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Full Length Research Paper

Determination of isoquinoline alkaloids contents in two Algerian species of *Fumaria* (*Fumaria capreolata* and *Fumaria bastardi*)

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This paper describes a fast and efficient procedure to separate and identify isoquinoline alkaloids from methalonic extract of two Algerian *Fumaria* (*Fumariacea*) species (*Fumaria capreolata* L. and *Fumaria bastardi* L.) used in traditional medicine in cases of hepatobiliary disfunction and diarrhoea. Total quinolizidine alkaloid contents were 426 mg/100 g (*F. capreolata)* and 521 mg/100 g (*F. bastardi).* The isoquinoline alkaloids, stylopine, protopine, fumaritine, fumaricine, fumarophycine, fumariline and fumarofine were determined by gas chromatography – mass spectrometry (GC-MS) in aerial parts of both *Fumaria capreolata* and *Fumaria bastardi.* In the first species, an ester of phtalic acid was identified, and in the second species a peak seems to be a benzophenanthridine, probably a dehydro derivative and three other peaks which were identified as phtalidisoquinoline, one of them seems to be dihydrofumariline. The chemotaxonomic significance of the results is discussed.

Key words: *Fumaria capreolata, Fumaria bastardi,* isoquinoline alkaloids, gas chromatography – mass spectrometry, chemotaxonomy.

INTRODUCTION

The genus *Fumaria* belongs to Fumariaceae family and encompasses about 40 species (Suau et al., 2001). These plants are annual herbs which have wide distribution in the Mediterranean region (Suau et al., 2005). The identification of *Fumaria* plants is subjective because they have very close morphological properties (Soušek et al., 1999). The chemotaxonomic evaluation of some types of isoquinoline alkaloids supports the differential of plants among these genera (Preininger, 1986). Several techniques have been used for the determination of isoquinoline alkaloids in plant extract (Soušek et al., 1999). However identification of tertiary bases is a problem and a screening tool based on GC-MS (gas chromatography coupled to mass spectrometry) technique for direct determination of alkaloids content in *Fumaria* was developed (Suau et al., 2002). The present paper deals with the GC-MS analysis of the alkaloids fraction from aerial parts of North East Algerian's indigenous plants, *Fumaria capreolata* (L.) and *Fumaria bastardi* (L.). Those 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 also considered useful to treat abdominal cramps, fever, diarrhoea as well as syphilis and leprosy (Gilani et al., 2005).

MATERIALS AND METHODS

Plant material

Aerial parts of *F. capreolata* and *F. bastardi* were obtained from

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Table 1. Identification by gas chromatography-mass spectrometry (GC-MS) of isoquinoline alkaloids in *F. capreolata* and *F. bastardi*.

Alkaloid type	Alkaloid	Rt (min)	EI-MS m/z (%)	*F. capreolata*	*F. bastardi*
Protopine	Protopine	20.55	148(100)163(20)354(5)	+	+
	Protoberberine	18.96	148(100) 369(4)	+	
Terahydroprotoberine	Stylopine	19.65	148(100)322(30)323(40)	+	+
	Phtalidisoquinoline	23.7	190(100)148(52)320(80)	–	+
	Phtalidisoquinoline	24.9	190(100)148(25) 320(32)	–	+
Spyrobenzylisoquinoline	Fumariline	20.92	322(100)336(20)351(25)	+	+
	Fumarophycine	2.11	322(100)337(85)	+	+
	Fumaritine	22.29	192(100)340(20)	+	+
	Fumarofine	30.25	369(100)354(56)192('34)	+	+
	Fumaricine	32.2	206(100)355(13)	+	+
	Dihydrofumariline	31.2	190(100)148(25)363(20)	–	+
Dehydrobenzophenanthridine		8.65	330(100)331(5)142(85)	–	+
Phtalic acid ester		7.13	149(100)167(25)317(12)	+	–
Total alkaloids (mg/100 g dry weight)				426±23	521± 36

– =component absent; + = component present.
Rt = Retention time.

their natural habitats. All specimens were collected in April and June 2005 when they were at the flowering and fruit setting stages. Plants were collected from Bejaia City in the north east of Algeria and identified by the Botany Laboratory of Bejaia University. The identification was confirmed latter by Professor Max Henry of the University of Nancy 1, France. Specimens are deposited in the herbarium of Bejaia University.

