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Phytochemical profile, total phenolic content and antioxidant activity of ethanolic extract of fumitory (*Fumaria capreolata* L.) from Algeria

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ABSTRACT: Fumitory or *Fumaria capreolata* L. is a medicinal plant, spontaneous and widely distributed in North Africa, particularly in Algeria. It has been recognized for centuries for its therapeutic virtues, and it is used in traditional medicine in the treatment of hepatobiliary diseases, gastrointestinal disorders and in the treatment of skin diseases. The phytochemical screening carried out on the aerial part of the species *F. capreolata* L., revealed the richness of this plant in secondary metabolites, such as alkaloids, catechic tannins, sterols and terpenes. On the other hand, we noticed the absence of cardinolides, leuco-anthocyanins, quinones and starch in all parts of the plant. Quantitative spectrophotometric analysis allowed us to detect the levels of total polyphenols using the reagent of Folin-Ciocalteu, according to the results obtained we find that the species *F. capreolata* is rich in these compounds (14.27 ± 1.65 mg GAE/g). The evaluation of the antioxidant activity was carried out using the DPPH method, indicated that the ethanolic extract of *F. capreolata* L. showed significant antioxidant activity, with an IC_{50} = 0.27 mg/ml. And it also has a strong inhibitory activity of the coupled oxidation of linoleic acid and β-carotene, with a percentage of 88.46 ± 1.02% at a concentration of 0.5 mg/ml. In addition, the crude extract of *F. capreolata* L., also exhibits a good iron reduction capacity, with a maximum optical density of 0.349 at a concentration of 0.5 mg/ml.

Keywords: *Fumaria capreolata* L.; Phytochemical screening; Antioxidant activity; DPPH; β-carotene; FRAP.

1. INTRODUCTION

Fumitory, *Fumaria capreolata* L. is a medicinal plant belonging to the Papaveraceae family, endemic to the Edough peninsula in Seraidi (Annaba Province), in Algeria. Commonly called by the local Algerian population "hechichate el siban". Fumitory is used in traditional Algerian medicine in hepatobiliary dysfunction, gastrointestinal disorders and for the treatment of skin pathologies. In addition, it has been reported that traditional medicine from many countries like Pakistan and India also use this herb as: cholagogue, diuretic, laxative, sedative, tonic and also considered useful to treat abdominal cramps, fever, diarrhea as well as syphilis and leprosy [1].

Medicinal plants of the genus *Fumaria* represent an inexhaustible source of natural antioxidants. Moreover, the number of studies carried out on these plants as well as antioxidants of plant origin undoubtedly reflects their importance in many areas, medicine or food. The anti-radical properties of these natural products are often linked to their ability to perpetrate stable radicals [2].

The objective of this study is the knowledge of Algerian natural resources in medicinal plants. In this context, the phytochemical composition, the content of phenolic compounds and the antioxidant activity *in vitro* of the species *F. capreolata* L., from the North-East of Algeria were studied.

2. MATERIALS AND METHODS

2.1. Plant material

Aerial parts of the species *Fumaria capreolata* L. were collected in full bloom and fruiting, between the years 2014 and 2015, from Edough in Seraidi, (Annaba Province, in Northeastern of Algeria). The samples were taken manually and randomly, the botanical identification was made according to the flora of Quezel and Santa [3], and validated by Doctor Hamel. T, teacher-researcher in plant physiology at the Biology Department of Annaba University, Algeria.

2.2. Phytochemical screening

The screening method by tube reactions allowed us to identify some major chemical families such as: alkaloids, flavonoids, quinones, sterols and terpenes. This is a qualitative analysis based on coloring and/or precipitation reaction compounds of the major chemical families present. This is carried out in the dry and/or fresh plants [4].

These preliminary tests were carried out according to the techniques of Solfo [5] and Harborne [6]. Table 1 indicates the different chemical groups sought and the specific reagents used.

Table 1. Reagents used in the characterization of chemical groups.

Chemical groups	Reagents	Reagent composition	Positive results
Alkaloids	Draragendorf	Nitrate of bismuth + acetic acid	orange-red precipitate
	Mayer	potassium iodide + mercury chloride	yellowish precipitate
Flavonoids	Shinoda	Ethanol 95° + HCl (N/2) + (Mg ou Zn)	orange color, red or purple
Steroids and Terpenoids	Lieberman Bouchard	Acetic anhydride + sulfuric acid	purple coloring, blue or green
Tannins	$FeCl_3$ 1%	$FeCl_3$ 1%	dark blue, green or black coloring.
Quinones	Bornstraëgen	/	red coloring or violet
Anthocyanins		HCl 20%	pink coloring, red-orange
Saponosides		Distilled water	Foam Index (MI): positive test if IM > 100

2.3. Preparation of the ethanolic extract

The ethanolic extract of *F. capreolata* L. was prepared according to the method of Rehman et al. [7]. 10 g of the powdered herbal drug (leaf, stem and flower) and 150ml of 90% ethanol were placed in a soxhlet apparatus (behr Labor) at 40°C for a period of 150 min (eleven extraction cycles). The extract was filtered and evaporated to dryness under reduced pressure with a Rotavap (Hei-Vap Ultimate, Heidolph), the latter is considered to be the crude extract.

