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Antioxidant activities of alkaloid extracts of two Algerian species of *Fumaria* : *Fumaria capreolata* and *Fumaria bastardii*

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Abstract: The antioxidant activities of the alkaloid extracts of *Fumaria capreolata* (L.) and *Fumaria bastardii* (L.) were determined by measuring their reducing power, their ability to inhibit linoleic acid peroxidation, and 2,2-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging activities. Total quinolizidine alkaloid contents were found to be 426 mg/100 g (*F.capreolata)* and 521 mg/100g (*F.bastardii)*. Both plant extracts exhibited strong total antioxidant activity, however, extract of *F.bastardii* was more potent then *F.capreolata*. Concentrations of 100, 300, and 500µg mL^{-1} showed 42, 55, 65 and 48.3, 60, and 67.8 % inhibition of lipid peroxidation of linoleic acid emulsion, for *F.capreolata* and *F.bastardii* extracts, respectively. On the other hand, 500µg mL^{-1} of the standard antioxidant butylated hydroxyianisole (BHA), quercetine, and caffeine exhibited 80, 56.2, and 64.3% inhibition of lipid peroxidation, respectively. In addition, the both extracts had effective reducing power, DPPH free radical scavenging activity at 250 and 50 µg mL^{-1}. The isoquinoline alkaloids, stylopine, protopine, fumaritine, fumaricine, fumarophycine, fumariline, fumarofine were determined by GC-MS from the aerial parts of *F. capreolata* and *F. bastardii.*

Key words: *Fumaria capreolata* (L.); *Fumaria bastardii* (L.); isoquinoline alkaloids; GC/MS; antioxidant activity.

1. Introduction

The species of *Fumaria capreolata* and *Fumaria bastardii* are the members of *Fumariaceae* family [1]. The species are annual herbs which have wide distribution in the Mediterranean region [2].

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The species of *Fumaria* plants are used in Algerian traditional medicine, in cases of hepatobiliary disfunction and gastrointestinal disorders. It was reported that the plant has local reputation in Pakistan and India as anthelmintic, antidyspeptic, blood purifier, cholagogue, diuretic, laxative, sedative, tonic, and is also considered as useful for the treatment abdominal cramps, fever, diarrhoea, syphilis, and leprosy [3]. Apart from regular thin layer chromatography (TLC) and column chromatography techniques, reverse phase HPLC, GC-MS, and capillary electrophoresis techniques have been reported for the determination of isoquinoline alkaloids from plant extracts [3-7]. The chemotaxonomic evaluation of some types of isoquinoline alkaloids supports the differential of species of two *Fumaria* has been evaluated [4, 8].

2. Materials and Methods

2.1. Plant Material

Aerial parts of *Fumaria capreolata* (L.) and *Fumaria bastardii* (L.) were collected when they were at the flowering and fruit setting phonological stages from Bejaia city in the north east of Algeria and identified by the Laboratory of Botanic of Bejaia University in April and June, 2005. The identification was confirmed later by Professor Max Henry of University of Nancy 1 – France. The voucher specimens were deposited in the Herbarium of Bejaia University.

Table 1. The MS fragments of identified components of from the *Fumaria* species by GC-MS