Extraction of alkaloids

Extraction was done as described by Suau et al. (2002a). 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 h, and then evaporated to 0.5 ml in vacuum. The methanol 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. Dichloromethane, analytical reagent grade and all other solvents were from Sigma Chemical Co.

GC-MS analysis

GC-MS Analysis was performed on a Fisons Trio 1000 mass selective detector with electronic impact ionisation (70 eV). The column used was a HP-1 (15 m x 0.25 mm i.d. and 1 µm phase thickness). Helium was used as a carrier gas at 1.0 mL min^{-1}. The injection temperature was 280°C. The column temperature was held initially at 200°C for 8 min, increased to 250°C at 10°Cmin^{-1} and then held at 250°C for 30 minutes. 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/s.

RESULTS AND DISCUSSION

GC-MS analysis

The results of GC-MS analysis of the different alkaloid extracts are on Table 1 and shows GC-MS experimental data, retention time and main fragments (their respective intensities) for indole alkaloids of *F. capreolata* and *F. bastardi.* Individual alkaloids (Figure 1) were identified from their Art values and their MS by comparison of their MNR data with those in literature (Suau et al., 2002a; Suau et al., 2002b; Suau et al., 2005; Zocoler et al., 2005; Sanchez et al., 2005; Yu et al, 1971; Pereira et al., 1999; Essential data of mass spectra, 1983). Some components remained unidentified due to the lack of reference substances and Library spectra. However we can identify protopine and stylopine as main alkaloids in both *F. capreolata* and *F. bastardi.* The presence of peaks at 355 m/z (M^+, 10%), 340 (M^+-CH_3, 20%) and 192 (base peak) was consistent with the occurrence of fumaritine, the peak at 369 m/z (100%) seems to be fumarofine. Others spirobenzyliso-quinolines as fumaricine, fumarophycine and fumaritine were identified in the two species. In *F. capreolata*, the peak at 149 m/z (100%), 167 m/z (30%), 279 m/z (6%) corresponds to a phtalic acid ester and the peak at 330 m/z (100%) observed in *F. bastardi* extract seems to be a benzophenanthridine, dehydroderivative (331 molecular ion), probably dihydrosanguinarine witch was found in different species of *Fumaria* by Suau et al. (2002b). The other benzophenanthridine which can correspond to this peak is chelidonine which was detected in the genus *Sarcapnos* (Suau et al., 2005). Three other peaks appear

