Phytoprotection

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Volume 102, Number 1, 2022

Received 2021-09-23; accepted 2022-04-08

URI: https://id.erudit.org/iderudit/1093946ar
DOI: https://doi.org/10.7202/1093946ar

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Publisher(s)

Société de protection des plantes du Québec (SPPQ)

ISSN

1710-1603 (digital)

Explore this journal

Cite this article

Dhaouadi, F., Mouna, S., Ismail, A., Fedia, M., Sana, K., Yassine, M., Lamia, H. & Naceur, M. (2022). Chemical composition, antioxidant and allelopathic activities of essential oils and crude extracts of *Cupressus arizonica* Greene. *Phytoprotection*, *102*(1), 21–29. https://doi.org/10.7202/1093946ar

Article abstract

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Received 2021-09-23; accepted 2022-04-08

PHYTOPROTECTION [102] : 21-29

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Keywords: *Cupressus arizonica*, essential oil, DPPH, ABTS, *Sinapis arvensis*, allelopathic activity.

[Composition chimique, activités antioxydantes et allélopathiques des huiles essentielles et extraits bruts de *Cupressus arizonica* Greene]

La composition chimique, les activités antioxydantes et allélopathiques de l'extrait de feuilles et de cônes de *Cupressus arizonica* de Tunisie ont été évaluées. Les valeurs des rendements en huiles essentielles (HE) étaient de 0,18 % pour les feuilles et de 1,07 % pour les cônes. Grâce à l'analyse par GC/MS des feuilles et des cônes de *C. arizonica*, les principaux composés identifiés dans l'HE des feuilles étaient l'umbellulone (19,4 %) et l'α-pinène (10,75 %) tandis que l'HE des cônes était caractérisée par sa richesse en α-pinène (81,3 %). Par rapport à différents extraits, la capacité antioxydante totale la plus élevée a été enregistrée avec les extraits éthanoliques des feuilles, suivie par celle des cônes. Cependant, l'activité antioxydante était plus forte avec l'extrait éthanolique des feuilles que celle des cônes en utilisant DPPH ou ABTS dans les deux tests d'activité de piégeage des radicaux libres. Les résultats de l'activité allélopathique ont montré que la réduction de la germination dépend de la nature des extraits et de leurs niveaux de concentration. Tous les extraits de *C. arizonica* étaient capables de diminuer la germination de *Sinapis arvensis* par rapport au témoin. Les HE des feuilles et des cônes avaient un impact plus important sur la réduction de la germination et l'inhibition de la croissance, suivi par l'extrait éthanolique puis par l'extrait aqueux. Les résultats de cette étude peuvent conduire à identifier de nouveaux composés phytotoxiques dans des extraits de *C. arizonica* utiles pour la lutte contre les mauvaises herbes.

Mots-clés : *Cupressus arizonica*, huile essentielle, DPPH, ABTS, *Sinapis arvensis*, activité allélopathique.

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INTRODUCTION

Today, the worldwide effort in modern agriculture is to scale back the use of chemical pesticides by introducing new biological and ecological methods (Barratt *et al*. 2017). One of these methods is the use of chemical interactions among plants (Jabran 2017). The competition involves the active absorption of limited resources by one organism, which ends up in decreased supply and subsequently the inhibition of other organism's expansion. Thus the event leading to the growth reduction of a species because of chemicals released from another species is termed allelopathy (Butcko and Jensen 2002). An oversized number of allelochemicals from various plant species are reported. Among them, the foremost important, are phenolic compounds, benzoxazinoids, sorogoleones, glucosinolates, terpenes, alkaloids, and mamilactones (Kaldi *et al*. 2013). Certain natural compounds, which are mostly safe for the environment and the human health, might cause allelopathy. The employment of allelopathic activity for effective weed control in agricultural systems can play a very important role in environmental and community safety. This approach is useful when we know that, every year, a brand new list of pesticide-resistant weeds is released. In addition to the 2017 report by the International Herbicide-Resistant Weed site, an inventory of 36 new cases of weed resistance to herbicides was released (Heap and Duke 2018). This prompted us to always rummage around for new herbicides to manage resistant weeds. Aromatic and medicinal plants have a special place among allelopathic plants because of their secondary constituents and their active ingredients (Kaldi *et al*. 2013).