2.4. Thin layer chromatographic analysis (TLC)

The analyzes by thin layer chromatography or (TLC) were performed on Silicagel aluminum plates 60 F254 (MARCHERY-NAGEL). The plates are developed in saturated glass vessels with the appropriate eluent. The mobile phase consists of a binary mixture of solvents. The solvent systems used are as follows (the proportions are given by volume and they are classified by increasing polarity):

- nonpolar extracts: dichloromethane/methanol (9:1)
- polar extracts: dichloromethane/methanol (9.5:0.5) (9.8:0.2)
- hexane/ethyl acetate (4:6) (2:8)

The TLCs are analyzed in visible light and under U.V. (254 and 356 nm), before and after revelation with appropriate reagents. Using reagents provides additional information about the type of a molecule (specific reagent cases). Three alkaloids were used as controls: the atropine, berberine and scopolamine, and a flavonoid: quercetin. The retention factors (Rf) of the spots resulting from the separation were calculated and compared to those of the controls, thus allowing the identification of the different compounds in the extract.

Table 2. Main reagents used for (TLC) development.

Reagents	Substances revealed	Method of preparation and use
Sulphuric anisaldehyde (Deleu-Quettier 2000)	Versatile reagent	- Prepare a 0.5% solution of p-anisaldehyde in a mixture of CH_3OH/Ac OH/H_2SO_4 (85:10:5). - Spray on the plate. After intense heating, the organic compounds appear as colored spots in daylight.
Mounir	Alkaloid revealer	- Prepare 10 ml of the stock solution and 20 ml of acetic acid and make up to 100 ml with distilled water. - Spray on the plate: the alkaloids appear as orange-colored spots in daylight.

2.5. Determination of total phenolic compounds by colorimetry

The method that we were able to adapt to our plant material, described by Juntachote et al. [8]. 0.5ml of the ethanolic extract of *F. capreolata* L. diluted in 5 ml of distilled water was mixed with 0.5 ml of the Folin-Ciocalteu reagent (FCR) in a test tube. Then 0.5 ml of 20% (w/v) anhydrous sodium carbonate solution (Na_2CO_3) was added to the mixture. After incubating the reaction mixture for one hour at room temperature in the dark, the absorbance is measured at 765 nm.

The phenolic content of the extract was determined from the regression equation of the calibration range established with gallic acid. The results are expressed in mg gallic acid equivalent per gram of dry plant material (mg GAE/g). All the measurements are repeated 3 times.

2.6. Evaluation of antioxidant activity in vitro

2.6.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity

The experimental protocol followed to measure the scavenging activity of DPPH is that by Benhammou et al. [9].

The DPPH is dissolved in methanol to obtain a solution of 0.3 mM. In tubes 1 ml of methanol and 1 ml of the ethanolic extract (at different concentrations 1 mg/ml in methanol) are introduced and 2 ml of the methanolic solution with DPPH is added. After vortexing, the tubes are placed in the dark at room temperature for 30 minutes. The reading is taken by measuring the absorbance with a spectrophotometer (Perkin-Elmer UV/Vis Lambada 35) at 517 nm.

The negative control is composed of 1 ml of the methanolic solution with DPPH and 2.5 ml of methanol. BHT, BHA and ascorbic acid have been used as standard synthetic antioxidants.

The percentage of antioxidant activity was determined according to the following equation:

% Anti-free radical activity = (Abs control - Abs sample / Abs control) × 100

The results are the mean of three separate measurements ± standard deviation. Calculation of IC_{50}: IC_{50} or 50% inhibitory concentration is the concentration of the test sample necessary to reduce 50% of the free radical DPPH.

2.6.2. β-carotene bleaching method

The experimental protocol followed is that of Ozsoy et al. [10]. The β-carotene/linoleic acid emulsion was prepared by dissolving 2 mg of β-carotene in 10 ml of chloroform, then 1 milliliter of this solution is mixed with 20 mg of purified linoleic acid and 200 mg of Tween 40, the chloroform was completely evaporated on a rotary evaporator at 40°C. And the residue obtained is taken up in 50 ml of water saturated with oxygen (H_2O_2), the resulting emulsion was stirred vigorously.

Tubes containing 5 ml of this emulsion are prepared, for which 200 µl of a solution of the ethanolic extract of the plant studied or of reference antioxidant (BHA) at different concentrations are added.

The mixture is stirred well and the absorbance reading at 470 nm is taken immediately against a blank, which contains the emulsion without the β-carotene. The covered tubes are placed in a water bath set at 50 ° C and the absorbance reading is taken after 120 minutes. A negative control is carried out in parallel, comprising 5 milliliters of the β-carotene emulsion and 200 µl of ethanol.

The results obtained are expressed as a percentage inhibition of β-carotene discoloration using the following formula:

Percent inhibition = $[1 - (A_o - At / A^0_0 - A^0_t)] \times 100$ [10].

where:

AA: Antioxidant activity;

$A°$: Absorbance of the sample at t_0

At: Absorbance of the sample after 120 minutes of incubation

A^0_0: Absorbance of negative control at t_0

A^0_t: Absorbance of negative control after 120 minutes of incubation.

