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Alkaloids of *Chronanthus orientalis* (Lois.) Heywood and Frodin (Fabaceae): An Endemic Species of Turkey

MERVE GÜNER, ISIN YAZICI*, FATIH KARABEY, SERDAR SENOL and HATICE DEMIRAY

Department of Biology, Faculty of Science, University of Ege, Section of Botany, Bornova 35100, Izmir, Turkey

*Corresponding author: Fax: +90 23 23881036; Tel: +90 23 23111798; E-mail: yaziciisin@hotmail.com

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Gas chromatography coupled with mass spectrometry has been used to analyze the alkaloids present in the aerial parts of *C. orientalis*. Sparteine, lidocaine, oxosparteine, lupanine alkaloids were the major compounds. From the identified four alkaloids, one lidocaine was found for the first time in this endemic plant. Almost all alkaloids belonged to the quinolizidine type.

Key Words: *Chronanthus orientalis*, Alkaloid, Cytisus, Quinolizidine.

INTRODUCTION

Turkey has about 10,750 vascular plant taxon and come to the fore in this aspect of geography. Floristic richness of this nearly 3,500 is endemic. This number forms 32 % of the total flora. Families contain more taxa of the flora of Turkey are: Compositae (1,215 taxa) and Leguminosae (Fabaceae) (1,071 taxa) and they constitute approximately one-fifth of our country's flora. In flora of Turkey, Leguminosae family are included in second place with around 400 endemic taxa. This study will form the basic material of the *Chronanthus orientalis* (Lois.) Heywood & Frodin includes also Leguminosae family. Genus *Chronanthus* contains only 2 species in the world. One of them, *Chronanthus biflorus* (Desf.) Koch, has the expansion in east-south Spain, Balarik Islands and the northwest Africa. Other species of in the single locality (Izmir-Bozdag) known to grow[1] and takes place in the category of vulnerable according to Turkey Red Book[2] is *Chronanthus orientalis* (Lois.) Heywood and Frodin. The name of *Chronanthus orientalis* is given to the plant as a result of new data provided to with the recent morphological and molecular systematic studies on the genus *Cytisus* by considering the nuclear and plastid DNA characters in mind[3]. According to the new nomenclature *Cytisus oritentalis* Desf.[4,1] is synonym of our plant nomenclatured as *Chronanthus orientalis* (lois). Heywood and Frodin. As a result of studies made so far on the phytochemistry of genus *Cytisus*: quinolizidine alkaloids (QA), (spartein, Lupa), fenetilaminler, isaflavonlar (genistein[5]), flavonoids, volatile oil, caffeic acid, *p*-coumaric acids, tannin and pigments were found. When used as dry and fresh, they have cardiotonic, cathartic, antioxidant, diuretic, emetic, purgative effects[6,7]. Quinolizidine alkaloids show a wide range of biological activities: they can inhibit the multiplication of viruses[8], the proliferation of bacteria[9-11] and the growth of certain fungi[9,12]. Some allelopathic (phytotoxic) effects of quinolizidine alkaloids have been described, including the inhibition of the growth of competing plants[13-15]. They can also deter a number of herbivores (nematodes, caterpillars, beetles, aphids, locusts, snails, rabbits and cows) but also pollinators such as bees[16]. Some are directly toxic or mutagenic[17,18]. Deterrent or toxic effects of quinolizidine alkaloids such as sparteine, lupanine, cytisine and 13-tigloyloxylupanine against phytophagous insects have been evaluated in some detail on some Lepidoptera. Several authors suggest that acetylcholine receptors and Na^+/K^+ channels are modulated by these compounds[19-22]. Tri- and tetracyclic quinolizidine (lupine) alkaloids have been used in folk medicine of eastern Asia and are nowadays of medical interest because of their oxytoxic and antiarrhythmic (sparteine, lupanine), hypoglycemic (lupanine), hallucinogenic (cytisine, N-methylcytisine), teratogenic (anagyrine) and inhibitory efects of natural killer cell growth[23,8]. Many publications have appeared in the last few years reporting the use of sparteine as a very efficient chiral diamine, demonstrating promising potential for asymmetric transformations of organometallic reagents to achieve enantioselective deprotonation, polymerization and carbonyl addition reactions[24-26]. The characteristics of quinolizidine alkaloids is known to be allelochemicals which are toxic to a variety of herbivores[27-32]. Whereas alkaloid profiles are usually rather constant within a species, some variation is found in the patterns of different

