldoňová *et al.* 2020).

Earlier studies of the chemical composition and biological activities of the essential oil extracted from the stem of *Olea europaea* sub sp. *africana* (Mill) showed that at the concentration of 0.15 mg/mL (149.090 μg/mL), the essential oil of *Olea europaea* exhibited the highest percentage of inhibition (95.03%) compared to the range given in the literature (Syamsir 2009). The crude essential oil of *Olea europaea* also gave strong antioxidant activities in DPPH radical scavenging test, with its IC50 values at 19.9 μg/mL and showed comparable antioxidant potential compared to ascorbic acid. Ascorbic acid showed 98.05% inhibition (IC50: 15.9 μg/mL), which serves as a standard (Syamsir 2009). Some essential stem oil of *Olea europaea* similarly was found in the extracts of fruit, leaf, and branchlet of *C. arizonica* such as spirohexane-5-carboxylic acid, 1,1,2,2-tetramethyl-, methyl ester, d-Limonene, catechol, and aromandendrene (Asfaw *et al.* 2022).

According to the findings of Mannai *et al.* (2021) regarding antifungal activities of *Raphanus raphanistrum*, it seems that benzeneacetonitrile, 4-fluoro as a glucosinolate product could be responsible of a part of antioxidant activity of *C. arizonica* leaf extracts.

According to the classification of percentage scavenging activities of DPPH radical by Syamsir (2009), the results of percentage scavenging activities of DPPH radical in the test solution at 5 mg/mL was strong when the scavenging percentage was between 71 and 100, moderate when the percentage scavenging activity was between 41 and 70, and weak when the scavenging activities were ≤ 40. Experimental findings showed that the results of percentage scavenging activities of DPPH radical in the all tested extracts was strong between 71 and 100.

Tepe *et al*. (2005) examined the antioxidant activity of the components of *Salvia tomentosa* Miller (Lamiaceae) essential oils (EOs). Of these, terpinene-4-ol, 1,8-cineole, camphor, borneol, p cymene, α-pinene, and β-pinene showed no activity. Furthermore, the main components of *Achillea millefolium* subsp. *millefolium* Afan EOs e.g., eucalyptol, camphor, β-pinene, borneol, terpinen-4-ol, and α-pinene, were all tested individually and none exhibited antioxidative activity in any of the assays employed (Candan *et al.* 2003). The reason that EOs showed much more activity than their constituents alone can be attributed to the high percentages of the main components, synergy among the different oil constituents, or to microcomponents acting as pro-oxidants (Viuda-Martos *et al.* 2010). According to the findings of Ruberto and Baratta (2000), oxygenated monoterpenes (OM), especially thymol and carvacrol, have high antioxidant activity. Although, monoterpene hydrocarbons (MH) may be considered as active antioxidants, none are stronger than oxygenated monoterpenes (OM). Sesquiterpene hydrocarbons (SH) and their oxygenated derivatives have very low antioxidant activity.

There is no data on the chemical composition and antioxidant activity of *C. arizonica* fruit, leaf, and branchlet extracts in the literature to be compared with the present results. However, a study on phenolic and other moieties contents in hydroethanolic extracts and antioxidant activity of its phylogenetically close taxa, *C. sempervirens* (CS), revealed the presence of high levels of terpenes and phenolic compounds and good antioxidant capacity in fruit, leaf, and branchlet.

It seems that diterpenes-types present in the fruit and branchlet hydroethanolic extracts of *C. arizonica* exhibited potent activity. The ethanolic extract of *C. sempervirens* fruit inhibited proliferation of human BPH-stromal cells, and the activity was localized to its chloroform-soluble, diterpene-rich fraction (Al-Snafi 2016). Diterpenes such as 6-deoxytaxodione (11-hydroxy-7, 9(11), 13-abietatrien-12-one), taxodione, ferruginol, sugiol, trans-communic acid, 15-acetoxy imbricatolic acid, and imbricatolic acid were isolated from *C. sempervirens* (Tumen *et al.* 2012).

CONCLUSIONS

The different parts of many plants and their extracts and essential oils have been used in folk medicine. In this research, the chemical composition and antioxidant capacity of hydroethanolic extracts of fruit, leaf, and branchlet of *C. arizonica* were studied to diversify herbal medicines. Preliminary screening of the phenolic and terpenic components and antioxidant activity of extracts from fruit, leaf, and branchlet of *C. arizonica* indicated the high potential of this tree for nutrition and pharmaceutical purposes. The important and general results of the current study are as follows:

1. Fruit and branchlet extracts of *Cupressus arizonica* exhibited a good antioxidant activity compared with leaf extracts, BHT, and vitamin C.
2. The main chemical constituents in the fruit extracts were communic acid, spirohexane-5-carboxylic acid, 1,1,2,2-tetramethyl-, methyl ester, and ferruginol; the main chemical constituents in the leaf extracts were hexadecanoic acid, 1H,5H-pyrrolo[1',2':3,4]imidazo[1,5-a]pyridine, octahydro-, and bicyclo[3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)-; the main chemical constituents in the branchlet extracts were communic acid, agatholic acid, and ferruginol.

3. The branchlet extracts from *C. arizonica* had better antioxidant activity compared with others and standard positive controls.
4. Fruit, leaf, and branchlet extracts of this species contain considerable amounts of phenols and terpenes and show good antioxidant activity, suggesting further investigation for isolation of the active components and biological characterizations and medicinal properties of the plant.

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APPENDIX

Table A1. Univariate Test Results of the Effect of Fruit, Leaf, and Branchlet Hydroethanolic Extracts and Their Concentrations on the Antioxidant Activity of *C. arizonica* Extracts Compared with BHT and Vitamin C

Source	Type III Sum of Squares	Df	Mean Square	F Value	Sig.
Corrected Model	5164.815	34	151.906	22.342	0.000
Intercept	629961.631	1	629961.631	92652.265	0.000
Treatment (T)	1161.921	4	290.480	42.723	0.000
Concentration (C)	471.667	6	78.611	11.562	0.000
T × C	3531.227	24	147.134	21.640	0.000
Error	475.944	70	6.799		
Total	635602.391	105			
Corrected Total	5640.759	104			