2.6.3. Ferric reducing antioxidant power (FRAP)

The crude ethanolic extract of *Fumaria capreolata* L. diluted (1 ml) at different concentrations was mixed with 2.5ml of the phosphate buffer solution (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide ($K_3Fe(CN)_6$) at 1%. The whole was incubated at 50°C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid (TCA) was added to the mixture to stop the reaction, then the tubes are centrifuged for 10 min at 3000 rpm. Distilled water (2.5ml) and ferric chloride ($FeCl_3$) (0.1%) were added to 2.5 ml of the supernatant. The absorbance reading was measured at 700 nm against a blank using a spectrophotometer (Perkin Elmer UV/Vis Lambada 35) [11].

Ascorbic acid was used as a positive control at the same chosen concentrations and under the same operating conditions as the samples.

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening of *Fumaria capreolata* L.

The results of the chemical screening carried out on the infused, the macerated and the powder of *Fumaria capreolata* L. from Eastern Algeria are shown in Table 3.

Table 3. The Phytochemical profile of the different organs of *Fumaria capreolata* L.

Phytochemical families		Fumitory *Fumaria capreolata* L.			
		Flower	Leaf	Stem	Root
Alkaloids		+	+	+	+
Flavonoids		-	-	-	-
Sterols and terpenoids		+	+	+	+
Tannins	gallic	+	+	+	-
	catechetical	-	+	+	+
Free quinones		-	-	-	-
Anthocyanins		+	-	+	-
Leuco anthocyanins		+	-	-	-
Saponosides		+	+	+	+
Starch		-	-	-	-
Cardinolides		-	-	-	-
Coumarins		+	-	-	-

According to the results of the phytochemical screening carried out on the species *F. capreolata* L., we were able to detect different families of chemical compounds co-existing in this species by staining and precipitation reactions.

Table 3 shows that the organs of the species *Fumaria capreolata* L. contain several chemical groups. We observed a significant presence of alkaloids, sterols, catechic tannins and saponins, in all organs of the plant. While coumarins could only be detected in the flowers.

We noted a virtual absence of anthocyanins and leuco-anthocyanins in all organs, except in the flowers and stems of climbing fumitory where they are present. However, testing for free quinones, cardinolides, flavonoids and starch produced a negative inference for all organs of the plant.

Much research has revealed the richness of European, Asian and African species of the genus *Fumaria* in different types of isoquinoleic alkaloids, in particular aporphine, protoberberine, protopine and benzophenanthridine. Also spiro-benzylisoquinoline alkaloids have been isolated such as fumaricin, fumarilin, fumaritin, fumarophycin, O-methylfumarophycin and parfumin [12-14]. Maiza-Benabdesselam and his collaborators [15] in Algeria identified the alkaloids contained in the methanolic extracts of the aerial part of two Algerian species: *F. capreolata* and *F. bastardi* by GC/MS (Gas Chromatography coupled with Mass Spectrometry). They showed the presence of a large number of alkaloids such as: stylopine, protopine, fumaritin, fumaricin, fumarophycin, fumarilin and fumarofin.

Gupta and Rao [16] in a phytochemical study of the methanolic extract of the species *Fumaria indica*, observe four major chemical groups: alkaloids, flavonoids, sterols and saponins. Several authors have also reported the presence of tannins, tri-terpenoids, saponins and flavonoids in different parts of the plant [17-19]. These results confirm the presence of these different chemical compounds in the organs of our plant: *Fumaria capreolata* L.

3.2. Yield of ethanolic extract of *F. capreolata* L.

The results we obtained indicate that from 10 g of the powder of aerial parts of the plant *Fumaria capreolata* L. and 150 ml of ethanol and following evaporation to dryness of ethanol. We obtained an ethanolic extract considered to be the crude extract of blackish green color and with a semi-solid appearance. This extract may contain chlorophyll, polyphenols and other compounds.

The yield is calculated on the total weight of dry and ground plant material, is expressed as a percentage. According to the results, the ethanolic extract of the plant studied has a low yield with a percentage of 8.4 ± 1.94%. This yield is higher than that obtained in another study carried out on the same species collected from the city of Constantine (Algeria), where the yield of the crude ethanolic extract is of the order of 2.5% [20].

Furthermore, the value we obtained in our study is consistent with the results obtained in other work carried out on species of the same genus, where the ethanolic extracts of *Fumaria officinalis* and *F. parviflora* gave similar yields with the values of the order of 8% and 7.4%, respectively [21, 22].

However, Mohajerani et al in 2019, mentioned that the ethanolic (80%) extract of the species *Fumaria vaillantii* L. has a very high yield with a percentage of 20.3% [23]. While other researchers have reported a much lower yield, with a percentage of 11% and 10.2%, for ethanolic extracts of *F. officinalis* and *F. indica* [24, 25].

The difference in yield noted in our study may be due to the chemical composition which differs from one species to another, the possible content of active ingredients, the plant material to be extracted, the nature of the solvent used and the extraction technique used without forgetting the nature and composition of the soil [26].

3.3. Thin layer chromatographic analysis (TLC)

In the analysis of plant extracts by thin layer chromatography, each substance is characterised by its fluorescence under UV light (254 nm and 365 nm), its "Rf" and its color after development with the appropriate chemical developer.

According to the results, we observe nine (9) spots with different colors and migration distances (Rf) under both wavelengths for the ethanolic extract of *F. capreolata* L. TLC plates developed in the sulphuric anisaldehyde (followed by heating) showed colored spots (green, blue and black spots), indicating the presence of organic compounds. On the other hand, the appearance of certain orange-yellow colored spots after the revelation of the plates by the Mounir reagent indicates the presence of alkaloids.