The plant of *Cupressus arizonica* Greene, subject of our investigation, is a medium-sized evergreen tree with a conic to ovoid-conic cones from Cupressaceae family, growing in southern and western United States (Fralish and Franklin 2002). It has been widely used in traditional medicine to treat various infections like colds where it combats coughing, parasitic infections, inflammation, hemorrhoids. The fruits of the plant were traditionally used as antiseptic compounds and to cure diabetes (S.A. Emami, Rabe *et al*. 2010). Nowadays extracts and EOs from *C. arizonica* were found to possess antifungal, antibacterial, antioxidant and insecticidal activities (Elansary *et al.* 2012). They are sometimes used for suffumigations, or in solution for laundry, and bandages for the treatment of circulatory diseases (E. Emami *et al.* 2013). To the best of our knowledge, there is no report on antioxidant and allelopathic activities of the crude extracts from *C. arizonica* grown in Tunisia.

The aim of the present study was first to determine the chemical composition of EOs from the leaves and the cones, the content of total phenolic in crude extracts and EO, and to evaluate their antioxidant activities. We also propose bioassay tests designed and implemented for the first time to investigate the allelopathic activity.

MATERIALS AND METHODS

Plant material and chemicals

C. arizonica were collected from the arboretum of Korbous (north of Tunisia) during spring seasons of 2020. The voucher specimen of the plant (No. Ca228) was prepared and deposed at the herbarium division of the National Research Institute of Rural Engineering, Water and Forests in Tunisia. The harvested material was air-dried at room temperature (20-25 °C) for one week and then stored in cloth bags.

Chemicals: DPPH, ABTS, Folin-Ciocalteu reagent and gallic acid, ascorbic acid and quercetin were purchased from Sigma-Aldrich. Sodium persulfate, ammonium molybdate, sulfuric acid, sodium hydroxide, methanol, ethanol, sodium phosphate, tannic acid, Butylated hydroxytoluene (BHT) were all of them purchased from the company Loba Chemie Pvt (India).

Preparation of the extracts

An amount of 30 g of the powdered sample was extracted separately with 300 mL of distilled water and 300 mL of ethanol at room temperature for 30 min in a shaking condition to obtain the aqueous extract and the ethanolic extract respectively. All extracts were filtered through Whatman filter paper (Bärenstein, Germany) and concentrated under reduced pressure in a Heidolph rotary evaporator (Schwabach, Germany). Extracts were stored at 4 °C in shady condition until use.

Essential oil distillation

Dried leaves and cones (100 g) were hydrodistilled for 4 h using a Clevenger-type apparatus according to the European Pharmacopoeia method (Council of Europe 2004). EO was dried over anhydrous sodium sulfate and stored in dark vials at 48 °C until analysis. For each sample, hydrodistillation was done in triplicate. The EO yield (%) was calculated based on the dried weight using the following formula:

$$\text{EO yield (\%)} = \frac{\text{mass of obtained EO}}{\text{mass of dry matter}} \text{ X } 100$$

Gas chromatography analysis/mass spectrometry conditions

Assessment of the chemical composition was carried out by gas chromatography/mass spectrometry (GC/MS) method. It was performed in a Hewlett Packard 5972 MSD type apparatus (capillary column HP-5 MS [30 m length and film thickness is 0.25 mm]) coupled to mass spectrometry. Helium was used as the carrier gas at a flow rate of 1.2 mL min^{-1}. The oven temperature was programmed as follows: 50 °C for 1 min; from 50 °C to 175 °C at 5 °C min^{-1} then, 175 °C for 10 min and from 175 °C to 250 °C at 15 °C min^{-1} followed by isothermal hold for 4 min. The temperature of the injector (250 °C), that of the detector (280 °C), injected solution (0.1 mL of 1%) was diluted in hexane in splitless mode; mass spectrometry: e HP5972 at 70 eV; scanning time: 1.5 s; gram mass: 40-300 amu. Spectra and mass chromatograms were managed by ChemStation.

The components were identified based on the comparison of their relative retention times and mass spectra with those of standards, Wiley and NIST library data of the GC/MS system, and literature data (Adams *et al.* 2008). Further confirmation was done from Retention Index data calculated from a series of alkane retention indices (relative to C9-C25) obtained on a ZB-5 MS, Zebron, Phenomenex capillary column (Berwind *et al*. 2018).

Determination of total polyphenol contents

The total phenolic content of extracts and the EOs were determined with a colorimetric quantification by using the Folin-Ciocalteu reagent according to the slightly modified method described by Dewanto *et al.* (2002). About 100 µL of each sample was mixed with 2 mL of sodium carbonate

(Na_2CO_3 [2%]) allowed for 3 min before adding 100 µL of Folin-Ciocalteu reagent. The mixture was thoroughly shaken and allowed for 30 min in darkness. The absorbance was measured at 760 nm. A standard curve of gallic acid was prepared. Total phenolic contents were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE g^{-1} DW).

