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Essential Oil and Quinolizidine Alkaloids of *Retama Raetam* (Forssk) Webb & Berthel (Fabaceae) Grown in Jordan

Suleiman Olimat[1]*, Hasan Y Muti[2], Tamam El Elimat[1] and Yara Al Tall[3]

[1]Faculty of Pharmacy, Department of Medicinal Chemistry and Pharmacognosy, Jordan University of Science and Technology, Jordan

[2]Faculty of Pharmacy and Medical Sciences, Al-Ahliyya Amman University, Jordan

[3]Faculty of Pharmacy, Department of Pharmaceutical Technology, Jordan University of Science and Technology, Jordan

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***Corresponding author:** Suleiman Olimat, Faculty of Pharmacy, Department of Medicinal Chemistry and Pharmacognosy, Jordan University of Science and Technology, Jordan, Email: smalkhalil@just.edu.jo

Abstract

Retama raetam essential oils (EOs) and quinolizidine alkaloids (QA) composition were studied. A quantitative analysis using a GC-MS analysis of the flowers revealed the presence of monoterpenes (47.62%), monoterpene hydrocarbons (28.6%) and oxygenated monoterpenes (14.3%) and sesquiterpenes (47.6%), exclusively sesquiterpenes hydrocarbon. The principal oil component was 49% mesitylene. The young twigs of *R. reatam* revealed the presence of quinolizidine alkaloids (QA). The alkaloids identified were 5,6-dehydrolupanine, β-isolupanine, 17-oxolupanine, anagyrine, β-isospartine, and spartine; 17-oxolupanine was the major alkaloid in the twigs.

Keywords: *Retama; Raetam*; Flowers; Essential Oil; Twigs; Alkaloids

Introduction

Fabaceae is a widely distributed family of flowering plants with 730 genera and 19,400 species. The Retama genus belongs to the family Fabaceae [1]. Retama raetam (Forssk.) Webb & Berthel. (Fabaceae) is a perennial and unarmed shrub with evergreen cladodes (photosynthetic stems). It is many-branched with simple and deciduous leaves which fall rapidly after emergence [2]. R. raetam, commonly known as 'raetam,' 'broom bush,' or 'white broom,' is a xerophytic shrub native to several countries of North Africa (e.g., Algeria, Egypt, Libya, Morocco, and Tunisia), temperate Asia (e.g., Jordan, Lebanon, Palestine, and Syria) and south-eastern Europe (e.g., Sicily in Italy) [1-3]. Analysis of the essential oil of R. raetam grown in Libya revealed the presence of an essential oil, which showed significant activity against microorganisms, especially Staphylococcus aureus [4]. Meanwhile, the analysis of R. raetam grown in Tunisia revealed that the essential oil of the flower possesses antibacterial, antifungal, and antioxidant capacity [5].

Oxygenated flavonoids and isoflavonoids isolated from *R. raetam* were responsible for the antidiabetic effect of the plant [5,6] *R. raetam* showed several biological activities, including antibacterial, anti-inflammatory, analgesic, antioxidant, anti-proliferative, anti-viral, and hepatoprotective activities [7,8].

Quinolizidine alkaloids (QA) are very common secondary metabolites of the Fabaceae family and are very abundant in *Retama* spp. [9]. A wide range of biological activities is attributed to pure (QA) alkaloids or a mixture of them [10].

Experimental

Plant Material

The flowers and young twigs of *R. raetam* were collected mid of April 2019, from Berin area (15 km northeast of Amman). The taxonomic identities of the collected plant material was confirmed by the assistance of

a plant taxonomist (Dr. Mohammad Gharaibeh, Faculty of Agriculture, Jordan University of Science and Technology) and by the comparison of a collected voucher specimen with those of known identity in the herbarium of the Faculty of Agriculture, Jordan University of Science and Technology. A voucher specimen (ID No.: Phar 9-35) of the collected plant was deposited in the research laboratory of the Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology.

Oil Distillation

The flowers of the collected plant material of *R. raetam* were air dried and ground to about 0.5 mm particle size (30-35 mesh). The essential oils were obtained by subjecting 420 g of the ground materials to hydrodistillation using the Clevenger-type apparatus (JSGW, India) for 4 h. The obtained oils (n = 2) were dried over anhydrous sodium sulfate, Na_2SO_4 (Analar, England), and stored in dry dark glass bottles at 4°C for later analysis.