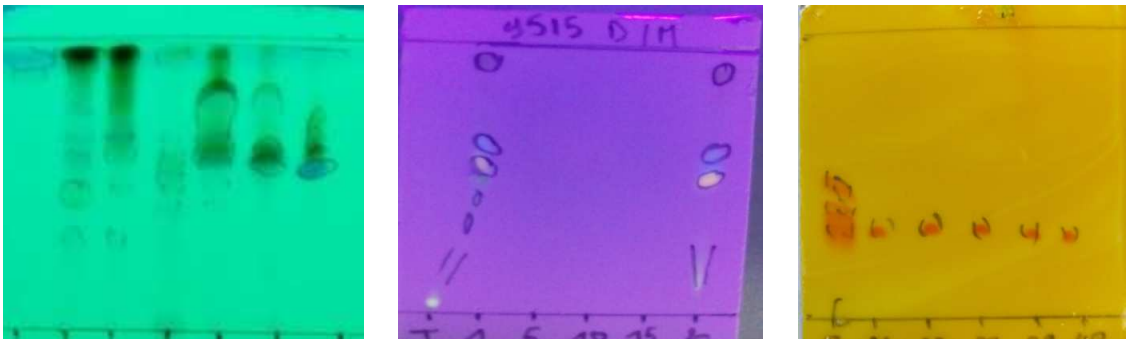

Figure 1. Chromatograms of the ethanolic extract of *F. capreolata* L. after development.

TLC showed the probability of the presence of isoquinoline alkaloid such as berberine (Rf = 0.80) and the absence of the atropine and scopolamine in the ethanolic extract of the studied plant. Also a large group of

phenolic compounds was detected. These results are consistent with those of Naz and co-workers in 2013 and Guna in 2017 in their studies on related species: *Fumaria parviflora* and *Fumaria indica* [27, 28].

3.4. Total phenolic content of ethanolic extract of *Fumaria capreolata* L.

The family of phenolic compounds includes a large number of secondary metabolic products which differ in their structures and reactivity. These compounds have been of great interest in recent years due to their beneficial effects on human health, their powerful antioxidant properties and their credible effects on the prevention of various diseases associated with oxidative stress [29].

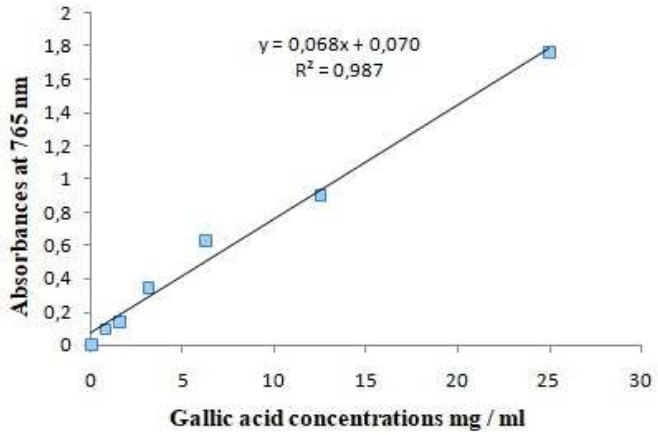

Figure 2. Gallic acid calibration curve for the determination of total polyphenols.

The content of phenolic compounds in the ethanolic extract of the species *F. capreolata* L. is 14.27 ± 1.65 mg GAE/g. While, the ethanolic extract of Romanian *F. capreolata* contains the highest amount of total phenolics (18.56 mg GAE/g d.w.) [30].

In addition, Ivan et al. [31], studied the levels of phenolic compounds in ethanolic extracts of five species of the genus *Fumaria*: *F. officinalis*, *F. thuretii*, *F. kralikii*, *F. rostellata* and *F. schrammii*. They showed that the polyphenol content in these species is between 20.20 ± 0.29 mg GAE/g (in the species *F. thuretii*) and 30.30 ± 0.31 mg GAE/g (in the species *F. officinalis*). These values are significantly higher than what we noted in our study on the species *F. capreolata* L. Significantly, the lowest values of phenolic compounds were recorded by Orhan et al. [32], in their study on four species of the genus *Fumaria* collected from Turkey: *F. cilicica*, *F. densiflora*, *F. kralikii* and *F. parviflora*. The values obtained varied between 0.05 and 0.09 mg GAE/g of dry extract. According to these results, we find that the species *F. capreolata* L. collected from Algeria is richer in phenolic compounds.

The variation noted in in the quantity of polyphenols from one extract to another and from one species to another is probably due to several parameters such as: the operating conditions of the extraction, the nature and the polarity of the solvent used. These variations are also considerable depending on the variety, the physiological stage of the plant and the nature of the plant tissues [33].

3.5. Antioxidant activity

3.5.1. DPPH radical scavenging activity

The anti-free radical activity of extract was evaluated by the DPPH spectrometry method. The DPPH molecule is a free radical, dark purple in color, characterized by an absorption band between 515-520 nm. The DPPH free

radical scavenging assay is based on the reduction of the latter when mixed with an antioxidant such as polyphenols, which leads to a loss of its violet color which turns pale yellow and to a reduction in its absorption at 520 nm [29].

Figure 3 reports the percentages of inhibition obtained from the ethanolic extracts of *F. capreolata* L., Compared to that of the positive controls used (BHA, BHT and ascorbic acid).

