Asian Journal of Chemistry; Vol. 24, No. 5 (2012), 1927-1930

ASIAN JOURNAL OF CHEMISTRY

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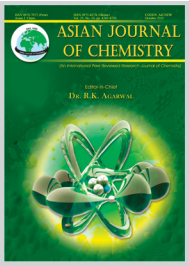

Composition and Antimicrobial Activity of Essential Oil from the Flower of *Rhododendron luteum* Sweet

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(*Received*: 5 March 2011; *Accepted*: 5 December 2011) AJC-10794

Essential oil from air-dried flower of *Rhododendron luteum* Sweet (Ericaceae), was obtained by hydrodistillation in a Clevenger-type apparatus and analyzed by GC-MS and GC-FID. Sixty-four components were identified in the oil. The most abundant components in the investigated essential oil from the flower of *R. luteum* was found to be α-cadinol (8.9 %), δ-cadinene (7.6 %), α-terpineol (7.2 %), benzyl salicylate (6.2 %), α-muurolene (4.1 %) and 1,6-germacradien-5β-ol (3.4 %). The antimicrobial activity of the isolated essential oil was investigated and it showed moderate antimicrobial activity against *Serratia marcescens*, *Enterococcus faecalis* and *Staphylococcus aureus*, but no antifungal activity was observed against yeast like fungi.

Key Words: *Rhododendron luteum*, Ericaceae, Essential oil, Antimicrobial activity, GC-MS.

INTRODUCTION

Turkey is divided into three major flora regions. These are Euro-Siberian, Irano-Turanian and Mediterranean. City of Rize stands at the Colchis part of Euro-Siberian flora region. It has been determined that *R. luteum* Sweet, *R. ponticum* L., *R. smirnovii* Trautv., *R. caucasicum* Pall. and *R. ungerni* Trautv. plants are also in the plant group of Colchis by the researches[1]. There are more than 600 *Rhododendron* species around the world. More than 400 of them are in Asia. The smaller percentage grows in high, cool and rainy regions of Europe, North America and Australia. *Rhododendron*, commonly known as rosage or by the folk names black poison or komar, are members of Ericaceae family. There are five *Rhododendron* species growing naturally in Turkey and especially in East Black sea region, namely *R. luteum*, *R. ponticum*, *R. smirnovii*, *R. caucasicum* and *R. ungerni*[1]. They are deciduous short trees with green leaves and have flowers of different colours and an aesthetically important role in landscape. Although popularly known to be toxic among public, previous studies on the pharmacological activities of *Rhododendron* species indicated that they contain potent antioxidative compounds[2,3]. The sap obtained from fresh branches of *R. ponticum* is dropped into tooth cavity against toothache in Turkish folk medicine[3,4]. The flowers of another *Rhododendron* species have also been recorded in ancient and modern monographs as analgesic and insecticides in Chinese traditional medicine[5].

Previous phytochemical studies on the *R. luteum* have shown the presence of many different natural compounds such as saponins[6], iridoid, monotropein[7], flavonoids[8-12], phenolic compounds[9,10,13,14], triterpenoids[15,16], ursolic acid[17] and fatty acids[18].

In literature survey, essential oils from *Azalea pontica* (*R. luteum*) and their toxicitiy were studied and reported solubility, optical rotation, acid, saponin and ester numbers and the yields of the essential oils obtained from fresh flowers, dried flowers and leaves of *A. pontica*[19]. Toxicity of essential oils from *A. pontica* was also mentioned and reported bacteriostatic activity, especially to *Bacillus anthracis*[19]. The antibacerial and antifungal activities of the crude extracts (leaves and flowers) of *R. ponticum* subsp. *ponticum*, *R. luteum*, *R. smirnovii* and *R. caucasicum* (Ericaceae) were investigated[20]. A food poisoning encountered in Turkey was traced to toxic honey made by bees from *Rhododendron* species[21]. However, literature search revealed no GC-MS research for the composition and antimicrobial activity studies on the essential oil from the flower of *R. luteum*. As part of this systematic research, the essential oil constituents of the plant were extracted by hydrodistillation in a Clevenger-type apparatus. The obtained crude essential oil was then investigated by GC-MS and GC-FID techniques[22-28]. The objective of this study was to identify and quantify the constituents of the essential oil from the flower of *R. luteum* and to investigate its biological activities[29,30].

EXPERIMENTAL

Rhododendron luteum flower were collected in Rize-Çamlihemsin (at height of *ca.* 1800 m) in the northern part of Turkey in August 2010. Voucher No: KATO 13371 (Herbarium of Karadeniz Technical University, Faculty of Forestry, Department of Forest Botany). The collected plant was identified immediately after collection[1] and the flowers were air-dried at room temperature for later analysis.

Isolation of the essential oil: The air-dried flowers (75 g) of *R. luteum* were hydrodistilled in a Clevenger-type apparatus using ice bath for cooling system (3 h). The oils were taken by HPLC grade *n*-hexane (0.5 mL) and kept at 4 ºC in the sealed brown vial. Then 1 µL of the extract was directly injected into GC-MS. The percentage yield of the oil was calculated on a moisture free basis (0.26 ± 0.1, v/w).