Analysis of the Essential Oils

A quantitative analysis using a gas chromatography with a flame ionization detector (GC-FID) was conducted using a Hewlett Packard HP-8590 gas chromatograph equipped with a split-splitless injector (split ratio 1:50) and an FID detector. The column (OPTIMA5 (5 % diphenyl 95 % dimethyl polysiloxane)) was a fused silica capillary column (30 m x 0.25 mm; 0.25 µm film thickness). The oils were separated under a linear temperature program set at 3°C/min heating rate from 60-250°C and then held at 250°C for 5 min. The temperature of the injector and detector were maintained at 250°C and 300°C, respectively. The relative peak area for each component of the oil was measured. The concentrations of the oil components were calculated as a percentage content using their relative peak areas assuming a unity response by all components. Each sample was analyzed twice.

GC-MS Analysis

A GC-MS analysis was performed on a Varian chrompack CP-3800 GC/MS/MS-200 equipped with a split-splitless injector and DB-5 GC column (5% diphenyl 95% dimethyl polysiloxane, 30 m x 0.25 mm ID, 0.25 µm film thickness). The injector temperature was set at 250°C with a split ratio of 1:10. Detector and transfer-line temperatures were 160°C and 230°C, respectively. A linear temperature program was used to separate the different oil components. Temperature programming was applied at 3°C/min heating rate starting from 60°C to 250°C and then held at 250°C for 5 min. The mass detector was set to scan ions between 40-400 m/z using full scan mode and electron impact (EI, 70 eV). Each sample was analyzed twice. A hydrocarbon mixture of n-alkanes (C_8-C_{20}) was applied separately on a GC-MS using the same chromatographic conditions. The Linear retention index (arithmetic Kovat's index) was calculated for each component separated by GC-MS using the values of its retention time and the retention times of the reference n-alkanes applying the Van Den Dool equation [9,11-13].

The identification of oil components was performed by matching their spectra with the data bank mass spectra (Wiley, Nist, and Adams 2007 libraries), and also by comparing their calculated arithmetic indices with the reported values in the literature [9-13].

Extraction of Alkaloids

The plant material of dried young twigs of *R. raetam* (50 g) was homogenized by an Ultra-turrax in 300 Ml (CH_2Cl_2: MeOH: NH4OH (25%), 15:5:1) and left to stand for at least 2 h. The extracts were concentrated under reduced pressure in a Rota Vapor and taken up in 20 mL of 0.5 N HCl. Basification of the aqueous acid solution with NH_4OH was followed by the extraction of a CH_2Cl_2 yield (8 mg) of alkaloid residue.

Analysis of Alkaloids

The alkaloid residue was dissolved in CH_2Cl_2 and injected into a GC-MS apparatus. Experimental conditions for the capillary GC-MS analysis were done under the following conditions.; capillary column HP-5 (cross-linked 5% phenyl methylsiloxane, 50 m x 0.32 mm (i.d.), with 0.17 µm film thickness, detector temperature 280 °C, injector temperature 250 °C, carrier gas He (1 mL/min.), split ratio 1/20, injection volume 0.2 µl, and mass range (*m/z*) 20-440. The GC oven temperature was kept at 120°C for 2 min, programmed to 300 °C at a rate of 6 °C /min, and kept constant at 300 °C for 10 min.

The identification of oil components was performed by matching their spectra with the data bank mass spectra (Wiley, Nist and Adams 20077 libraries) and also by comparing their calculated arithmetic indices with reported values in literature [8-10,12-17].

Results and Discussion

The simultaneous use of mass spectral and retention (Kovat's) index matching allowed for an unequivocal identification of more than 99% of the components of the collected oil, obtained from the aerial parts of the plant under study, as determined by GC and GC-MS. The oil yield (expressed as % v/w of dried material) was 0.35%. The analyses permitted the identification of 21 compounds in the oils of *R. raetam*. The identified components and their corresponding contents are presented in Table 1.

The oil was characterized by equal percentage levels of monoterpenes and sesquiterpenes. Monoterpenes (47.62 %), monoterpene hydrocarbons (28.6 %) and oxygenated monoterpenes (14.3%) and sesquiterpenes (47.6%), exclusively sesquiterpenes hydrocarbon.

Butylfuran (1), carene (2), mesitylene (3), *p*-cymene (4), *trans*-beta-ocimen (5), *cis*-beta-ocimen (6), dehydrolinalol (7), *trans*-verto citral (8), *cis*-rose oxide (9), allocimene (10), isogeijerene (11), silphirol-5-ene (12), presilphiperfol-7-ene (13), siliphinene (14), silphiperfol-5-ene (15), cyclosativene (16), longicyclene (17), sativene (18), beta-caryophyllene (19), alpha-guaine (20) and aristolochene (21).

The principal oil component was 49% mesitylene (3), 22.5% *trans*-β-ocimene (5), 8.9%, *trans*-verto citral (8) 3.3%, dehydrolinalol (7) 3.2%, longicyclene (17) 2.3% presilphiperfol-7-ene (13), and 2.1% *cis*-rose oxide (9), (as shown in bold in Table 1).