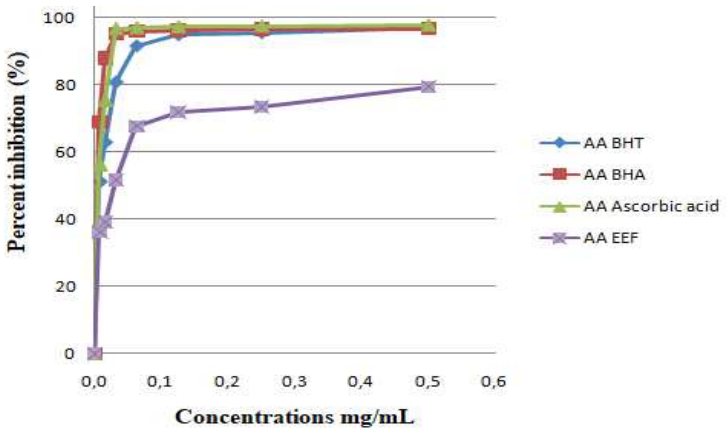

Figure 3. Anti-free radical activity of the ethanolic extract of *Fumaria capreolata* L.

Each value represents the mean of three tests ± SD.

Figure 3 shows that the percentages of inhibition are important at different concentrations; which increase in anti-free radical activity proportional to the increase in the concentration of the extract tested. At a concentration of 0.5 mg/ml the ethanolic extract of *Fumaria capreolata* L. shows significant anti-free radical activity with a high DPPH radical scavenging power (72.35 ± 0.27%).

Table 4. IC_{50} found in the extract of the plant studied.

Extracts	IC_{50} expressed in mg/ml
Ethanolic extract of *F. capreolata* L.	0.0300
BHT	0.0078
BHA	0.0058
Ascorbic acid	0.0071

From the results shown in the Table 4, we note that the three positive controls used have a potent anti-free radical activity and superior to that of the extract of the plant studied. Indeed, BHA is the most active with an IC50 = 0.0058 mg/ml, then ascorbic acid, then BHT (0.0071 mg/ml and 0.0078 mg/ml). We also note that the ethanolic extract of *F. capreolata* L. showed a still high IC_{50} value (0.030 mg/ml).

Much research describes the antioxidant activity by trapping the free radical DPPH of species of the genus *Fumaria*. Previously, Bribi et al. [34] evaluated the antioxidant activity of the extract of total alkaloids of the same species *Fumaria capreolata* L., by the DPPH radical scavenging method in a concentration range between 0 and 800 µg/ml. The strong anti-free radical effect of the extract tested was estimated at 68.31 ± 0.35% at a concentration of 100 µg/ml. And the IC50 values found in the tested extract and the BHA were in the order of 28.87 µg/ml and 8.21 µg/ml. This result is clearly superior to that which we obtained in our study, where the ethanolic extract of the aerial part of the species *Fumaria capreolata* L. collected from the Edough region (Annaba, Algeria), seems to have anti-radical activity with a DPPH radical scavenging power of around 72.35 ± 0.05% at a concentration of 0.5 mg/ml.

In the same context, Maiza Benabdesselam and these collaborators [15], studied the antioxidant activity of alkaloids extracts of the two species *Fumaria capreolata* L. and *Fumaria bastardii* native to Algeria. The high inhibitory effect of the free radical DPPH was exerted by the extract of the total alkaloids of the species *F. bastardii* with a percentage of 86%, followed by the extract of the total alkaloids of the species *F. capreolata* L. with a percentage inhibition of 45.6%, only at a concentration of 50 µg/ml.

Several other studies have demonstrated the anti-free radical activity of the ethanolic extract of *F. indica* by this same method, and each time the extract has shown a significant inhibitory power of the order of 61.8% [19], and with an IC_{50} of 11 mg/ml [25].

In the work of Orhan et al. [34], the antioxidant activity of several types of extracts from four species of the genus *Fumaria*: *F. cilicica*, *F. densiflora*, *F. kralikii* and *F. parviflora* was studied by several methods. The results obtained show that the extracts tested have a significant DPPH radical scavenging activity at the concentrations of 250, 500 and 1000 µg/ml. And the fraction of ethyl acetate and dichloromethane of the species *F. cilicica* exhibits the highest activity with a percentage of inhibition of the order of 76.16 ± 0.12% and 51.86% respectively, at a concentration of 1000 µg/ml.

Theremore, Ivan et al. [31], observed that the ethyl acetate extract of the species *F. vaillantii* has the highest antioxidant activity compared to the rest of the extracts tested, with a percentage inhibition of the DPPH radical of 83.41%. This result joins that of Moghaddam et al. [35], who noted that the highest antioxidant power of the same species was observed at the vegetative stage, with an IC_{50} value of the order of 1217.85 ± 1.02. These authors reported that this activity is probably due to the presence of phenolic compounds and flavonoids.

This antioxidant property of the species *F. capreolata* L. may be due to protopine, which is an isoquinoline alkaloid present in the ethanolic extract of this plant as a major compound. The latter is endowed with several biological activities. Species of this genus also contain a number of fatty acids with an antioxidant effect, such as: linoleic acid, oleic acid, palmitic acid and myristic acid [30].