Determination of flavonoid contents

The total content of flavonoids in *C. arizonica* leaves and cones extracts was determined using the aluminum chloride method according to the procedure of Dewanto *et al*. (2002).

Briefly, 250 µL of *C. arizonica* extract from leaves (aqueous, ethanolic and EOs) was appropriately diluted and added to 75 µL of $NaNO_2$ solution (5%) and mixed for 6 min before adding 150 µL of $AlCl_3$ (10%). After 5 min of incubation, 500 µL of 1 M NaOH solution was added to the mixture that was adjusted to 2.5 mL with distilled water. The absorbance of samples and the prepared blank was determined at 510 nm. Total flavonoid contents of *C. arizonica* leaves extracts (three replicates per treatment) were expressed as milligrams of quercetin equivalents per gram of dry weight and EO weight (mg QE g^{-1} DW) using the calibration curve with quercetin.

Total condensed tannins content

The method of San Miguel-Chávez (2017) was adopted for the determination of total condensed tannins content. In presence of concentrated sulfuric acid, condensed tannins were transformed with vanillin to anthocyanidols. A volume of 5 µL of the extract appropriately diluted was mixed with 3 mL of vanillin (4%) and 1.5 mL of concentrated sulfuric acid. After 15 min, the absorbance was measured at 500 nm.

DPPH radical scavenging assay

According to the slightly modified method described by Hatano *et al*. (1988), the antioxidant activity of the individual EO and extracts were tested using 1,1-diphenyl-2- picrylhydrazyl (DPPH) assay. The reaction mixture contained 50 µL of each extract with a concentration ranging from 1.25 to 20 µg mL^{-1} and 1.9 mL of the methanolic solution of DPPH (0.2 mM). The mixture was incubated at room temperature for 30 min. The absorbance was read at 517 nm. The antiradical activity was expressed as IC_{50} (mg mL^{-1}), the concentration required to cause a 50% DPPH inhibition. Butylated hydroxytoluene (BHT) was used as the standard antioxidant for the positive control.

ABTS scavenging assay

ABTS scavenging activity was assessed according to the method of Re *et al*. (1999). First, 19.2 mg of ABTS (7 mM) dissolved in distilled water is prepared and mixed with 3.3 mg of potassium persulfate (2.45 mM). After incubation in the dark at 25 °C for 16 h, a volume of 1 mL of diluted ABTS solution was added to 10 µL of plant extract. The water was used for the negative control and ascorbic acid for the positive control. The scavenging activity was determined by an assessment of the absorbance at 734 nm after 6 min of the initial mixing. The percentage of inhibition was calculated by the equation:

$$\text{Inhibition percentage (\% IP)} = \frac{(Ac - As)}{Ac} \times 100,$$

where Ac is the absorbance of the control and As is the absorbance of the sample.

The amount of sample necessary to decrease the absorbance of ABTS by 50% (IC_{50}) was calculated graphically using for each extract (% IP vs. sample concentrations).

Determination of the total antioxidant capacity

The total antioxidant capacity (TAC) of the plant extracts was evaluated by the phosphor-molybdenum method of Prieto *et al*. (1999). A volume of 0.1 mL aliquot of the plant extract was mixed with 1 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). For the blank, 100 µL of deionized water was used instead of the sample. The tubes were incubated in a boiling water bath at 95 °C for 90 min. Then samples were cooled at room temperature, and the absorbance of the aqueous solution of each sample was measured at 695 nm in the spectrophotometer. The total antioxidant activity was calculated by the following equation:

$$\text{TAC (\%)} = \frac{(A_{\text{sample}} - A_{\text{control}})}{A_{\text{blank}}} \times 100,$$

where A_{sample} is the absorbance of the sample mixed with the reagent solution, A_{control} is the absorbance of deionized water mixed with the sample, and A_{blank} is the absorbance of the reagent solution mixed with water. Gallic acid concentration ranging from 0.001 to 0.1 mg mL^{-1} was used to depict the curve of the positive control. The antioxidant activity of the samples was expressed as milligrams of gallic acid equivalents per gram of dry weight.

The allelopathic activity

Allelopathic effects of *C. arizonica* extract and EOs were evaluated against the selected agricultural weed species *Sinapis arvensis* L. The herbicidal activity of the ethanolic extract, the aqueous extract and EOs had been studied at the germination stage. This is achieved by the direct contact method (Tworkoski 2002). A volume of 5 mL taken separately from solutions of EO and plant extracts prepared with Tween-20 (final concentration 0.1%) was used to moisten a Petri dish. Petri dishes were then sealed with adhesive tape to prevent escaping of volatile compounds. The number of seeds germinated was counted in each Petri dish and the length of emerged seedlings was measured.

