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# Chemical composition of *Rhododendron aureum* (gold rosebay) essential oil from Pribaikal'e (Russian Federation)

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*Abstract*: The essential oils from five samples of leaves of *Rhododendron aureum* from the Irkutsk region, Pribaikal'e, Russian Federation, were isolated by hydrodistillation and analyzed by a combination of GC and GC/MS. Compounds representing 70.5–78.3 % of the oils were identified. Twenty-seven compounds were identified according to their chromatographic retention indices and mass spectra. The major components of the oils were calarene (10.4––66.4 %), $\beta$-bourbonene (0.5–27.4 %), $\alpha$-selinene (2.1–8.0 %) and kaur-16-ene (2.0–6.3 %). It was found that the chemical composition of *Rh. aureum* essential oil depends on the altitude of the growing plants.

*Keywords*: *Rhododendron aureum*; gold rosebay; essential oil; calarene.

## INTRODUCTION

*Rhododendron* is a genus from the Ericaceae family which includes about eight hundred species of evergreen, half-deciduous and deciduous shrubs and trees. Rhododendrons are distributed mainly in the temperate zone of the northern hemisphere, with the greatest diversity of species occurring in southern China, the Himalayas, Japan, Southeast Asia and North America.[1] A resent feasibility study revealed several opportunities for the commercialization of *Rhododendron* as mulch, biofuel, charcoal, foliage, a source of phytochemicals and wood for turnery.[2]

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The leaves of *Rhododendron* species contain essential oils, the volatile compounds of which have been widely studied by different scientists. The following presents information about the dominant components and their contents in the essential oils from some *Rhododendron* species: *Rh. adamsii* – *trans*-nerolidol (18.2––29.5 %) and *β*-farnesene (6.9–17.1 %); *Rh. anthopogon* – *α*-pinene (37.4 %) and *β*-pinene (16.0 %); *Rh. aureum* – hexanoic acid (10.0 %) and carvacrol (7.8 %); *Rh. calophytum* – 3,7,11,15-tetramethyl-2-hexadecen-1-ol (5.68 %) and 1,3,5-trimethylbenzene (5.53 %); *Rh. dauricum* – *β*-caryophyllene (28.3 %) and *α*-humulene (15.0 %); *Rh. ledebouri* – *β*-myrcene (13.3 %) and bornyl acetate (11.8 %); *Rh. sichotense* – *α*-pinene (44.3 %) and *β*-pinene (13.1 %); *Rh. sutchuenense* –– caryophyllene (12.5 %) and guaiol (8.8 %); *Rh. simsii* – phytol (15.2 %) and 3,7-dimethyl-1,6-octadien-3-ol (12.6 %); *Rh. mucronulatum* – *α*-humulene (28.8 %) and *β*-caryophyllene (14.6 %); *Rh. naamkwanense* – [*Z*,*Z*,*Z*]-9,12,15-octadecatrienoic acid (45.3 %) and phytol (8.6 %).[3–8] Tasdemis *et al*. analyzed the volatile components of hexane- and $CH_2Cl_2$-extracts from five *Rhododendron* species growing in Turkey.[9] They found that the dominate compounds in the hexane-extract of *Rh. ponticum* leaves were 5,15-rosadiene (42.8 %) and 2-ethylhexanol (13.3 %) and in the $CH_2Cl_2$-extract, 1-butanol (17.0 %) and *γ*-butyrolactone (13.5 %); in the hexane-extract of *Rh. luteum* leaves, ethyl acetate (13.3 %) and 6-methyl-5-heptene-2-one (11.1 %) were dominant and in the $CH_2Cl_2$--extract, 1-butanol (58.7 %) and benzyl alcohol (17.1 %); in the hexane-extract from *Rh.*×*sochadzeae* leaves, 6-methyl-5-heptene-2-one (11.9 %) and calarene (7.1 %) were dominant while, in the $CH_2Cl_2$-extract, phenethyl alcohol (100 %); in the hexane-extract of *Rh. ungernii* leaves, 6-methyl-5-heptene-2-one (29.4 %) and 2-ethylhexanol (24.4 %) dominated while in the $CH_2Cl_2$-extract, 2-(2--ethoxyethoxy)ethanol (12.8 %) and phenethyl alcohol (11.5 %); in the hexane--extract of *Rh. smirnovii* leaves, 6-methyl-5-heptene-2-one (21.7 %) and viridiflorol (15.4 %) were dominant. The total content of identified compounds was 75.3–100 % in the hexane-extracts and 60.1–100 % in the $CH_2Cl_2$-extracts.