3.5.2. β-carotene bleaching method

The antioxidant power of our extract has also been tested by the β-carotene bleaching method. The oxidation of linoleic acid generates peroxide radicals, and conjugated diene hydro peroxides. This test is based on the fact that these freed radicals will subsequently oxidize the highly unsaturated β-carotene, which loses its double bonds, thus causing the disappearance of its red color, which is measured spectrophotometrically at a wavelength λ = 490 nm. However, the presence of an antioxidant could neutralize free radicals derived from linoleic acid, and therefore prevents the oxidation and bleaching of β-carotene [37].

From our results, we clearly notice that the ethanolic extract of the studied plant and BHA exert a powerful inhibitory effect on the oxidation of β-carotene, after 120 minutes of incubation. The ethanolic extract of *F. capreolata* L. shows the greatest inhibitory activity of the coupled oxidation of linoleic acid and β-carotene with a percentage of 88.46 ± 1.02% at a concentration of 0.5 mg/ml, followed by BHA with a percentage of 82.69 ± 0.03%.

According to the literature, two species of the genus *Fumaria* native to Algeria were investigated for their ability to inhibit the peroxidation of linoleic acid. Maiza-Benabdesselam et al. [15] studied the antioxidant activity extracts of the total alkaloids of *F. capreolata* L. and *F. bastardii* L. The alkaloids extracts of both plants expressed strong antioxidant activity; however, the activity of *F. bastardii* extract was more potent than of *F. capreolata* L.

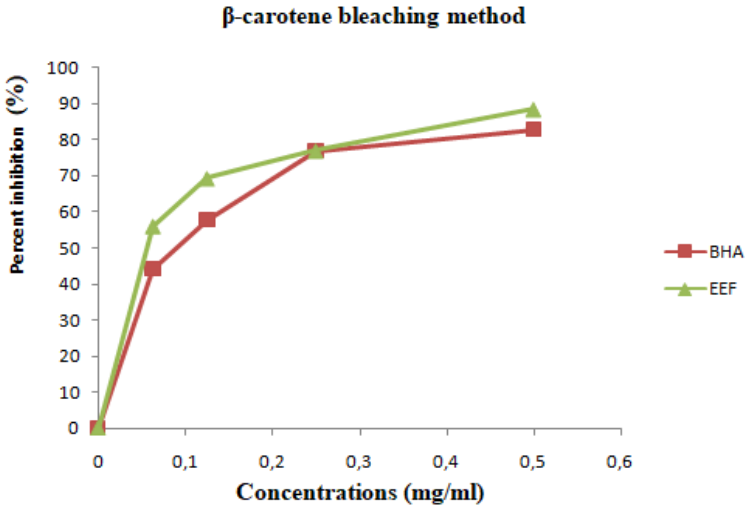

Figure 4. The antioxidant power of the ethanolic extract of *Fumaria capreolata* L. tested by β-carotene decoloration method.

At a concentration of 500 µg/ml, the two extracts tested showed a percentage of 65 and 67.8% inhibition of the peroxidation of linoleic acid, for the species *F. capreolata* L. and *F. bastardii* respectively. On the other hand, at the same concentration the antioxidant butylated hydroxyanisole (BHA), quercetin, and caffeine have an inhibition rate of 80, 56.2 and 64.3%, respectively.

3.5.3. Ferric reducing antioxidant power (FRAP)

The FRAP method is a simple, inexpensive and robust spectrophotometric technique. It is based on the ability of polyphenols to reduce ferric iron Fe^{3+} to ferrous iron Fe^{2+} [40]. From the results obtained, we note that the increase in ferric iron reducing absorbances is proportional to the concentrations used.

The crude extract of *F. capreolata* L. expressed a very low reducing power, with observed values of optical densities not exceeding 1 (OD = 0.349 ± 0.0062) at a concentration of 0.5 mg/ml. From the graphs shown in figure 3, we can clearly observe the low capacity of the crude extract of *F. capreolata* to reduce iron by comparing the latter with the reducing power of ascorbic acid which is of the order from 2.52 ± 0.0052. This potential is related to the nature of the reducing substances existing in the extract tested.

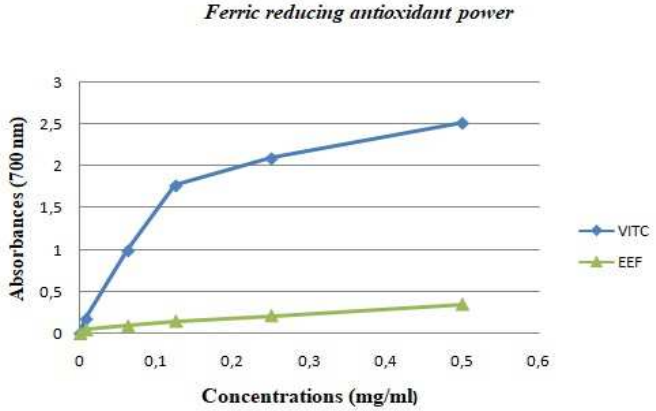

Figure 5. Reducing power of the ethanolic extract of the plant studied and of the ascorbic acid tested by the FRAP method. Each value represents the average of three trials.

A compound's reducing capacity can serve as an indicator of its antioxidant potential. The presence of reducing agents (such as antioxidants) causes the conversion of Fe^{3+} ferricyanide complex in the ferrous form Fe^{2+}. Although iron is essential for oxygen transport for respiration and enzyme activity, it is a reactive metal that catalyzes oxidative damage in living tissues and cells [41].

