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Gas chromatography-mass spectrometry (GC-MS) analysis of the crude alkaloid extract of *Ziziphus mauritiana* Lam., grown in Algerian

Elyacout Chebouat[1], Belkhir Dadamoussa[1], Azeddine Kabouche[2], Messaouda Allaoui[1], Messaoud Gouamid[1], Abdelkrim cheriti[3] and Noureddine Gherraf[2]*

[1]Kasdi Merbah University, Ouargla 30000, Algeria.
[2]Process Engineering Department, Larbi ben M'hidi University, Oum Elbouaghi 04000, Algeria.
[3]Phytochemistry and Organic Synthesis Laboratory, University of Bechar , Bechar 08000, Algeria.

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The identification of the crude alkaloid extract of the Algerian *Ziziphus mauritiana* Lam. was carried out using gas chromatography-mass spectrometry (GC-MS) analysis. The identified ten alkaloids seem to be reported for the first time in this plant species.

Key words: Rhamnaceae, alkaloids, gas chromatography-mass spectrometry (GC/MS), *Ziziphus mauritiana.*

INTRODUCTION

Jujube (*Ziziphus* spp.) belongs to the Rhamnaceae family and involves approximately 40 species. Jujube is overspread mainly in tropical and subtropical parts of the world (Mukhtar et al., 2004). *Ziziphus mauritiana* and *Ziziphus jujuba* are cultivated deliberately in many parts of the world for economic purpose. Jujube fruits are consumed fresh dried, and processed (jams, loaf, cakes, jelly, etc.) throughout the world (Pareek, 2002). The jujube fruits exhibit high sugar content and high levels of vitamins A, C and B complexes, in addition to phosphorus and calcium (Pareek, 2002). Fruits, leaves, seeds, roots and bark of jujube have been commonly used as a source of simple remedies in folk medicine (Belford, 1994). Flowers of Chinese jujube have been reported to contain high-quality nectar, and the leaves are consumed as tea (Zhao et al., 2008). Alkaloids, flavonoids, sterols, tannins, saponins, and fatty acids have been detected in many species of the genus *Ziziphus* (Croueour et al., 2002). Some phenolics, such as chlorogenic acid, caffeic acid, catechin, epicatechin and rutin, were isolated from jujube fruit (Croueour et al., 2002). Phenolics play a major physiological role in fruit, especially in resistance to various stress factors, and in fruit quality. In addition, phenolic compounds have significant levels of antioxidant activity and are free radical scavengers (Li et al., 2005). Moreover, the phytochemicals used in the treatment of diabetes are generally polysaccharides, including mucilages and glycans (Perez et al., 1998).

As far as *Z. mauritiana* is concerned, it was reported that the leaves can be used as healing, hemostatic, antiseptic, stimulant, tonic, anti-diarrhea (Malgras, 1992) and diabetes agents (Yansambou, 2002). Various activities of the plant have been confirmed (Fortin et al., 1997): antiscorbutic, anti-inflammatory, diuretic, emollient, hypotensive and sedative. Some anti-complementary polysaccharides (arabinans) were isolated from the leaves of *Z. mauritiana*(Yamada et al., 1985). Previous phytochemical studies reported that seven cyclopeptide alkaloids were isolated from root

*Corresponding author. E-mail: gherrafn@yahoo.com.

Table 1. Alkaloids of *Ziziphus mauritiana*.