Gold rosebay, kashkara (*Rhododendron aureum* Georgi) is a small evergreen shrub of the Ericaceae family with a dark-brown bark, stems procumbent usually crooked and lifted at 20–100 cm from the ground branches; the blossom period is May–June and fruiting in July–August. It grows on stony slopes and rocks in the mountainous regions of eastern Siberia and the Far East, reaching the Western Sayan and the Abakan Range, mainly in the alpine zone.[10] The harvesting season of rhododendron is in August–September. In Russia, mass harvesting occurs in the Pribaikal'e (Irkutsk region) and Zabaikal'e (Buryatia and Chita region).

In Tibetan medicine, *Rh. aureum* is used under the names *shu-mkhan* or *da-li* for the treatment of rheumatism and for cardiovascular and diuretic means.[11,12] Experimentally, it was found that *Rh. aureum* drugs have pronounced effects on the heart in patients with cardiovascular insufficiency, reducing shortness of breath,

palpitations, edema and venous pressure, and increasing the speed of blood flow and diuresis. *Rh. aureum* extracts have bactericidal effects against pathogenic microflora, especially streptococcus and staphylococcus, due to the presence of phenols and essential oil.[13]

Chemical studies on *Rh. aureum* found the presence of: diterpenes (andromedotoxin), triterpenes (campanulin, simmiarol, uvaol, oleanolic acid), simple phenols (rhododendrol, rhododendrin, hydroquinone, arbutine, 1-*O*-*β*-glucopyranosyl-5-methoxy-3-hydroxy-benzene, 5-methoxy-1,3-dihydroxybenzene) and flavonoids (quercetin, hyperin, avicularin, polystachoside).[14–17] Earlier research on the essential oil from Zabaikal'e *Rh. aureum* samples has already been realized[6] but not the study of the essential oil from Pribaikal'e samples.

This paper reports the results of an analysis of the essential oil of *Rh. aureum* growing in the Irkutsk region of Pribaikal'e.

## EXPERIMENTAL

### *Plant material*

The plant materials of *Rh. aureum* leaves were collected from different locations on Pribaikal'e (Irkutsk region, Russia), in August 2008 (Table I). The samples were gathered after the flowering period. A voucher specimen is kept at the Siberian Institute of Plants Physiology and Biochemistry, Siberian Division, Russian Academy of Sciences.

TABLE I. Characteristics of the *Rh. aureum* samples

| Sample No. | Locality | Altitude, m a.s.l. | Collected date | Essential oil content / % |
|---|---|---|---|---|
| RA-1 | Village Rechka Vydrinnaia | 460 | 23 VIII 2008 | 0.25 |
| RA-2 | Village Sliudianka | 1172 | 16 VIII 2008 | 0.20 |
| RA-3 | Village Sliudianka | 1350 | 16 VIII 2008 | 0.15 |
| RA-4 | Village Sliudianka | 1402 | 16 VIII 2008 | 0.14 |
| RA-5 | Village Sliudianka | 1452 | 16 VIII 2008 | 0.09 |

### *Isolation of the essential oil*

Fresh leaves (200 g) were subjected to hydrodistillation in a Clevenger-type apparatus for 150 min, which gave the essential oil. The amounts of essential oil extracted from the different samples are given in Table I.