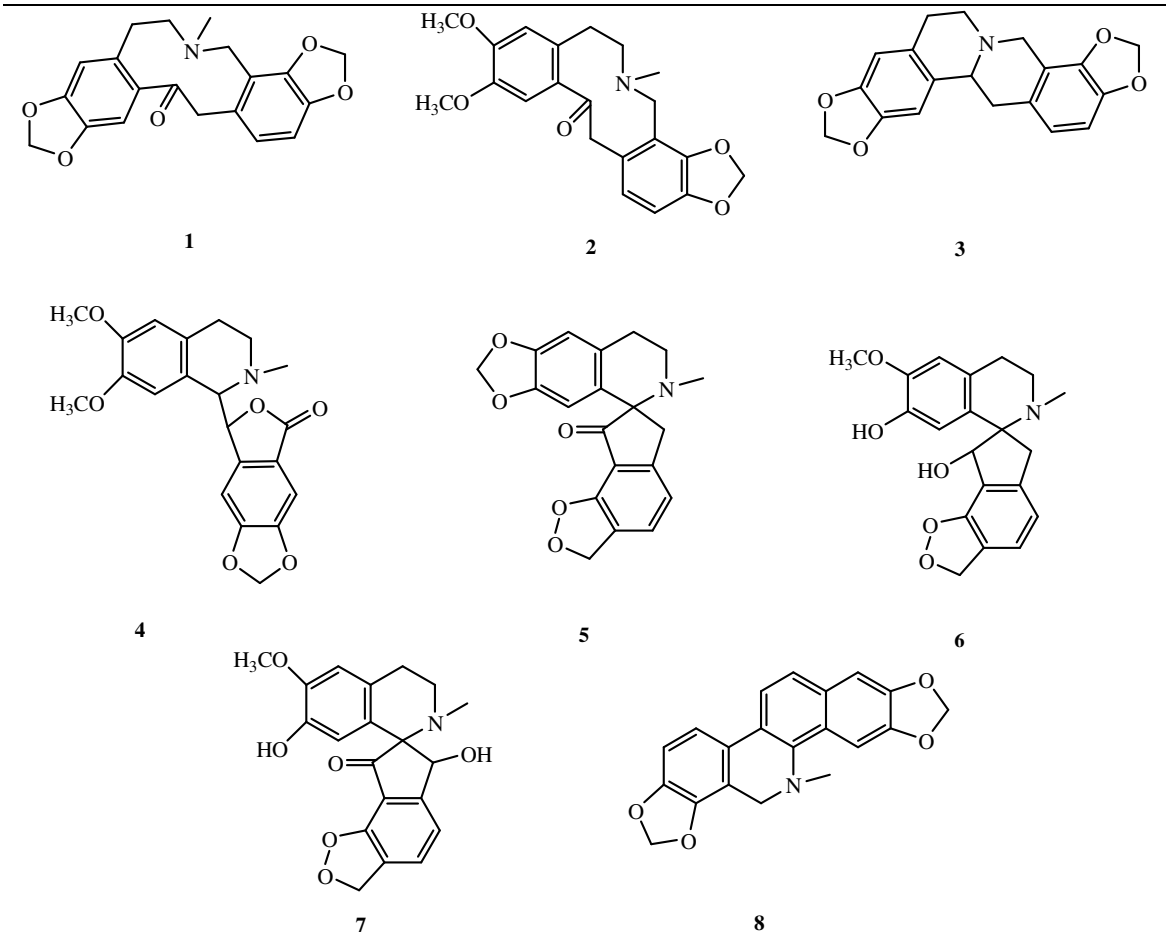
RT	Alkaloid type	Compound	EI-MS m/z (°/°)	F. *capreolata* *(%)*	F. *bastardii* *(%)*
20.5	Protopine	Protopine	148(100)163(20)354(5)	50.6	61.5
	Tetrahydroprotoberine	Protoberberine	148(100) 369(4)	3.2	2.4
19.6		Stylopine	148(100)322(30)323(40)	22.9	17.4
23.7		Phthalideisoquinoline	190(100)148(52)320(80)	-	t
24.9	Spirobenzylisoquinoline	Phthalideisoquinoline	190(100)148(25) 320(32)	-	5.0
20.9		Fumariline	322(100)336(20)351(25)	8.0	6.4
22.1		Fumarophycine	322(100)337(85)	t	t
22.3		Fumaritine	192(100)340(20)	3.7	2.2
30.2		Fumarofine	369(100)354(56)192('34)	t	t
32.2		Fumaricine	206(100)355(13)	2.0	4.0
31.2		Dihydrofumariline	190(100)148(25)363(20)	-	t
8.6	Dihydrobenzophenanthridine		330(100)331(5)142(85)	-	t
7.1		Phtalic acid ester	149(100)167(25)317(12)	t	–
	Total alkaloids (mg/100g dry weight)			426 ± 23	521 ± 36