Statistical analysis

The obtained data were analyzed using a one-way analysis of variance (ANOVA) and the statistical significance was determined by post hoc test (*p*-value of 0.05). All the analyses were conducted using SPSS 17 Student–Newman–Keuls test. In all tables, the means of the same column with different letters correspond to statistically significant differences according to the Student–Newman–Keuls test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Essential oil composition

The EOs from *C. arizonica* leaves were characterized by a light-yellow colour, while those of cones were colourless with values of yield of 0.18% and 1.07% respectively. Our results showed significant differences in the EO yields among parts of the plant. In confirmation of this quantitative variability, the EOs extracted from the leaves and the cones of *C. arizonica* from Morocco have yields of 2.018% and 0.18% respectively (Bouksaim *et al*. 2018). However, Iranian EOs extracted from the leaves and the cones have yields of 0.75% and 0.8%, respectively (Sedaghat *et al.* 2011). The yield of EOs in aromatic and medicinal plants varies considerably depending on several factors; climatic conditions (temperature, humidity, duration

of sunshine, etc.), extraction methods, drying or even the origin and the part of the plant used for extraction (Khammassi *et al*. 2018). The chemical composition of the leaves and cones of *C. arizonica* from Tunisia are reported in Table 1. Chemical analysis of the EOs samples conducted according to their retention index (RI), and the mass spectral data revealed the presence of 45 compounds, with 92.5% and 90.2% of total compounds of oil respectively for leaves and cones.

Table 1. Chemical composition of *C. arizonica* essential oils (%) from leaves and cones analyzed by GC/MS

N°	Main chemical classes	Compound	RI	Leaves	Cones
1	Hydrocarbon monoterpenes	α-Thujene	930	0.3	-
2		α-Pinene	939	10.75	81.3
3		α-Fenchene	952	0.2	-
4		Camphene	954	1.9	-
5		Sabinene	975	1.2	-
6		β-Pinene	979	0.1	0.7
7		β-Myrcene	990	0.9	-
8		α-Phellandrene	1002	0.4	-
9		p-Cymene	1024	0.8	-
10		Limonene	1029	5.08	6.9
11		δ-Terpinene	1059	1.8	-
12		Terpinolene	1088	1.5	-
13	Oxygenated monoterpenes	Linalool	1096	0.3	0.2
14		β-Fenchol	1121	0.5	-
15		allo-Ocimene	1132	0.4	-
16		Camphor	1146	0.7	-
17		Umbellulone	1171	19.4	-
18		Terpinen-4-ol	1177	3.9	-
19		Borneol	1184	0.4	-
20		α-Terpineol	1188	0.2	-
21		(E)-Piperitol	1196	0.3	-
22		Verbenone	1205	0.2	-
23		Carvone	1243	0.2	-
24		Isobornyl acetate	1285	0.3	-
25		Piperitone	1343	0.8	0.1
26	Hydrocarbon sesquiterpene	β-Cubebene	1388	6.2	-
27		β-Bourbonene	1389	1.3	-
28		β-Elemene	1390	0.6	-
29		α-Cedrene	1411	8.3	0.8
30		β-gurjunene	1433	0.2	-
31		Aromadendrene	1441	3.3	-
32		α-Humulene	1454	0.2	0.2
33		δ-Muurolene	1479	1.2	-
34		αr-Curcumene	1480	0.4	-
35		Germacrene D	1485	2.9	-
36		β-Selinene	1490	0.4	-
37		(E)-Calamanene	1529	1.8	-
38		α-Calacorene	1545	1.2	-
39	Oxygenated sesquiterpenes	α-Cedrol	1600	4.9	-
40		τ-Cadinol	1640	1.1	-
41		β-Acorenone	1692	0.9	-
42	Hydrocarbon diterpenes	Manoyloxide	2010	2.3	-
43		Abietatriene	2056	1.1	-
44	Oxygenated diterpenes	Nezukol	2133	0.6	-
45		(Z)- Totarol	2314	1.1	-
	Total (%)			92.5	90.2