### *Gas chromatography*

The GC analysis was performed on an Agilent 6890N series gas chromatograph fitted with a HP-5MS fused capillary column (30 m×0.25 mm, film thickness 0.50 μm, 5 % diphenyl- and 95 % dimethylpolysiloxane stationary phase). The oil solutions (0.20 μl) in hexane (≈ 1 %) were injected in the split mode (1:20). The carrier gas was helium at a flow of 1.0 ml $min^{-1}$. The column temperature was programmed from 150–250 °C at 2.0 °C $min^{-1}$; the injector temperature was 250 °C and the detector (FID) temperature was 300 °C.

### *Gas-chromatography/mass spectroscopy*

The GC/MS analysis was performed on the same gas chromatograph but instead of the FID detector, it was coupled to an Agilent Technologies 5973 N mass selective/quadrupole

detector. The same column and chromatographic conditions as above were employed. The ion source temperature was 230 °C. The EIMS spectra (70 eV) were obtained in the scan mode in the *m*/*z* range 41–450.

*Component identification and quantification*

Identification of compounds was realized by comparison of the peak retention times with those of analytical standards of available terpenoids and by comparison of the mass spectra with those found in the literature,[18] the mass spectrometry data bank (NIST 05) and a computer search of the Wiley library. For quantification purposes, the relative percent peak areas registered using the FID were used.

## RESULTS AND DISCUSSION

The chemical compositions of the essential oils of *Rh. aureum* leaves are presented in Table II. In the samples of essential oils, forty components were detected, of which twenty-seven were identified. The identified components accounted for 70.5–78.3 % of the total oils. The content of hydrocarbons was 60.5–78.2 % and of oxygenated terpenes 0–11.6 %; the dominant constituents were sesquiterpene compounds.

TABLE II. Chemical compositions (area, %) of the essential oils from *Rh. aureum* leaves from Pribaical'e

| No. | Compound | RA-1 | RA-2 | RA-3 | RA-4 | RA-5 |
|---|---|---|---|---|---|---|
| 1 | *cyclo*-Sativene | – | – | – | 0.1 | 0.8 |
| 2 | *α*-Ylangene | 0.6 | 0.4 | 0.5 | 0.7 | 6.0 |
| 3 | *α*-Copaene | 0.4 | 0.1 | 2.0 | 1.2 | 10.3 |
| 4 | *α*-Bourbonene | 0.7 | – | – | – | – |
| 5 | *β*-Bourbonene | 4.1 | 0.5 | 12.9 | 7.8 | 27.4 |
| 6 | Aristolene | 0.5 | 1.3 | 0.6 | 0.9 | – |
| 7 | n.i.[a] ($C_{15}H_{24}$) | 2.1 | – | 3.3 | – | – |
| 8 | Calarene | 34.4 | 66.4 | 26.2 | 41.3 | 10.4 |
| 9 | *cis*-Calamene | 0.6 | – | – | – | 0.2 |
| 10 | n.i ($C_{15}H_{24}$) | – | 8.2 | – | 3.4 | – |
| 11 | *trans*-*α*-Bergamotene | 0.1 | – | – | – | 0.6 |
| 12 | n.i. ($C_{15}H_{24}$) | 2.0 | – | – | – | – |
| 13 | *α*-Humulene | 0.3 | – | – | – | 0.3 |
| 14 | Aromadendrene | 11.1 | – | 0.5 | 7.1 | 0.8 |
| 15 | *α*-Amorphene | 0.2 | 0.3 | | | 0.4 |
| 16 | Germacrene D | 0.1 | | 1.6 | 0.5 | 1.7 |
| 17 | *β*-Selinene | 1.6 | 1.0 | 1.6 | 1.5 | 0.3 |
| 18 | *α*-Selinene | 8.0 | 3.5 | 3.8 | 4.9 | 2.1 |
| 19 | Isoledene | 0.3 | – | – | – | – |
| 20 | n.i. ($C_{15}H_{24}$) | 2.4 | – | – | – | – |
| 21 | *α*-Muurolene | – | – | – | 0.4 | – |
| 22 | *γ*-Cadinene | 1.1 | 0.9 | 2.8 | 0.9 | 1.9 |
| 23 | *δ*-Cadinene | 0.1 | 0.5 | 1.1 | 0.3 | 1.2 |
| 24 | *β*-Cadinene | 1.2 | 1.2 | 0.7 | 1.2 | 2.9 |
| 25 | *α*-Cadinene | – | – | – | 0.2 | 0.4 |