t < 0.2

2.2. Extraction of Alkaloid extract

Extraction of the alkaloids from the species has been reported in the literature [7-9]. Briefly; dried (at 40°C) samples (5-6 g) of the aerial parts from several individuals of each population, were powdered and extracted with methanol (100 mL) in a Soxhlet apparatus for 3 hours, and then evaporated to 0.5mL *in vacuo*. The methanolic residue was taken up in 10 mL of 2.5% hydrochloric acid and filtered. The aqueous acid solution was adjusted to pH=8 with concentrated ammonium hydroxide and extracted with dichloromethane (3 x 10 mL). The extracts were dried over magnesium sulphate and the solvent evaporated to afford a crude extract of alkaloids. After evaporation the yield of each fraction was calculated.

Figure1. Identified Alkaloids from the *Fumaria capreolata* and *Fumaria bastardii**



*Protopine **(1),** cryptonine **(2)**, Stylopine **(3)**, phtalidiisoquinoline**(4)**, Fumariline **(5)**, Fumaritine **(6)**, fumarafne **(7)** and dehydrobenzophenanthridine **(8)**

2.3. GC-MS conditions

The alkaloids contents of methanolic extract were analyzed using Fisons Trio 1000 GC-MS spectrometer. HP-1 (15 m x 0,25 mm x 1 µm film thickness) was used. The carrier gas was helium at a rate of 1mL/min. The injection temperature was 280 °C. The GC oven temperature was kept at 200°C for 8 min, and programmed to 250 °C at 10 °C/min and kept constant at 250 °C for 30 min. The MS source temperature was operated at 250 °C and the transfer line was maintained at 280 °C. The mass range scanned was 125 – 450 g and scan rate was 26 scans/sec.

2.4. Antioxidant activity

2.4.1- Free Radical Scavenging Activity

The stable 2,2-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging activity effect of alkaloids were carried out as described by Shirwaikar *et al* [10]. 1 mL of various concentrations of alkaloid solution was mixed with 1 mL of DPPH solution (0.1 mM). An equal amount of methanol and DPPH served as control. After incubation in the dark for 20 min, absorbance was recorded at 517 nm. The percentage scavenging was calculated as according to the following equation:

$$\% \text{ Scavenging} = \frac{A_c - A_S}{A_c} \times 100\%$$

Ac: absorbance of control
A_S: absorbance of sample

2.4.2- Reducing power

The total reducing power of the alkaloid extracts from *Fumaria bastardii* and *Fumaria capreolata* was determined according to the method of Oyaizu [11]. Briefly, 1mL of different concentrations of our methanolic extracts (5, 10, 15, 20, and 25 mg mL^{-1}) were mixed with phosphate buffer (1mL, 0.2 M, pH=6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1 mL, 1%). The mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (1 mL, 10%) was added to the mixture, which was then centrifuged for 10 min at 3000 × g. The upper layer of the solution (1.5 mL) was mixed with distilled water (1.5 mL) and $FeCl_3$ (0.3 mL, 0.1%), and the absorbance was measured at 700 nm using UV-VIS spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

2.4.3 Effect of alkaloids on the peroxidation of linoleic acid

The antioxidant activity of the alkaloid extracts from *F.bastardii* and *F.capreolata* was determined according to the ferric thiocyanate method in linoleic acid emulsion [12]. Different amounts of samples dissolved in 120 µL of MeOH were added to a reaction mixture in a screw cap vial. Each reaction mixture consisted of 2.88 mL of 2.5 % linoleic acid in Ethanol (EtOH) and 9 mL of 40 mM phosphate buffer (pH 7.0). The vial was placed in an oven at 40 °C in the dark. At intervals during incubation, 0.11 mL aliquot of the mixture was diluted with 8 mL of 75 % EtOH, which was followed by adding 0.1 mL of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 mL of 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm and every 24 h.