The major compounds of the EOs of the leaves are umbellulone (19.4%) followed by α-pinene (10.75%), α-cedrene (8.3%) and limonene (5.08%). Our findings are in accordance with results registered by Amri *et al.* (2017) and Mannai *et al.* (2021), where low variation was shown in the major components obtained from leaves of Tunisian *C. arizonica*. However, in the Italian *C. arizonica*, umbellulone was represented by far with the highest value of 45.1% (Flamini 2003). While the Iranian *C. arizonica* analysis showed among the major compounds the presence of α-pinene (19.87%), thriphenyphosphine oxide (13.12%) and umbellulone (11.08%) (Shafaie *et et al.* 2019). The EOs of the cones showed a total of seven compounds representing 90.2% of the total compounds of the oils. These EOs were characterized by its richness in hydrocarbon monoterpenes especially α-pinene (81.3%), while the other compounds such as β-pinene (0.7%), limonene (6.9%) and linalool (0.2%) were presented in small quantities. Likewise, other studies from Iran showed that the most dominant compound was α-pinene with a content ranged between 54.3% and 57.6% (Sedaghat *et al*. 2011).

Total phenols, flavonoids, and condensed tannins

Polyphenols are known for their biological activity related to their chemical properties as antioxidants (San Miguel-Chávez 2017). Typically, the number of polyphenols depends on the part of the plant and the solvent used. Indeed, our results showed that ethanolic extracts from the leaves and cones of *C. arizonica* had a high content of total phenols, reaching a value of 4.08 ± 0.19 mg GAE g^{-1} DW and 2.66 ± 0.09 mg GAE g^{-1} DW respectively (Table 2). While the lowest phenol content was registered in the aqueous extract. It presented 2.53 ± 0.35 mg GAE g^{-1} DW from leaves, and 1.6 ± 0.1 mg GAE g^{-1} DW from cones. The estimation of total phenol content varies depending on several factors among them the polarity of the solvent used for the extraction (Sepahpour *et al*. 2018). It is worth noting that, the higher phenolic contents in the leaves compared to the cones could be due to the fact that the leaves are more exposed to environmental stress factors such as ultraviolet rays from the sunlight (Stephen and Ayalur 2017).

The determination of total flavonoids revealed a wide range of flavonoids contents as a function of the solvent and organ. As shown in Table 2, the ethanolic extracts presented the highest flavonoid content with 0.14 ± 0.005 mg QE g^{-1} DW from leaves, and 0.135 ± 0.011 mg QE g^{-1} DW from cones, followed by the aqueous extract which presented 0.12 ± 0.002 mg QE g^{-1} DW, and 0.06 ± 0.005 mg QE g^{-1} DW from leaves and cones respectively. Generally, the flavonoid content depended on the polarity of the solvents (Souihi *et al*. 2020). As for total phenols and total flavonoids, the maximum level of total condensed tannins, was registered in the ethanolic extract from leaves (144.63 ± 30.16 mg CE g^{-1} DW), followed by that from cones (94.10 ± 20.06 mg CE g^{-1} DW). The minimum level of this phenolic class was assessed in the aqueous extract from leaves and cones with values of 26.53 ± 15.82 mg CE g^{-1} DW and 94.10 ± 20.06 mg CE g^{-1} DW respectively (Table 2).

Table 2. Results of spectrophotometric analysis of total polyphenol content in *C. arizonica* dry plant material. The values were expressed as the mean of three replications ± SD.

	Polyphenols		
Samples	Total phenols (mg GAE g^{-1} DW)	Total flavonoids (mg QE g^{-1} DW)	Total tannins (mg CE g^{-1} DW)
AEL	2.53 ± 0.35 b	0.12 ± 0.002 b	26.53 ± 15.82 c
EEL	4.08 ± 0.19 a	0.14 ± 0.005 a	144.63 ± 30.16 a
AEC	1.6 ± 0.1 c	0.06 ± 0.005 c	69.30 ± 17 .10 b
EEC	2.66 ± 0.09 b	0.135 ± 0.011 ab	94.10 ± 20.06 b

AEL: aqueous extract of the leaves; EEL: ethanolic extract of the leaves; AEC: aqueous extract of the cones; EEC: ethanolic extract of the cones.

Table 3. Antioxidant activity of different extracts from leaves and cones of *C. arizonica.* Each value represents the mean datum of three replicates ± SD.