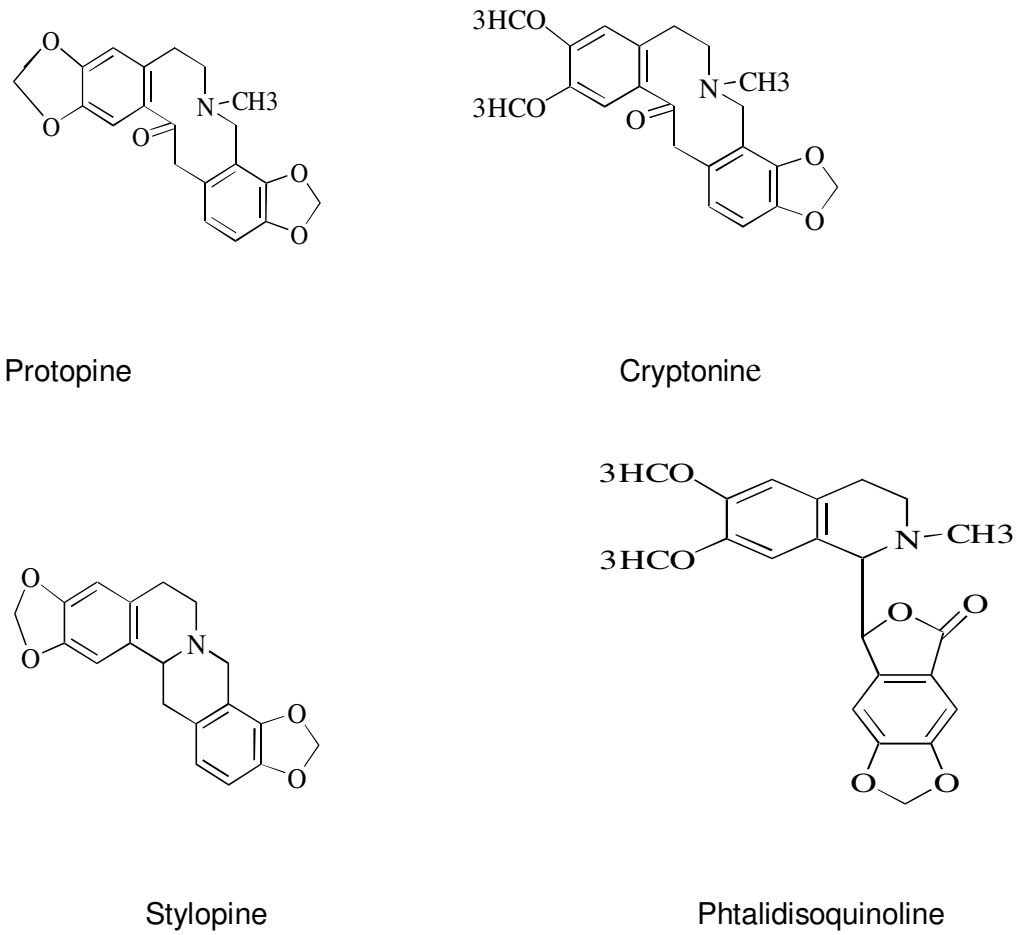


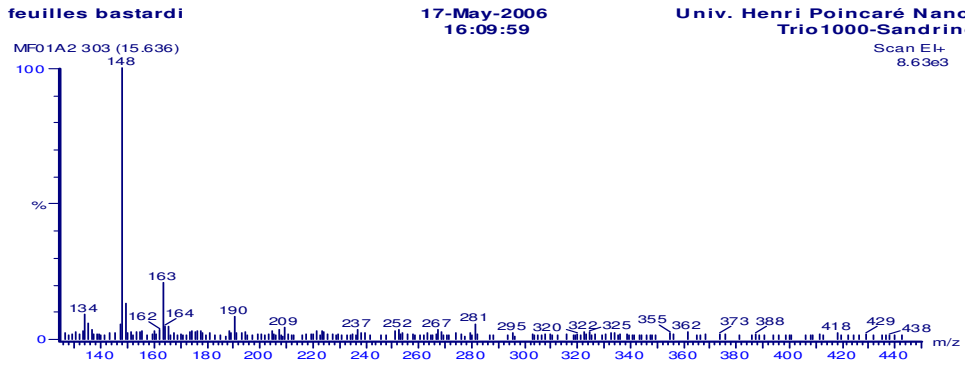
Figure 1. Isoquinoline alkaloids identified in *F. capreolata* and *F. bastardi*.

only in *F. bastardi*. Although these peaks could not be fully characterised, they were identified as phtalidisoquinoline alkaloids based on the presence of a prominent ion at 190 m/z as base peak (Blaskó et al., 1982); one of them can be identify as dihydrofumariline. However there are several unresolved peaks, perhaps components derived from aporphines (proeminent ion at m/z 340 and 335) (Pereira et al.1999).

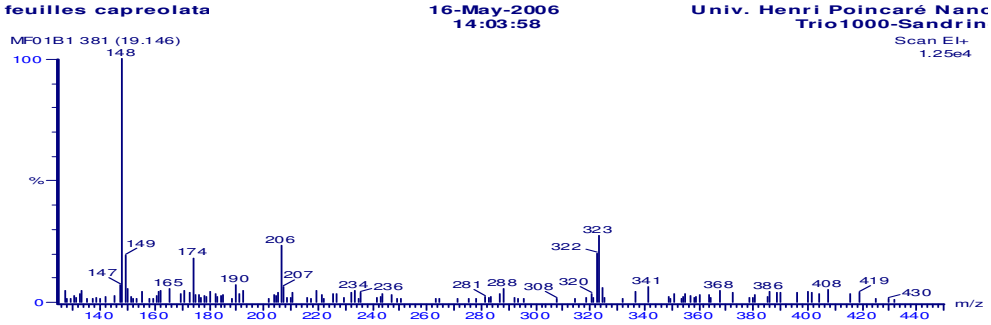
Algerian species of *Fumaria* seems to have highest concentrations of alkaloids than Spain species studied by Suau and his collaborators (Suau et al., 2002a). The total alkaloids (mg/100 g dry weight) of *F. bastardi and F. capreolata* are 521 and 426, respectively, in Algerian species while the amounts founded in the same species from Malaga are 425 and 412, respectively (Suau et al. 2002a). Moreover, phtalidisoquinoleine found in our *F. bastardi* extract was absent in *F. bastardi* of Malaga and the spirobenzylisoquinoline alkaloids were different. In the Algerian species we found fumarilline, fumaritine, fumarofine and dehydrofumaritine, while in *Fumaria* from Malaga, the alkaloids are parfumine, fumariline and fumarophycine in *F. capreolata* and parfumine, fumariline and parfumidine in *F. bastardi*. (Suau et al., 2002a). The traditional use of the two species of *Fumaria* in the North of Algeria in several gastrointestinal diseases and skin diseases can be explained by the fact that the main constituents of the crude extracts are indole alkaloids, a class of substances which a wide range of pharmacological activities, cholinesterase inhibitors, analgesic, anti-inflammatory, stimulant and depressant of the Central Nervous System (CNS) (Zocoler et al., 2005). Figure 2 shows the chromatographic profiles of the main alkaloids founded in the two Algerian species of *Fumaria.*