organs such as leaves as compared to seeds[33-36,21,30]. Quantitatively alkaloid levels vary diurnally and during the growth cycle[37]. A previously reported postulate concerning the evolution of quinolizidine alkaloids and the detailed consideration of the chemical composition led to a revised dendrogram showing proposed phylogenetic relations within the subfamily Papilionoideae in general and the tribe Genisteae in particular[38]. In the genera *Genista* and *Cytisus* (both commonly called broom) as well as *Laburnum*, quinolizidine alkaloids, including cytisine and sparteine, are common[39]. The hepatotoxic pyrrolizidine alkaloids are found in this family (*e.g.*, in membres of the genus *Crotalaria*)[40]. Due to the presence of endemic plant phytochemical investigation of new compounds are likely to provide contributions to the literature.

EXPERIMENTAL

Chronanthus orientalis were collected from its natural habitat, Ödemis (Bozdag) in May 2009, by Serdar G. Senol (Department of Biology, Facuty of Science, Ege University, Izmir, Turkey). Voucher specimens have been deposited at the herbarium of the department of Botany, Faculty of Science, Ege University, Izmir, Turkey.

Extraction: The aboveground plant organs of plant has been turn to drog material by drying in the air and shade. Dried and powdered material (150 g) of *C. oriantalis* were extracted for 4 days with ethanol (7 L) in cold perculator by stirring at room temperature. The solvent was removed by rotary evaporation at 40 ºC, yielding 14,96 g of extract.

Total alkaloid isolation: The residue was resolved with 1 % HCl (200 mL.) in a water bath of 60 ºC and filitrated. Obtained filitrate was seperated with petroleum ether (200 mL) to two phases. 25 % NH_3 (25 mL) was added on the lower acidic phase. pH was regulated to 10-11 with 13 mL NH_3. Alkaloids were collected with 900 mL of chloroform by extraction in a separation funnel. Chloroform extract was dried in rotovapor to afford (260,28 mg) and than passed through the anhydrous Na_2SO_4.

Open column chromatography: Total alkaloid extract was subjected to open column chromatography (Si gel 60 (Kieselgel 60, 70-230 mesh, Merck), 120 g, 2 cm × 60 cm) as stationary phase and employing $CHCl_3$/MeOH (95:5, 870 mL) gradient mixtures (90:10, 570 mL; 80:20, each 570 mL) and $CHCl_3$/MeOH/H_2O (70:30:3, each 300 mL) for elution to afford 170 fractions totally. These fractions were chromatographed on TLC (chromatography tank:Camag 22 cm × 23 cm × 8 cm Adsorban: Silica gel: Kiesegel 60 F_{254}, 0.2 mm, Merck, Art. 5554) with the solvent of EtOAc-MeOH-H_2O (100:20:15). Fractions were combined as A:82-98 (53,7 mg), B: 30-80 (69,3 mg), C:81-170 (40,3 mg), D: 20-28 (37,3 mg), E: 6-18 (25,6 mg). Total alkaloid extract were chromatographed with (20:75:15) benzene-chloroform-diethylamine solvent and six different bands were observed. All of them were isoquinoline alkaloids (literature). All of the fractions were examined by GC-MS in AUBIBAM (Anadolu University Plant Drug Scientific Investigation Center).

GC-MS analysis: Alkaloids were investigated by GC MS on Shimadzu GCMS QP 5050A linked to system, equipped with a HP-1 fused silica capillary column (25 m, 0.32 mm i.d., 0.17 µm film thicknes % dimethylpolysiloxane). The temperature was programmed from 80 °C min, 10 ºC/min to 220 ºC 1 min, 5 ºC/min and 250 ºC 10 min (80- 250 ºC at a rate of 5 ºC min^{-1} with a 10 min hold). Helium was used as a carrier gas with a constant flow at 1.2 mL min^{-1}. The ionization voltage was 70 eV. Evaluations were made according to Wiley GC/MS Library. Extract were analyzed after dissolved in (9:1) $CHCl_3$:MeOH solvent. Injection temperature was 250 ºC, interface temperature was 280 ºC and ionization mode was EI, mass range was 40-500 m/z.

Identification of compounds: The alkaloid identification was performed by comparisons of retention time and a mass spectra with autentic samples. When such samples were not available tentative structures were proposed on the basis of the mass spectral fragmentation.