Samples	TAC (mg AGE g^{-1} DW)	DPPH (IC_{50} mg mL^{-1})	ABTS (IC_{50} mg mL^{-1})
AEL	325.30 ± 5.93 e	5.35 ± 0.15 d	6.04 ± 0.97 b
EEL	184.00 ± 10.16 b	2.20 ± 0.01 a	2.87 ± 0.86 a
AEC	215.43 ± 18.16 c	3.41 ± 0.16 c	6.20 ± 0.18 b
EEC	199.76 ± 8.22 bc	2.96 ± 0.05 b	3.55 ± 0.33 a
EOL	210.70 ± 7.38 a	5.38 ± 0.1 d	5.52 ± 0.44 b
EOC	253.76 ± 9.80 d	10.4 ± 0.1 e	9.81 ± 0.69 c
BHT	-	0.047 ± 0.01	-
AA	-	-	0.035 ± 0.01

AEL: aqueous extract of leaves; EEL: ethanolic extract of leaves; EAC: aqueous extract of cones; EEC: ethanolic extract of cones; EOL: essential oil of leaves; EOC: essential oil of cones; BHT: Butylated hydroxytoluene; AA: ascorbic acid.

Total antioxidant capacity (TAC)

The total antioxidant assay based on the reduction of molybdenum is just a quantitative estimation method of total antioxidant capacity of the *C. arizonica* extracts leaves and cones. Total antioxidant capacity was determined as gallic acid equivalents in milligrams per gram of dry weight (mg AGE g^{-1} DW). Results in Table 3 showed appreciable antioxidant activity of various samples. The phenolic compounds are well known by their antioxidant activity. Indeed, the ethanolic extract of *C. arizonica* that have the highest total polyphenol content, have also the highest total antioxidant capacity with the values of 184.00 ± 10.16 mgAGE g^{-1} DW and 199.76 ± 8.22 mg AGE g^{-1} DW for leaves and cones respectively, followed by the EOs (253.76 ± 9.80 mg AGE g^{-1} DW, 210.70 ± 7.38 mg AGE g^{-1} DW), then the aqueous extract (325.30 ± 5.93 mg AGE g^{-1} DW, 215.43 ± 18.16 mg AGE g^{-1} DW). The best antioxidant activities were recorded with leaves for the ethanolic extract and the EO, while aqueous extract of cones showed the highest antioxidant capacity (215.43 ± 18.16 mg AGE g^{-1} DW) compared to the aqueous extract of leaves (325.30 ± 5.93 mg AGE g^{-1} DW). The variability in antioxidant capacity depends on the plant part and the nature of the solvent. In accordance to this finding, Ben Nouri *et al.* (2015) showed that the antioxidant capacity of the EOs from *Cupressus sempervirens* L. depended of the nature of the solvent used.

Free radical scavenging capacity of DPPH

The DPPH scavenging assay is broadly applied to assess the free radical scavenging of plant extracts because it is sensitive, simple and rapid (Kim *et al*. 2002). Results of the DPPH assay showed pronounced differences between the investigated extracts. There was a significant disparity between ethanolic and aqueous extract. The ethanolic extract of leaves showed the highest antioxidant capacity with an IC_{50}: 2.2 ± 0.01 mg mL^{-1}, followed by the ethanolic extract of cones IC_{50}: 2.96 ± 0.05 mg mL^{-1}. While the aqueous extract presents a low activity with an IC_{50}: 5.35 ± 0.15 mg mL^{-1} and IC_{50}: 3.41 ± 0.16 mg mL^{-1} for leaves and cones respectively (Table 3). We also note that the EOs of *C. arizonica* showed the weakest antioxidant activity, with 5.38 ± 0.1 mg mL^{-1} and 10.4 ± 0.1 mg mL^{-1} for leaves and cones respectively (Table 3). The variability of this activity might be due to the presence of phenolic and flavonoids compounds (Limam *et al*. 2021). The moderate antioxidant activity of *C. arizonica* EO is due to the synergistic effect of some components existing in low amounts, such as sabinene, myrcene, terpinene. These components were proved to have DPPH radical-scavenging activities (S.A. Emami, Fakhrjafary *et al*. 2010).