No	RI exp	RI lit	Content %	Compound
1	891	885	1.5	2-Butylfuran
2	995	995	0.3	Carene
3	993	995	49	Mesitylene
4	1020	1024	0.75	p- Cymene
5	1037	1037	22.5	trans-β-Ocimene
6	1050	1050	0.73	Cis-β-ocimene
7	1090	1090	3.3	Dehhydrolinalol
8	1104	1106	8.9	trans-Reto citral
9	1111	1108	2.1	cis-Rose oxide
10	1130	1132	0.7	Allocimene
11	1147	1149	0.5	Isogeijerene
12	1323	1328	0.3	Silphirol-5-ene
13	1334	1336	2.3	Presilphiperfol-7-ene
14	1341	1346	0.76	Siliphinene
15	1346	1348	0.33	Silphiperfol-5-ene
16	1371	1371	0.3	Cyclosativene
17	1374	1374	3.2	Longicyclene
18	1391	1394	0.32	Sativene
19	1421	1419	1	β-Caryophyllene
20	1440	1439	0.38	alpha-Guaine
21	1485	1488	0.67	Aristolochene
			47.62	Monoterpenoids
			28.6	Monoterpenoids Hydrocarbons (MHC)
			14.3	Oxygenated Monoterpenoids (OM)
			47.6	Sesquiterpene Hydrocarbons (SHC)
			4.76	Furano derivatives

Table1: Chemical composition the essential oil hydro-distilled from the flowers parts of Jordanian *R. raetam*.
RI exp: Linear (arithmetic) retention index calculated on DB-5 equivalent column.
RI lit: reference retention index value from literature
*Average% content of 4 determinations (2 oil samples, 2 replicates each), for which the standard deviation (SD) values were within 2% (+2%) of the mean
Compounds in bold are the major components (≥1.0%)

Analysis of the essential oil of *R. raetam* grown in Libya revealed the presence of β-linalool (51%), 2-decen-1-ol (6.6%) and limonene (7.4%) as the major components [4]. Meanwhile, the analysis of the essential oil of *R. raetam* grown in Tunisia revealed the presence of a total of 50 components representing 98.58% of the oil. The major components were nonanal (35.75%), alpha-humulene (29.29%), acetaldehyde (7.84%), linalool (5,62%), myrcene (3.38%), tridecanal (2.21%), beta-caryophyllene (1.79%), alpha-terpinyl acetate (1.46%), terpinolene (1.26%) and methyl anthranilate (1.06%) [5].

The variations in the concentration and of the components of the essential oils of *R. raetam* in Jordan, Libya and Tunisia and this might be due to the time of the flowers are collected in the vegetative, flowering, or fruiting stage [1,15].

R. raetam grown in Tunisia, in the vegetative stage, composed of 24 components with the major constituents of this essential oil being 2-methoxy-4-vinylphenol (23.89%), eugenol (14.45%), and linalool (11.12%). In the flowering stage, 31 compounds were identified, including linalool (32.68%) and heptanal (8.35%), as the main components, while there were 23 constituents identified in the essential oil obtained from the fresh fruiting stage with the main compounds represented by 1-octen-3-ol (33.11%), linalool (9.73%), phenyl ethyl alcohol (9.29%), and *cis*-linalool oxide (8.67%) [15].

The relative contents of the % alkaloids were determined via the areas under the peaks from the total ion chromatography. The structure of the alkaloids was identified based on a comparison of their Kovats index and mass spectral fragmentation (shown in Table 2). This comparison revealed and confirmed the presence of quinolizidine alkaloids previously reported by other authors [9, 13-18]. The alkaloids identified were 5,6-dehydrolupanine (1), alpha-isolupanine (2), 17-oxolupanine (3), anagyrine (4), β- isospartine (5), and spartine (6). 17-Oxolupanine (3) was the major alkaloid in the twigs. Sparteine, β-isospateine, α-isolupanine, and anagyrine were previously detected in *R. raetam* [9, 18, 19].

No.	RI (Exp.) a	RI (Lit.) b	% Content c	Compound	Ref
1	2132	2128	14.9	5,6-Dehydrolupanine	14
2	2111	2105	5.5	α-Isolupanine	9, 17
3	2055	2070	34.7	17-Oxolupanine	10, 13
4	2473	2390	24	Anagyrine	10, 13
5	1824	1830	11.5	β-Isospartine	10, 14
6	1776	1785	3.7	Spartine	10, 14

Table2: Chemical composition the alkaloids from the young twigs parts of Jordanian *R. raetam.*
[a]RI (Exp.): Linear (arithmetic) retention index calculated on DB-5 equivalent column
[b]RI (Lit.): reference retention index value from literature
[c]Average % content of 4 determinations (2 oil samples, 2 replicates each), for which the standard deviation (SD) values were within 2% (+2%) of the mean

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