Compound	Found in	[M+] (%)	m/z
4-Methoxyquinoline (1)	Flowers	159.07 (100.0)	159, 160, 161, 144, 129, 116, 89, 63, 28
N-ethyl-3-phenylbicyclo[2.2.1]heptan-2-amine(2)	Roots	215.17 (100.0)	215, 216, 217, 187, 172, 96, 92, 91, 43
(Z)-4-((3,4-dihydro-2H-pyran-2-ylidene)methyl)morpholine (3)	Roots	181.11 (100.0)	181, 182, 153, 113, 69, 56
2,3,5-Trimethylpyrazine (4)	Aerial parts	122.08 (100.0)	122, 123, 124, 81, 54, 39
2-Fluoro-3-(1-hydroxy-2-(methylamino)ethyl)phenol (5)	Flowers	185.09 (100.0)	167, 119, 44
3-Fluoro-4-(1-hydroxy-2-(isopropylamino)ethyl)benzene-1,2-diol(6)	Roots and flowers	229.11 (100.0)	215, 72, 32, 28
1,4-Diphenyl-1,2-dihydrophthalazine (7)	Fruits	284.13 (100.0)	284, 208, 207
Methyl 3-propionamidobenzoate (8)	Fruits	207.09 (100.0)	207, 208, 209, 148, 28
9-Amino-1-methyl-1H-purine-2,6(8H,9H)-dione (9)	Leaves	181.06 (100.0)	181, 182, 183, 153, 113, 69
(2-(4-Amino-2-methylphenyl)-7-methyl-1H-indol-3-yl)(4-chlorophenyl)methanone (10)	Flowers	374.12 (100.0)	374, 376, 181, 165

barks of Z. *mauritiana* (Yamada et al., 1985). Gas-chromatography/mass-spectrometry (GC/MS) proved to be a suitable method to investigate complex mixtures of different alkaloid groups (Kreh et al., 1995). In order to increase the volatility of the alkaloids and make them suitable for GC/MS investigation, the alkaloid mixtures can be silylated before analyses, but the spectra obtained gave limited information (Wink et al., 1983). Much more informative appeared to be the spectra of underivatized alkaloids. There are only a few reports on GC/MS of underivatized alkaloid mixtures from Rhamnaceae plants which showed that the alkaloids retain their characteristic lectronic impact mass spectrometry (EIMS) fragmentation pattern under GC/MS conditions (Wink et al., 1983).

In the present report, the alkaloid composition of *Ziziphus mauritiana* plant from South Algeria (Ouargla city) has been carried out using GC/MS analysis of the alkaloid fractions from leaves, flowers, fruits, stump, twigs and roots. To the best of our knowledge no studies have been reported dealing with alkaloid composition of *Ziziphus mauritiana* from Algeria Southern part.

MATERIALS AND METHODS

Plants

Samples of *Z. mauritiana* were collected in September, 2011 from Ouargla Algeria. A voucher specimen was kept at the Herbarium of VPRS Laboratory, University of Ouargla, under the code Number CE 7.

Isolation of the alkaloid fractions

Dry powder plant parts were dampened with a weak base (ammonia 25% NH_4OH) which leads to the liberation of alkaloids from their salts. Then, the samples were extracted with chloroform (3×). After filtration, the organic extracts containing alkaloids and impurities were concentrated in a rotary evaporator, acidified with 3% sulfuric acid to reach a pH of 1 to 2 and defatted with chloroform (3×). After that, the acidic aqueous phase was alkalized with 25% NH_4OH to pH = 9 and the alkaloids were extracted three times with chloroform. The chloroform extracts were combined, dried over anhydrous Na_2SO_4 and than evaporated. The residues obtained were dissolved in methanol and subjected to GC/MS analysis.

GC/MS analysis

The GC/MS were recorded on a Hewlett Packard 5890/MSD 5972A instrument operating in EI mode at 70 eV. A HP5 MS column (30 m × 0.25 mm × 0.25 µm) was used. The temperature program was 80 to 280°C at 10°C min^{-1} and held for 10 min at 280°C. The injector temperature was 280°C. The flow rate of carrier gas (He) was 0.8 ml min^{-1}. The identification of the alkaloids was confirmed by comparing the mass spectral data with those of authentic compounds and with data obtained from the literature.