Free radical scavenging capacity of ABTS

Proton radical scavenging is a fundamental function of antioxidants. ABTS is a protonated radical, which has an absorption maximum at 734 nm. It plays a vital role in determining the antioxidant capacity. The ABTS scavenging ability of *C. arizonica* extracts showed that the best antioxidant activities were recorded with the ethanolic extract from leaves (2.87 ± 0.86 mg mL^{-1}) and cones (3.55 ± 0.33 mg mL^{-1}) (Table 3). The lowest antioxidant activity was presented by the EO of the cones (IC_{50}: 9.81 ± 0.69 mg mL^{-1}). This variability depends on the solvent and plant part used for the extraction. Using the same plant part, the best activities have been obtained with ethanolic extracts, which were proved to be quick and effective scavengers of the ABTS radical (S.A. Emami, Fakhrjafary *et al*. 2010). Moreover, ethanol is the most suitable solvent for the extraction of phenolic compounds (Souihi *et al*. 2020). Therefore, these compounds can be considered as potential components contributing to the strong antioxidant activity of *C. arizonica* (Anka *et al*. 2020). Studies on the relationship between the chemical structure of phenolic compounds and their scavenging power for free radicals have shown that the anti-radical activity is dependent on the number, the position and the nature of the substituents, in particular the type of hydroxyl groups, methoxyls, oxoferryl radical, superoxide ions as well as the degree of polymerization (Popovici *et al*. 2011). For example, the most hydroxylated flavonoids are the most easily oxidized (Hagerman *et al.* 1998). Online with our results ABTS assays indicated that some components such as α-pinene and limonene have relatively weak radical scavenging activities (Sharopov *et al*. 2015). The Low ability to scavenge free radicals (ABTS) by EO extracted either from leaves or cones suggested that *C. arizonica* EOs did not have significant amounts of specific compounds that could successfully scavenge free radicals.

Allelopathic activity

C. arizonica EOs and extracts effect on germination of *S. arvensis* was studied. Different concentrations were used and germination rate was recorded daily over six days. The germination of *S. arvensis* showed significant differences following treatments with different extracts. Results showed that a dose of 1 mg mL^{-1} of the EO oil from leaves presented the best allelopathic effect with a total inhibition of germination (Table 4). While a dose of 4 mg mL^{-1} of EO from cones was needed to reduce the germination to 43.33% in comparison to control. The EO of leaves was superior to that of cones in inhibiting germination but the ethanolic extract from leaves was slightly inferior to that of cones while a dose of 4 mg mL^{-1} from both extracts inhibited totally the germination. At this dose, the aqueous extract of leaves and cones reduced drastically the germination to 13.33% and 6.66% respectively.

In comparison with control, all extracts of *C. arizonica* decreased the aerial part length. The length of aerial part that has 11.2 mm as control was totally reduced with 5 mL of leaves EO solution (4 mg mL^{-1}). However, ethanolic extract and in less extend aqueous of leaves and cones reduced aerial part length. The highest reduction was obtained with a dose of 4 mg mL^{-1} of either ethanolic extract from leaves or cones. While the same dose from aqueous extracts of leaves and cones reduced the length to 5.10 ± 1.64 mm and 6.30 ± 1.01 mm respectively.

In comparison with control, radicle length was also affected by different amounts of *C. arizonica* extracts. The growth of the radicle that measured initially 11.2 ± 1.27 mm was found to be totally inhibited after treatment with 1 mg mL^{-1} of EO from leaves followed by the ethanolic extract of cones with a total inhibition at 4 mg mL^{-1}. However, 4 mg mL^{-1} of aqueous extract from leaves and cones decreased radicle growth to 3.20 ± 0.65 mm and 1.20 ± 1.08 mm respectively. Taken together all different extracts prepared from leaves and cones reduced the germination and seedling growth of *S. arvensis.* However, the pronounced inhibitory effects were registered with EO of *C. arizonica* leaves. Aqueous extract followed by ethanolic extract from cones were revealed slightly superior to EO in inhibiting the germination as well as the seedling growth of *S. arvensis.* Our data is in agreement with previous finding where the seeds germination was totally inhibited by the leaf essential oil at the highest doses tested (0.8 and 1 mg mL^{-1}) (Amri *et al.* 2014). However, 3 µL of pure EO from Iranian *C. arizonica* injected into a cotton swab deposited in the vial were ineffective in the germination of lettuce seed (Mirmostafaee *et al.* 2020).

Table 4. The effective concentration of different extracts from *C. arizonica* on radicle, aerial part, and germination of *S. arvensis*