RESULTS AND DISCUSSION

The underivatized alkaloid mixture extracted from herb of *Chronanthus orientalis* Heywood and Frodin was investigated by capillary GC/MS for the first time. Among the known Fabaceae alkaloids of four structure types were identified. We identidied 11 alkaloids (one of them tentatively) (Table-1) (Fig. 1). Some components remained unidentified due to the lack of reference substances and library spectra. The main alkaloids were sparteine (61.43 %) (Fig. 2), lidocaine (5.32 %) (Fig. 3), oxosparteine (4.65 %) (Fig. 4), lupanine (0.72 %) (Fig. 5). Spartein found (61.43 %) (Fig. 2) in *C. orientalis* comprised the major alkaloid of plant. The second major one was lidocaine. Here we can take attention to the lidocaine as being the new compound special to this species in the fabaceae family. By the way the alkaloids which are derived from ornithine and lysine are named "quinolizidine alkaloids"[41]. They are characteristic secondary metabolites of the family of Fabaceae (Leguminosae) and are especially abundant in the tribes genisteae, sophoreae and thermopsideae[42]. The genus *Cytisus* is known to produce this type of alkaloids[39].

TABLE-1
GC-MS ANALYSIS OF *C. orientalis* ALKALOIDS

Retention time	Relative (%)	Compare with Wiley GC/MS library*	Wiley GC/MS library (%) comparison*
10.773	61.43	Sparteine	91
11.002	7.70	–	–
11.227	1.54	–	–
11.357	5.54	–	–
11.559	1.88	–	–
11.842	5.32	Lidocaine	96
12.845	1.26	–	–
14.039	4.65	Oxosparteine	88
14.875	0.72	Lupanine	90
17.959	2.83	–	–
18.351	1.33	*n*-Octylphtalate	94

*Compounds with high comparison in Library scanning are identified.

We subjected to GC-MS analysis the underivatized alkaloid mixture, encouraged by the excellent results of Erdemoglu *et al.*[43]. These authors applied for the first time GC-MS to underivatized fabaceae alkaloids (from *G. vuralii* L. growing in Turkey) and demonstrated its advantages over the analysis of silylated samples, especially in identifying minor components.

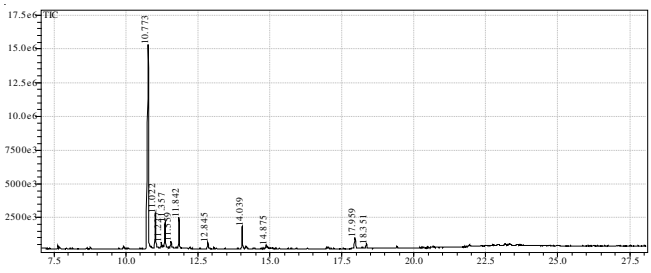

Fig. 1. GC/MS chromatogrammme of *C. orientalis* extract

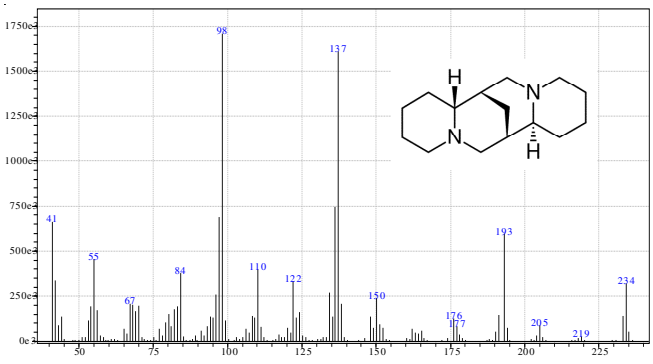

Fig. 2. Mass spectrum of spartaine (Rt: 10.773)

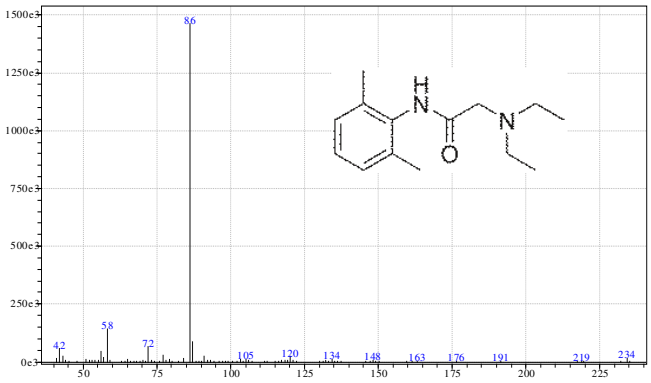

Fig. 3. Mass spectrum of lidocaine (Rt: 11.842)