The reducing activity of the ethanolic extract of the plant *Fumaria capreolata* L. from the Edough region was moderate and significantly lower than that of ascorbic acid, with an optical density of 0.349 ± 0.03 at a concentration of 0.5 mg/ml.

Our results join those obtained by Maiza-Benabdesselam et al. [15], where the extracts of the total alkaloids of *F. bastardii* and *F. capreolata* showed a low activity for the reduction of iron compared to the standards used, in the following order: quercetin > BHA > gallic acid followed by the extracts of the total alkaloids of *F. bastardii* and *F. capreolata.* In another study, the best reducing capacity of the extract of the total alkaloids of *F. capreolata* was obtained at a concentration of 800 µg / ml with an optical density of around 0.57 ± 0.005 [33]. On the other hand, the ethanolic extract and the fractions of four plants belonging to the genus *Fumaria* from Turkey were tested for their reducing capacity. All of the extracts tested exerted a low reducing capacity compared with the positive control. And the greatest reducing activity was obtained from the ethanolic extract of *Fumaria kralikii* with an absorbance of 0.390 ± 0.04 at a concentration of 1000 µg/ml [37]. Further, the antioxidant activity of *F. vaillantii* extracts reported by FRAP assay, demonstrates that the reducing power of BHT (585.91 Fe^{2+}/mg extract) was significantly higher than vegetative, budding and flowering stages (359.48 and 248.87 µmol Fe^{2+} per mg EO, respectively) [38].

Generally, the variation in the reducing activity is attributed to the chemical composition of the extracts tested. However, it may be due to one of the majority constituents or to other minority constituents or also to a synergy between them.

4. CONCLUSIONS

We can conclude that, the plant that we studied as well as various other species of the same genus, were markedly different with regard to their phytochemical composition, in particular alkaloids and phenolic compounds. As a result, they also differ in their anti-oxidant activities. However, we noted that the antioxidant properties of the species *Fumaria capreolata L* from Edough region (Annaba Province, Algeria), correlated with their phytochemical composition.

Authors' Contributions: SI: collecting plant samples, extraction, determination of total phenolic content, studied antioxidant activity of the plant and wrote the manuscript. SR: Supervised the findings of this work. All authors are read and approved the final manuscript.

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REFERENCES

1. Gilani AH, Bashir S, Janbaz KH, Khan A. Pharmacological basis for the use of *Fumaria indica* in constipation and diarrhea. J Ethnopharmacol. 2005; 3: 585-589.
2. Rice-Evans CA, Packer L. Flavonoids in Health and Disease, 2nd edn. CRC Press. 2003.
3. Quezel P, Santa S. La nouvelle flore de l'Algérie et des régions désertiques méridionales. Ed. Centre national de la recherche. Paris. 1963.

4. Ngakegni-Limbili AC. Etude de synergie des effets chimiques et biologiques des lipides de réserves et des huiles essentielles des fruits et graines saisonniers de la sous-région Afrique Centrale. Institut National Polytechnique. Université de Toulouse. 2012; 170-171.
5. Solfo R. Etude d'une Plante Médicinale Malgache *Buxusma dagascarica* Bail et ses variétés. Ed. O.R.S.T.O.M. 1973.
6. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. Chapman et Hall Thomson Science (UK). 1998.
7. Rehman S, Abdul L, Shamim A, Asad UK. Antibacterial activity of Shahtra (*Fumaia officinales Linn*) extracts against MRSA (Methicillin Resistant *Staphyloccocus aureus*). Unani Medicus Int J. 2010; 1: 57-61.
8. Juntachote T, Berghofer E, Siebenhandl S, Bauer F. The ant-oxidative properties of Holy basil and Galangal in cooked ground pork. Meat Sci. 2006; 72: 446-456.
9. Benhammou N, Atik-Bekkara F, Kadifkova PT. Antiradical capacity of the phenolic compounds of *Pistacia lentiscus* L. and *Pistacia atlantica Desf*. Adv Food Sci. 2007; 155-161.
10. Ozsoy N, Can A, Yanardag R, Akev A. Antioxidant activity of *Smilax excelsa* L. leaf extracts. Food Chem. 2008; 571-583.
11. Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr. 1986; 413-419.
12. Sousek J, Guedon D, Adam T, Bochorakova H, Taborska E, Valka I, Šimanek V. Alkaloids and organic acids content of eight *Fumaria* species. Phytochem Anal. 1999; 10: 6-11.
13. Bentley KW. β-Phenylethylamines and the isoquinoline alkaloids. Royal Soc Chem. 2000; 247-268.
14. Suau R, Cabezudo B, Rico R, Najera F, Lopez RJM. Direct Determination of Alkaloid Contents in *Fumaria* Species by GC-MS. Phytochem Anal. 2002; 13: 363-367.
15. Maiza BF, Khentache S, Bougoffa K, Chibane M, Adach S, Chapeleur Y, et al. Antioxidant activities of alkaloid extracts of tow Algerian species of *Fumaria*: *Fumaria capreolata* and *Fumaria bastardii*. Rec Nat Prod. 2007; 1: 28-35.
16. Gupta P, Rao CHV. Morpho-anatomical and physicochemical studies of *Fumaria indica* (Hausskn.) Pugsley. Asian Pac J Trop Biomed. 2012; 830-834.
17. Shayka A, Chatterjee SS, Kumar V. Holistic psychopharmacology of *Fumaria indica*. Chinese Med. 2012; 3: 182-199.
18. Rao CV, Verma AR, Gupta PK, Kumar MV. Anti-Inflammatory and Anti-Nociceptive Activities of *Fumaria indica* Whole Plant Extract in Experimental Animals. Acta Pharm. 2007; 57(4): 491-498.
19. Gupta P, Sharma N, Rao CHV. A review on ethnobotany, phytochemistry and pharmacology of *Fumaria indica* (Fumitory). Asian Pac J Trop Biomed. 2012; 665-669.
20. Mezhoud K, Benmokhtar M, Smati D. Contribution to the study of *Fumaria capreolata* L. (Papaveraceae), in the Wilaya of Constantine: phytochemical characterization and research of the anti-inflammatory activity. Batna J Med Sci. 2018; 5(1): 26-28.
21. Pehlivan KF, Yildirim A, Türker A. Biological screening of various medicinal plant extracts for antibacterial and antitumor activities. Turk J Biol. 2012; 36: 641-652.
22. Al qasoumi SI, Al dosari MS. Evaluation of the hepatoprotective effect of *Fumaria parviflora* and *Momordica balsamina* from Saudi folk medicine against experimentally induced liver injury in rats. Res J Med Plant. 2009; 3: 9-15.