Chemotaxonomic significance

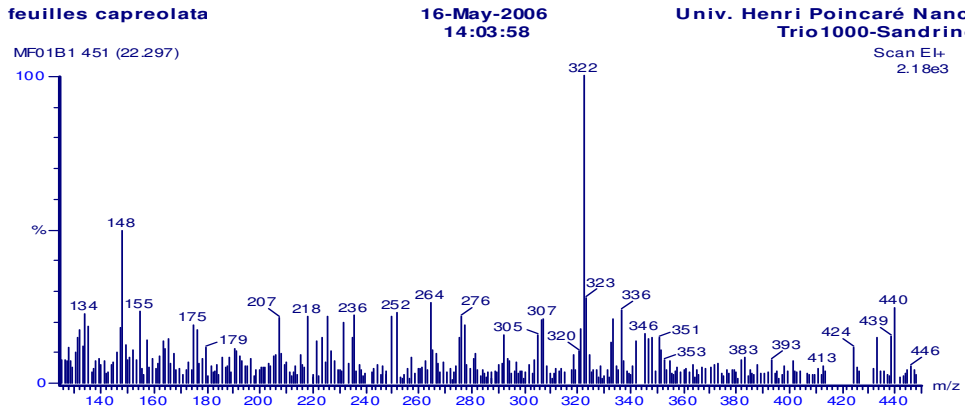
Most of the European Fumariacae can be associated with three chemotypes based on alkaloids contents. Chemotaxonomic investigations of several populations of different species of the genus *Fumaria* resulted in the definition of two different chemotypes based on the presence of either the protoberberine Stylopine (group I) or the protopine Cryptonine (group II) in the plant (Suau et al., 2002a). The group I species (*Fumaria agraria, F. bastardi, F. capreolata and Fumaria sepium*) included the tetrahydro-