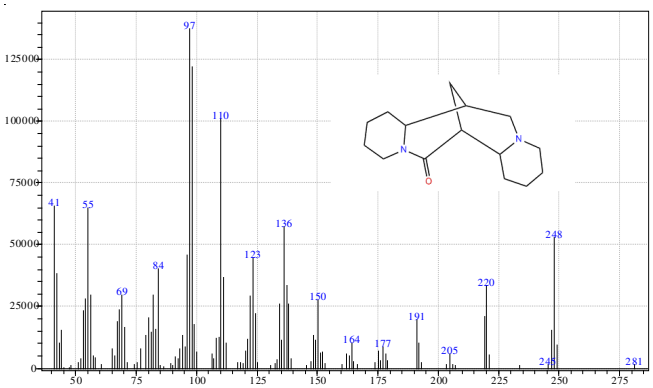

Fig. 4. Mass spectrum of oxospartaine (Rt: 14.039)

Ten quinolizidine alkaloids were identified by capillary GC-MS, namely, N-methylcytisine, cytisine, tetrahydrorhombifoline, 17-oxosparteine, 5,6-dehydrolupanine, lupanine, 17-oxolupanine, anagyrine, baptifoline and 13α-tigloyloxylupanine from *Genista vuralii*. 5 alkaloids (anagyrine, cytisine, N-formylcytisine, N-methylcytisine and lupanine) have been isolated and identified in aerial parts of *Genista tenera*[44]. In

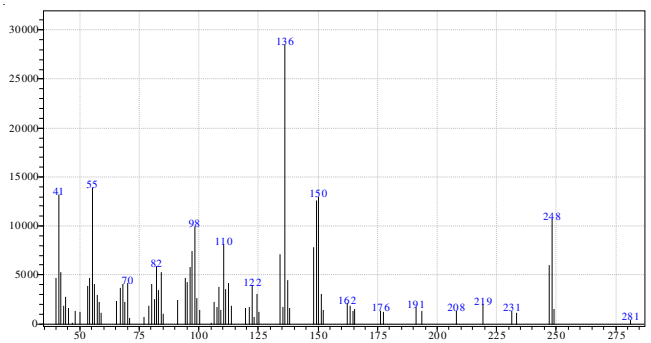

Fig. 5. Mass spectrum of lupanine (Rt: 14.875)

our samples, we found only two of them: lupanine and oxosparteine. According to Martinez-Herrera *et al.*[45]; more than 25 alkaloids were detected from *L. flavoculatus*, *L. kingii*, *L. odoratus*, *L. pusillus* and *L. shockleyi* and sparteine, β-isosparteine, isolupanine, 5,6-dehydrolupanine, lupanine and anagyrine were found while lupanine and sparteine were detected as major alkaloids in *C. orientalis*. Ghania[46] have determined the alkaloid profile of the plant *Cytisus purgans* growing in Algeria by GC-MS and improved the presence of lupinine, camoensidine, lupanine, sparteine, multifloine, aphylline, angustifoline, isolupanin, anagyrine, martine, ammodendrine, retamine, alkaloids. 44 quinolizine alkaloids were isolated from Egypt spreaded various species of *Lupinus* and *lupanine* and spartein was also identified in *C. orientalis*[47,48].

Quinolizidine alkaloids are the systematic markers of Papilionaceae subfamily and *Genisteae tribus* and this phylogenetic association was made by drawing the dendrograms of two plants[49]. Alkaloid pattern of *Cytisophyllum sessilifolium* (Fabaceae-Genisteae) was revealed both the quinolizidin and adenocarpin alkaloids as being the important chemotaxonomic character for this species[50]. Alkaloid patterns of *Genista cinerea* was demonstrated chemical dichotomy for the species of the section Spartioides: one group of species contained the α-pyrydone alkaloids cytisine. Spartioides: one group of species contained the α-pyridone alkaloids cytisine, N-methylcytisine and anagyrine as major alkaloids, while the other group contained lupanine, 13-hydroxylupanine and its esters as main compounds[51]. The quinolizidin type alkaloids as common in *Chronanthus orientalis* as: 17-oxospartein and lupanin contained *Genista vuralii* demonstrated an antibacteraial effect against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans*, *Candida krusei* antifungal effect[43].

When we examined all above mentioned studies by different investigators included the structure of 170 quinolizidine alkaloids of *Orbanche rapumgenistea*[52], did not coincide any results of lidocaine alkaloid except found as a major alkaloid used as topical anaesthesia[53] in *Chronanthus orientalis* endemic species.

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