The percentage of linoleic acid inhibition was calculated following the equation:
Inhibition of peroxidation:

IP (%) = [1-(absorbance of sample at 500 nm/absorbance of control at 500 nm)] × 100.

3. Results and Discussion

3.1. GC-MS analysis

The results of GC-MS analysis of the species of *Fumaria* alkaloid extracts were given in Table 1, which show GC-MS experimental data, retention time (RT), and main fragments of the compounds for alkaloids of *Fumaria capreolata* and *Fumaria bastardii.* Individual alkaloids (Figure 1) were identified from RT, mass data, and by comparison of the data of the standard compounds with those of in the literature [2, 6, 9, 13-17]. Some components remained unidentified due to the lack of reference substances and library spectra. We have identified protopine **(1)** and stylopine **(3)** as main alkaloids in both of the species. The other protoberberine founded was determined as cryptonine **(2)**. The presence of peaks at *m/z* 355 (M^+, 10 %), 340 (M^+-CH_3, 20%) and 192 (base peak) was consistent with the occurrence of Fumaritine **(6)**, the peak with *m/z* at 369 (100 %) indicated fumarofine **(7)**. Other spirobenzylisoquinolines such as fumaricine, fumarophycine, and fumarilline **(5)** were identified from the methanol extract of the two species. From the *F.capreolata*, the peaks at *m/z* 149 (100 %), 167 (30 %), 279 (6 %) corresponds to a phthalic acid ester and the peak at *m/z* 330 (100 %) observed in *F.bastardii* extract seems to be a benzophenanthridine probably a dihydroderivate **(8)** [6, 8]. The other benzophenanthridine which can correspond to this peak is chelidonine which was detected in the genus *Sarcapnos* [2]. Three other peaks were founded only in only in *F. bastardii*. Although these peaks could not be fully characterised, they were identified as phthalidisoquinoline alkaloids based on the presence of a proeminent ion at 190 *m/z* as base peak [18], one of them can be identified as dihydrofumariline. However, there are several unresolved peaks, perhaps components derived from aporphines (proeminent ion at *m/z* 340 and 335) [16, 17]. The detailed information about the identified compounds can be seen in Table 1.

Algerian species of *Fumaria* seems to have highest concentrations of alkaloids than species from Spain studied by Suau and his collaborators [7, 8]. The total alkaloids (mg/100g dry weight) of *F.bastardii and F.capreolata* are respectively 521 and 426 in Algerian species while the amounts founded in the same species from Malaga are, respectively 425 and 412 [8].

3.2. Antioxidant activity

3.2.1. Free Radical Scavenging Activity

The scavenging effects of our extracts on DPPH radical are shown in the Figure 2. Maximum scavenging activity was found at concentration of 50 $\mu g.mL^{-1}$ whilst the minimum scavenging activity was found at 5μg mL^{-1}. *F.capreolata* presents a lower effect (45, 6%) than *F.bastardii* (86%), whereas the antioxidant efficiency of the standards was better (99% scavenging effect at the concentration of 50 $\mu g.mL^{-1}$). Caffeine did not show any antioxidant effect. This result can be related to some studies showing that caffeine has a prooxidant effect [19].

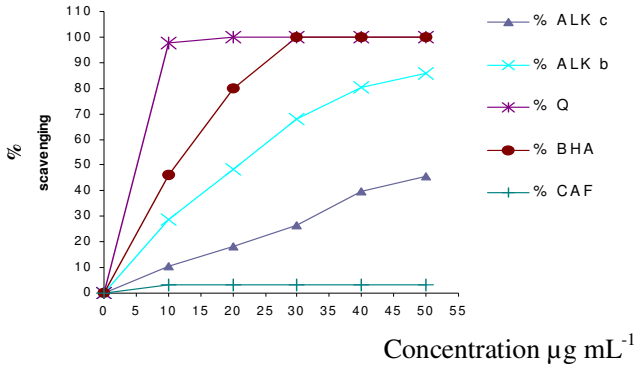


Fig 2: Antioxidant activity (% scavenging of DPPH free radical) of different concentrations of alkaloid extracts from *F.bastardii* and *F. capreolata* and standards.
ALKc (alkaloid extract of *F. capreolata*), ALKb (alkaloid extract of *F. bastardii*), Q (quercetine), CAF (caffeine), BHA.