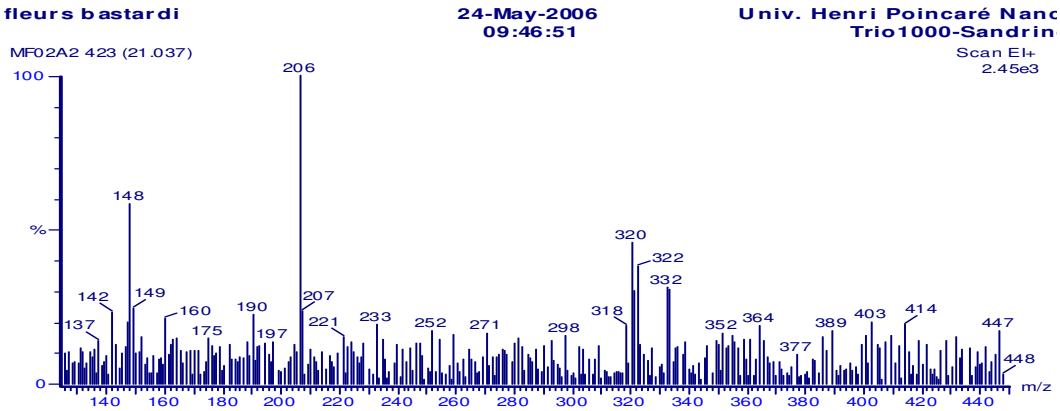
Spectra of Protopine



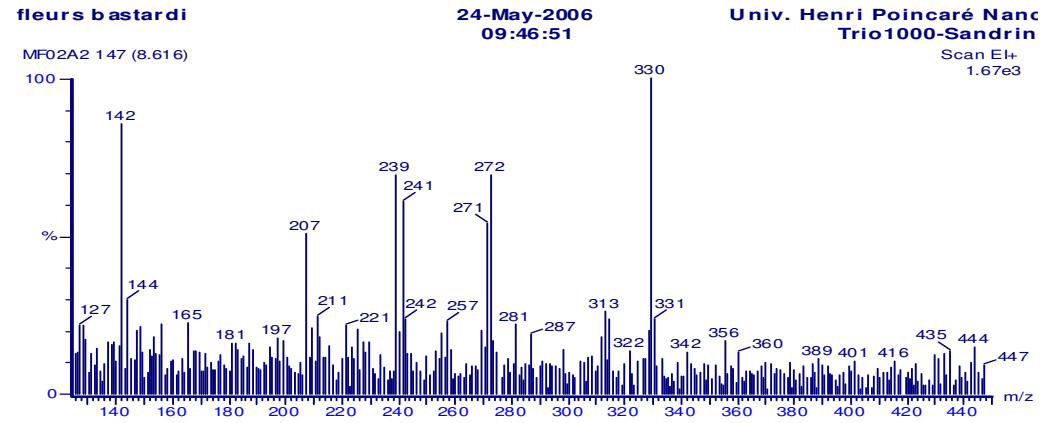
Spectra of Stylopine



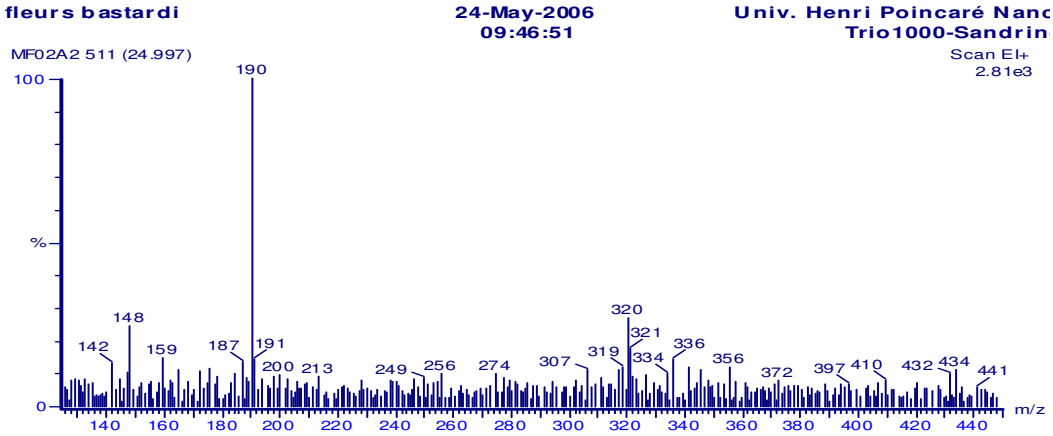
Spectra of fumariline



Spectra of fumaricine



Spectra of dehydrophenanthridine.



Spectra of phtalidisoquinoline

Figure 2. Gas chromatography-mass spectrometry (GC-MS) profiles of methanolic extracts of *F. capreolata* and *F. bastardi.*

protoberberine, stylopine, the biogenic precursor of protopine and cryptonine as main alkaloid (Suau et al., 2002b). We can confirm that our species are well identified and belong to the chemotaxonomic group I. Furthermore, Fumariaceae and Papaveraceae are closely related and the evaluation of some type of isoquinoline alkaloids allows the differentiation among these genera. For example *Fumaria* is rich in spirobenzylisoquinolines, while these components are absent from the other genus which contains aporphines and morphinanes (Suau et al., 1991, 2002b).

Conclusion

This study shows that the crude extracts of aerial parts of *F. capreolata* and *F. bastardi* contain isoquinoline alkaloids which explain their pharmacological properties. Gas Chromatography coupled to Mass spectrometry (GC-MS) is proven to be a valuable tool for the analysis of *Fumaria* indole alkaloids, but this method is useless for quaternary alkaloids with base peak lower than 125 m/z. However a successful method based on reversed phase HPLC allows the detection of quaternary alkaloids (Soušek et al, 1999; Gerasimenko et al., 2001) and exact quantification of *Fumaria officinalis* isoquinoline alkaloids was been determinate by nonaqueous capillary electrophoresis-electrospray (Sturn et al., 2005).

Soxhlet extraction combined with the GC-MS method is a direct and fast analytical approach for identification of the various tertiary bases present in alkaloid extracts and only a few grams of plant material are required.

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