Gas chromatography and gas chromatography-mass spectrometry analysis: GC-FID and GC-MS analyses were done as described previously[23,24].

Identification of constituents: The components of the oil was characterized by comparison of their mass spectra with those of a computer library or with authentic compounds (linalool, borneol, α-terpineol, geraniol, tetradecane, hexadecane, heptadecane, octadecane, nonadecane, docosane, tricosane, tetracosane, pentacosane, hexacosane, heptacosane and nonacosane) and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature[22-28] (Table-1).

TABLE-1
IDENTIFIED COMPONENTS IN THE OIL OF *R. luteum*

Compounds	Area[a] (%)	Exp. RI[b]	Lit. RI
1,3-Dimethylbenzene	0.1	865	867
1-Octen-3-ol	0.3	979	979
6-Methyl-5-hepten-2-ol	0.2	990	992
trans-Linalool oxide	0.2	1074	1073
cis-Linalool oxide	0.2	1085	1087
Benzoic acid methyl ester	0.2	1091	1091
Linalool[c]	1.1	1096	1095
Nonanal	0.5	1100	1101
Borneol[c]	0.3	1169	1169
1-Nonanol	0.2	1170	1169
α-Terpineol[c]	7.2	1187	1189
Myrtenol	1.4	1194	1196
Decanal	0.2	1202	1202
β-Cyclocitral	0.2	1222	1224
Geraniol[c]	0.2	1253	1253
Isobornylacetate	0.2	1287	1286
2E,4E-Decadienal	0.1	1317	1317
Eugenol	0.1	1360	1359
α-Ylangene	0.1	1378	1375
β-Elemene	0.5	1393	1391
Tetradecane[c]	0.2	1400	1400
Methyl eugenol	0.5	1405	1404
Longifolene	0.3	1407	1408
α-Gurjunene	0.2	1411	1410
(E)-Caryophyllene	2.5	1420	1419
(E)-α-Ionone	0.3	1429	1430
Aromadendrene	0.4	1441	1441
Calarene	0.7	1442	1444
γ-Muurolene	0.7	1480	1480
(E)-β-Ionone	1.2	1488	1489
α-Muurolene	4.1	1501	1500
δ-Amorphene	2.0	1516	1512
γ-Cadinene	0.9	1514	1514
δ-Cadinene	7.6	1526	1523
trans-Cadina-1(2),4-diene	0.5	1534	1535
α-Calacorene	0.2	1546	1546
Elemol	1.9	1552	1550
Germacrene B	1.1	1560	1561
Ledol	0.6	1568	1569
1,6-Germacradien-5β -ol	3.4	1579	1579
Caryophyllene oxide	2.0	1585	1583
Viridiflorol	1.2	1595	1593
Hexadecane[c]	0.4	1600	1600
Tetradecanal	1.1	1613	1613
Unknown	1.9	1616	MS
Isoaromadendrene epoxide	1.4	1617	1616
γ-Eudesmol	2.6	1634	1632
Tau-muurolol	6.7	1645	1642
α-Cadinol	8.9	1656	1654
Heptadecane[c]	0.5	1700	1700
14-Hydroxy-α-muurolene	1.3	1780	1780
Octadecane[c]	0.2	1800	1800
Hexahydro farnesylacetone	1.1	1847	1847
Benzyl salycilate	6.2	1868	1866
Nonadecane[c]	0.3	1900	1900
Farnesyl acetone	0.8	1920	1919
Heneicosane[c]	0.3	2100	2100
(Z,Z)-9,12-Octadecadienoic acid	0.1	2147	2150
Docosane[c]	0.1	2200	2200
Tricosane[c]	1.0	2300	2300
Tetracosane[c]	0.2	2400	2400
Pentacosane[c]	1.4	2500	2500
Hexacosane[c]	0.1	2600	2600
Heptacosane[c]	0.6	2700	2700
Nonacosane[c]	0.2	2900	2900

	Area[a] (%)	Number of compounds
Monoterpenoids	10.8	8
Sesquiterpene hydrocarbons	21.1	14
Sesquiterepenoids	28.7	9
Terpen related compounds	4.3	6
Others	15.3	26
Unknown	1.9	1
Total isolate	82.1	64

[a]Percentages obtained by FID peak-area normalization. [b]RI calculated from retention times relative to that of *n*-alkanes (C_6-C_{32}) on the non-polar HP-5 column. [c]Identified by authentic samples. Unknown (RI: 1616): EIMS, 70 eV, m/z (%): 222(5), 207(18), 189(12), 179(6), 161(19), 137(22), 119(56), 109(30), 93(32), 79(32), 69(100), 55(35).

Antimicrobial activity assessment: All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Yersinia pseudotuberculosis* ATCC 911, *Serratia marcescens* ATCC 13880, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 60193, *Candida tropicalis* ATCC 13803. Essential oil was weighed and dissolved in acetone to prepare extract stock solution of 1000 µg/mL.