3.2.2. Reducing power

Figure 3 shows the reductive capabilities of the alkaloid extracts compared to BHA, gallic acid, and quercetine. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [18]. The reducing power of the alkaloid extracts from *F. bastardii*, *F. capreolata,* and the standard compounds followed the order: quercetine > BHA > gallic acid > ALKb > ALKc.

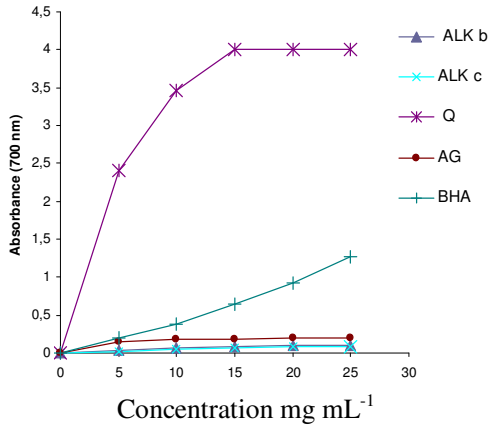


Fig 3: Reducing power of alkaloid extracts from *Fumaria bastardii* and *Fumaria capreolata* and standards by spectrophotometric detection of the Fe^{3+}-Fe^{2+} transformation: ALKc (alkaloid extract of *F. capreolata*), ALKb (alkaloid extract of *F. bastardii*), Q (quercetine), AG (gallic acid), BHA

3.2.3. Inhibition of the peroxidation of linoleic acid

The antioxidant activity of the alkaloids increased with their concentration (Table 2) but decreased at the higher one of 500 µg mL^{-1}. This may be explained by the fact that at higher concentrations of alkaloid acts as oxygen-carrying agent and serves as a pro-oxidant in the co-oxidation of linoleic acid. The thiocyanate test showed that at a concentration of 500 µg mL^{-1} the inhibition of peroxidation of linoleic acid produced by the alkaloid extracts of *F.bastardii* and

F.capreolata, the quercetine and BHA were 67.8, 65, 64.3, 56.2 and 80 %, respectively as compared with the blank.

Table 2. Inhibition of linoleic acid peroxidation (**IP %**)

	Inhibition of peroxidation (IP %)		
	100 µg mL^{-1}	**300 µg mL^{-1}**	**500 µg ml^{-1}**
ALKb	48.3	60	67.8
ALKc	42	55	65.0
Quercetine	50	60	64.3
Caffeine	46	50	56.2
BHA	71	75.3	80

ALKb (alkaloid extracts of *F.bastardii*), **ALKc** (alkaloid extracts of *F.capreolata*).

In conclusion, this study shows that the Algerian species of *Fumaria*, *Fumaria capreolata,* and *Fumaria bastardii* contain isoquinoline alkaloids, which explain their pharmacological properties. Gas chromatography coupled to mass spectrometry (GC-MS) is proved to be a valuable tool for the analysis of *Fumaria* alkaloids, however, this method is not applicable to quaternary alkaloids and the alkaloids with base peak $m/z < 125$. Soxhlet extraction combined with the GC-MS method is a direct and fast analytical approach for identification of the various tertiary bases present in the alkaloid extracts and only a few grams of plant material is required. The antimicrobial, antimalarial, cytotoxic, and anti HIV activities of the isoquinoline alkaloids have been reported and the possible chemopreventive antitumor promoters are probably related to their radical scavenging activity against DPPH radical [20]. Both alkaloid extracts of the two species of *Fumaria* showed a strong antioxidant activity, especially a strong radical scavenger power, so they can be used as natural and good sources of natural antioxidants for medicinal and commercial needs.

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