Organs	Extracts	Dose (mg mL^{-1})	Germination (%)	Seedlings growth (mm)	
				Aerial parts	Roots
Leaves	Ethanolic extract	0	83.33 ± 5.77 a	11.20 ± 1.27 a	11.20 ± 1.08 a
		1	63.33 ± 5.77 b	7.70 ± 4.36 ab	10.46 ± 1.68 a
		2	16.66 ± 15.27 c	4.36 ± 3.80 b	10.36 ± 0.47 a
		4	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
	Aqueous extract	0	83.33 ± 5.77 a	11.20 ± 1.27 a	11.20 ± 1.08 a
		1	73.33 ± 5.77 a	11.20 ± 1.17 a	9.10 ± 0.10 b
		2	43.33 ± 15.27 b	10.86 ± 0.80 a	7.33 ± 0.94 c
		4	13.33 ± 5.77 c	5.10 ± 1.64 b	3.20 ± 0.65 d
	Essential oil	0	83.33 ± 5.77 a	11.20 ± 1.27 a	11.20 ± 1.08 a
		1	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b
		2	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b
		4	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b
Cones	Ethanolic extract	0	83.33 ± 5.77 a	11.20 ± 1.27 a	11.20 ± 1.08 a
		1	53.33 ± 5.77 b	7.33 ± 1.19 b	10.03 ± 1.17 a
		2	13.33 ± 5.77 c	7.26 ± 0.70 b	4.40 ± 3.85 b
		4	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
	Aqueous extract	0	83.33 ± 5.77 a	11.20 ± 1.27 a	11.20 ± 1.08 a
		1	66.66 ± 5.77 b	10.23 ± 0.87 a	6.66 ± 0.81 b
		2	36.66 ± 11.54 c	7.60 ± 1.15 b	3.50 ± 0.78 c
		4	6.66 ± 5.77 d	6.30 ± 1.01 b	1.20 ± 1.08 d
	Essential oil	0	83.33 ± 5.77 a	11.20 ± 1.27 a	11.20 ± 1.08 a
		1	56.66 ± 5.77 b	11.06 ± 1.30 a	9.36 ± 1.78 a
		2	46.66 ± 5.77 b	8.50 ± 0.78 b	4.46 ± 1.79 b
		4	43.33 ± 11.54 b	8.03 ± 1.05 b	3.63 ± 0.35 b

Apart of EO, our study reveals for the first-time phytotoxic effects of ethanolic and aqueous extracts prepared either from leaves or cones of Tunisian *C. arizonica*. This inhibition could be ascribed to the closer contact of the roots with the extracts or even due to the higher permeability of the root cells (Chung *et al*. 2001). It is reported that allelochemicals may change numerous of metabolic processes, such as photosynthesis, stomatal physiology, gas exchange, cell division, membrane permeability and production of reactive oxygen species (ROS) leading to a reduction in plant size and biomass production (Chung *et al*. 2001; Omezzine *et al*. 2014). Many phytotoxins alter the morphology and the anatomy of seedlings by modifying the function of some plant hormones, such as auxin, thereby leading to the emergence of anomalies responsible for the delay or the prevention of the development of the plants (Habermann *et al.* 2017). The aforementioned studies emphasized the importance of allelopathy on the control of weeds. This study confirms the phytotoxic potential of *C. arizonica* crude extract.

The results revealed a great difference between the total phenols, flavonoids, total condensed tannins contained in the extracts of leaves and cones of *C. arizonica* and their antioxidant capacity. Taking into account the highest content of phenols, flavonoids and condensed tannins in ethanolic extract of leaves, the high value of the total antioxidant capacity was most probably correlated with total phenolic content. Our study showed for the first time that EO of *C. arizonica* leaves provoked a so strong allelopathic effect that a low dose of 1 mg mL^{-1} inhibited totally *S. arvensis* germination. The allelopathic property of EOs was most likely due to the presence of a high number of monoterpenes (52.53%). Monoterpenes triggered the accumulation of ROS leading to membrane lipid peroxidation and resulting in the loss of membrane function. The allelopathic activity of *C. arizonica* compounds could be ascribed to synergetic activities of *α*-pinene, limonene, *p*-cymene, sabinene, myrcene, germacrene D and spathulenol. These minor components were proved to have allelopathic activities (Abd-El Gawad *et al*. 2020; Dayan *et al*. 2000). The allelopathic activity is consonant with the antioxidant activity. The generation and the clearing of ROS in the cell play an important role in allelopathic effects. Plants exposed to allelochemicals may rapidly produce ROS which alter the activity of antioxidant enzymes such as superoxide dismutase, peroxidase and ascorbic acid peroxidase. The imbalance between the production of reactive oxidant species and antioxidant capabilities impeded plants to resist oxidative stress (Cheng and Cheng 2015). The functional analysis of EOs *C. arizonica* components may provide us with molecules useful in several biological activities among them the biological herbicide effect against *S. arvensis.*

ACKNOWLEDGEMENTS

This work is part of the doctoral thesis of Ferjani Dhaouadi. It was supported by the budget of the laboratory of biotechnology and nuclear technology. The authors thank the employees from the National Research Institute of Rural Engineering, Water and Forests (INRGREF) in Tunisia, for their availability and their engagement.

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