RESULTS AND DISCUSSION

We investigated the alkaloid composition of leaves, flowers, fruits, stump, twigs and roots of *Z. mauritiana* so as to highlight the presence of biologically active alkaloids in different tissues and to confirm the traditional use of the plant in folk medicine. Ten compounds were identified and to the best of our knowledge they are reported for the first time in *Z. mauritiana* (Table 1 and Figure 1). Alkaloids are: (1) 4-methoxyquinoline, (2) N-ethyl-3-phenylbicyclo[2.2.1]heptan-2-amine, (3) (Z)-4-((3,4-dihydro-2H-pyran-2 ylidene)methyl)morpholine, (4) 2,3,5-trimethylpyrazine, (5) 2-fluoro-3-(1-hydroxy-

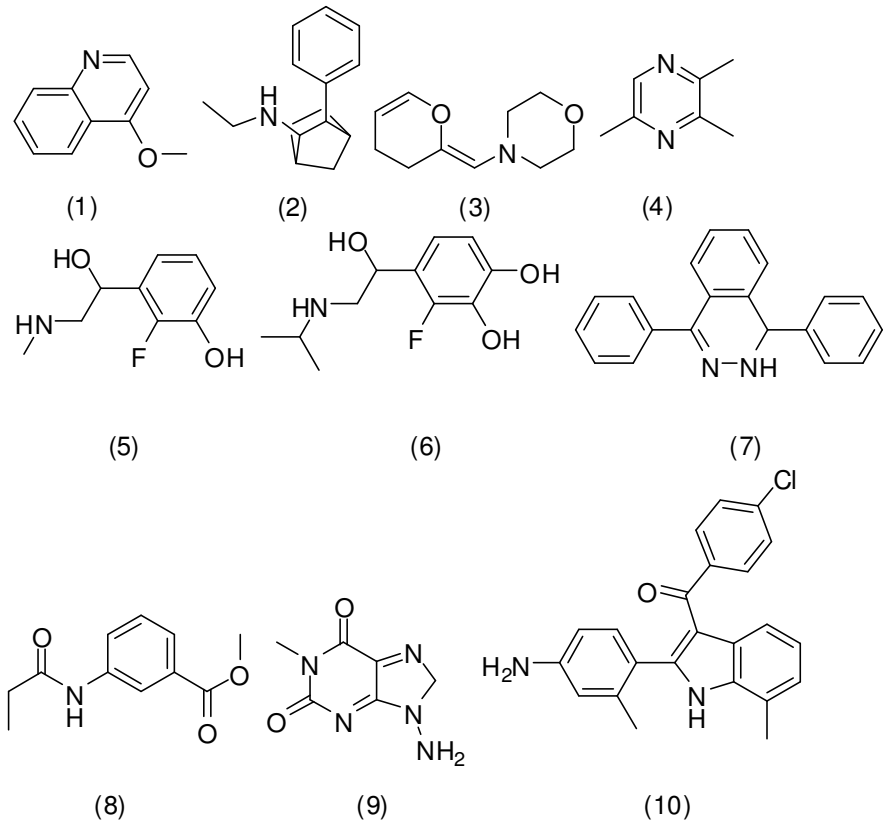

Figure 1. Structures of alkaloids identified in *Ziziphus mauritiana*. Alkaloids were generally present in trace amounts in the alkaloid mixtures extracted from plants and it seems that the most effective method for their identification is GC/MS rather than the ordinary separation. In the study at hand, the crude extract of alkaloids has undergone GC/MS analysis to yield ten alkaloids whose characteristic mass spectral fragmentations are listed in Table I.

2(methylamino)ethyl)phenol, (6) 3-fluoro-4-(1-hydroxy-2-(isopropylamino)ethyl)benzene-1,2diol, (7) 1,4-diphenyl-1,2-dihydrophthalazine, (8) methyl,3-propionamidobenzoate, (9) 9-amino-1-methyl-1H-purine-2,6(8H,9H)-dione, (10) (2-(4-amino-2-methylphenyl)-7-methyl-1H-indol-3-yl)(4-chlorophenyl)methanone.

Conclusion

Taking into account the complexity of the alkaloid fractions is the method of choice for a rapid analysis which requires minimum of plant material and allows the identification of numerous compounds, some of them of pharmacological interest. GC/MS analysis of *Z. mauritiana* alkaloids afforded ten alkaloids which confirm the use of the plant in healing many ailments and incite further investigations seeking natural bioactive products.

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