23. Mohajerani F, Pourjabbar Z, Mazdeh FZ, Rahimi R, Amin GR, Toliyat T, et al. Quantitation of phytochemical constituents of *Fumaria vaillantii* L. with different extract methods. Tradit Med Res. 2019; 4(5): 237-245.
24. Wasu SJ, Muley BP. Antioxidant Activity of *Fumaria officinalis Linn* and Its study on ethanol induced-immuno-supression. Res J Pharm Tech. 2009; 2: 405- 408.
25. Pandey GG, Kumar GR, Gupta ShS, Ojha SK, Rao Ch V. Wound Repair and Anti-inflammatory Potential of *Fumaria indica* in Excision Wound-induced Rats. Br J Pharm Res. 2014; 4: 257-266.
26. Duraffourd C, Lapraz JC. Traité de phytothérapie Clinique: endobiogénine et médecine. Elsevier Masson, 2002.
27. Naz I, Palomares-Rius JE, Saifullah, Blok V, Khan MR, Ali S. In vitro and in planta nematicidal activity of *Fumaria parviflora* (Fumariaceae) against the southern root-knot nematode *Meloidogyne incognita*. Plant Pathol. 2013; 62: 943-952.
28. Guna G. Pharmacological activity of *Fumaria indica,* A review. J Phytopharm. 2017; 6(6): 352-355.
29. Waston RR. Polyphenols in Plants: Isolation, Purification and Extract Preparation. Academic Press, 2014.
30. Paltinean R, Mocan A, Vlase L, Gheldiu AM, Crisan G, Ielciu I, et al. Evaluation of polyphenolic content, antioxidant and diuretic activities of six *Fumaria* species. Molecules. 2017; 22 (639): 1-14.
31. Ivan GI, Radka ZV, Andrey SM, Nadezhda TP, Ina YA, Panteley PD. Antioxidant activities and phenolic compounds in Bulgarian *Fumaria* species. Int J Curr Microbiol App Sci. 2014; 3(2): 296-306.
32. Orhan I, Sener B, Musharraf S. Antioxidant and hepatoprotective activity appraisal of four selected *Fumaria* species and their total phenol and flavonoid quantities. Exp Toxicol Pathol. 2010; 1-5.
33. Macheix JJ, Fleuriet A, Allemand CJ. Les composes phénoliques des végétaux: un example de metabolites secondaires d'importance économique. PPUR presses polytechniques. 2005.
34. Bribi N, Bouguezza Y, Maiza BF. Evaluation of erythrocytes toxicity and antioxidant activity of alkaloids of *Fumaria capreolata*. Int J Pharma Bio Sci. 2013; 2: 770-776.
35. Orhan I, Sener B, Choudhary MI, Khalid A. Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some Turkish medicinal plants. J Ethnopharmacol. 2004; 57-60.
36. Moghaddam M, Seyed Naser Khaleghi Miran SN, Mehdizadeh L. Total phenolic content and antioxidant activity of *Fumaria vaillantii* extract at three phenological stages assayed by various methods. Int J Hortic Sci. 2018; 5(1): 93-102.
37. HabibiTirtash F, Keshavarzi M, Fazeli F. Antioxidant components of *Fumaria* species. World Acad Sci Engin Technol. 2011; 74: 238-241.
38. Kaur C, Kapoor HC. Antioxidant activity and total phenolic content of some Asian vegetables. Food Sci Technol. 2002; 37: 153-161.
39. Yang J, Guo J, Yuan J. In vitro antioxidant properties of rutin. LWT. 2008; 41: 1060-1066.
40. Rosa LA, Alvarez PE, Gonzalez GA. Fruit and vegetabale phytochemicals: Chemistry, Nutritional Value and Stability. John Wiley and Sons. 2009.
41. Bourgou S, Ksouri R, Bellila A, Skandrani I, Falleh H, Marzouk B. Phenolic composition and biological activities of Tunisian *Nigella sativa L* shoots and roots. C. R. Biologies. 2008; 48-55.