Agar well diffusion method: Simple susceptibility screening test using agar-well diffusion method[29] as adapted earlier[30] was used. Each microorganism was suspended in Brain

TABLE-2
SCREENING RESULT FOR ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *Rhododendron luteum*

Extract	Stock (µg/mL)	Microorganisms and inhibition zone (mm)								
		Ec	*Yp*	*Kp*	*Sm*	*Ef*	*Sa*	*Bs*	*Ca*	*Ct*
R. luteum	1000	-	-	-	+	+	+	-	-	-
Ceftazidime	10	+++	+++	+++	+++	+++	+++	+++		
Triflucan	5								+++	+++

Results were interpreted in terms of the diameter of the inhibition zone: (-): < 5.5 mm; (+): 5.5-10 mm; (++): 11-15 mm; (+++): ≤ 16 mm. *Ec: E. coli, Pa: Yp: Y. pseudotuberculosis, Kp: K. pneumoniae, Sm: S. marcescens, Ef: E. faecalis, Sa: S. aureus, Bs: B. subtilis, Ca: C. albicans, Ct: C. tropicalis*

Heart Infusion (Difco, Detroit, MI) broth and diluted approximately 10^6 colony forming unit (cfu) per mL. They were flood-inoculated onto the surface of Brain Heart Infusion agar and Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) and then dried. For *C. albicans*, *C. tropicalis*, Sabouraud Dextrose Agar were used. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer and 100 µL of the extract substances were delivered into the wells. The plates were incubated for 18 h at 35 ºC. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ceftazidime (Fortum) (10 µg) and Triflucan (5 µg) were standard drugs. Acetone was used as solved control. The tests were carried out in duplicate. Results were interpreted in terms of the diameter of the inhibition zone: (-): < 5.5 mm; (+): 5.5-10 mm; (++): 11-15 mm; (+++): ≤ 16 mm (Table-2).

RESULTS AND DISCUSSION

The essential oil was obtained by hydrodistillation in a Clevenger-type apparatus from the flowers of *R. luteum*. The hydrodistillation of the flowers of *R. luteum* gave pale yellow oils with the yield of 0.26 ± 0.1 (v/w) on dry weight basis. The general chemical profiles of the essential oil, the percentage content and retention indices are summarized in Table-1. Essential oil from the flowers of *R. luteum* analyzed by GC-MS from HP-5 column and 76 components were identified on the basis of a typical library search match exceeding 80 %, which represented about 82.1 % of the total detected constituents[22-28]. α-Cadinol (8.9 %), δ-cadinene (7.6 %), α-terpineol (7.2%), benzyl salycilate (6.2 %), α-muurolene (4.1 %) and 1,6-germacradien-5β-ol (3.4 %) were found as the major compounds in the essential oil from the flower of *R. luteum*. Unidentified component was present in very low amount (1.9 %) and there were no matches in the libraries (NIST, WILLEY) used. The chemical class distribution of the essential oil from the flower of *R. luteum* components were separated into four classes, which were monoterpenoids, sesquiterpenes, sesquiterpenoids and others (Table-1). Monoterpenoids constituted 10.8 % and the major compound of monoterpenoids was α-terpineol (7.2 %), the ratio of sesquiterpenes was 21.1 % and the main component of sesquiterpenes was δ-cadinene (7.6 %) and sesquiterpenoids constituted 28.7% and the major representative of sesquiterpenoids was α-cadinol (8.9 %). The ratio of the other compounds was 15.3 % in the essential oil from the flower of *R. luteum*. The results of the terpene analyses showed that sesquiterpenoids are the main constituents (28.7 %) for the essential oil from the flower of *R. luteum*.

Some species and hybrids of plants were noted to be valuable sources of essential oils, from which sesquiterpenes, aldehydes, phenols, carbohydrates and lipids and such individual compounds as carvacrol, citronellol, engenol, geraniol, coumarin, linalool, citral, nerol, safrole, linalyl acetate, terpineol, lavandulol, patchoulene, isomenthol, borneol, methyl anthranilate, benzoic acid, citronellic acid and camphor were isolated[31]. In our case we observed similar result with different ratios which could be due to geographical origins and the climates.

The antimicrobial activity for the essential oil from the flower of *R. luteum* was tested in vitro using the method of diffusion on disc with the microorganisms as seen in Table-2 and showed antimicrobial activity against *S. marcescens, E. faecalis* and *S. aureus* but, no activity against *E. coli*, *Y. pseudotuberculosis, K. pneumoniae, B. subtilis, C. albicans* and *C. tropicalis*. The antibacterial and antifungal activities of the crude extracts (leaves and flowers) of *R. ponticum subsp. ponticum*, *R. luteum, R. smirnovii* and *R. caucasicum* were more active then essential oils of the plant, because of more polar components like the grayanotoxin[19-21].

ACKNOWLEDGEMENTS

The authors thank Prof. Dr. Salih Terzioglu for characterization of plant material. This work was supported by grants from Karadeniz Technical University Research Fund and State Planning Agency (DPT) of Turkey.

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