TABLE II. Continued

| No. | Compound | RA-1 | RA-2 | RA-3 | RA-4 | RA-5 |
|---|---|---|---|---|---|---|
| 26 | $\alpha$-Calacorene | 0.4 | 0.2 | – | 0.3 | 0.4 |
| 27 | n.i. ($C_{15}H_{26}O$) | 2.7 | – | – | 0.4 | – |
| 28 | Spathulenol | – | – | 11.6 | – | – |
| 29 | Caryophyllene oxide | 0.3 | – | – | 0.3 | – |
| 30 | n.i. ($C_{15}H_{26}O$) | 1.9 | – | – | 3.0 | – |
| 31 | n.i. ($C_{15}H_{24}O$) | – | 2.2 | – | – | – |
| 32 | $\tau$-Muurolol | — | – | – | 0.5 | – |
| 33 | $\alpha$-Cadinol | – | – | – | 0.2 | – |
| 34 | n.i. ($C_{20}H_{24}$) | – | – | – | 0.9 | – |
| 35 | n.i. ($C_{15}H_{24}O$) | – | – | – | 14.1 | – |
| 36 | n.i. ($C_{15}H_{26}O$) | 2.7 | 3.1 | – | – | – |
| 37 | n.i. ($C_{15}H_{24}O$) | 4.8 | 3.0 | 11.7 | 1.3 | 16.7 |
| 38 | n.i. ($C_{20}H_{32}O$) | 8.9 | 5.2 | 12.8 | 3.8 | 11.7 |
| 39 | n.i. ($C_{20}H_{32}O$) | 2.0 | – | – | – | – |
| 40 | Kaur-16-ene | 4.4 | 2.0 | 6.3 | 2.8 | 3.5 |
| Total identified compd. | | 70.5 | 78.3 | 72.2 | 73.1 | 71.6 |
| Total unidentified compd. | | 29.5 | 21.7 | 27.8 | 26.9 | 28.4 |

[a]Not identified

The essential oils from the different samples of *Rh. aureum* leaves were characterized by a high level of variability. In the essential oil of sample RA-1, the major compounds were calarene (34.4 %), aromadendrene (11.1 %), $\alpha$-selinene (8.0 %), kaur-16-ene (4.4 %) and $\beta$-bourbonene (4.1 %); in sample RA-2, they were calarene (66.4 %), $\alpha$-selinene (3.5 %) and kaur-16-ene (2.0 %); in sample RA-3, they were calarene (26.2 %), $\beta$-bourbonene (12.9 %), spathulenol (11.6 %) and kaur-16-ene (6.3 %); in sample RA-4, they were calarene (41.3 %), $\beta$-bourbonene (7.8 %), aromadendrene (7.1 %) and $\alpha$-selinene (4.9 %); in sample RA-5, they were $\beta$-bourbonene (27.4 %), calarene (10.3 %), $\alpha$-copaene (10.3 %) and ylangene (6.0 %).

A comparative study of altitude and the composition of the essential oils revealed an important feature, *i.e.*, with changing altitude the terpene synthesis also changed. In samples RA-1–RA-4, the aristolane derivative calarene was dominant but in sample RA-5, the prevailing compound was $\beta$-bourbonene, which represents a group of bourbonane derivatives. The content of essential oil was inversely related to the value of the altitude, *i.e.*, with increasing altitude, the oil content decreased from 0.25 to 0.09 % (Table I). There was also a change in the appearance of the essential oils. The essential oils from samples RA-1–RA-4 were solid, poorly-colored substances with a weak odor, while the oil from sample RA-5 was a green liquid with a strong specific smell. The reason for this is likely to be the change in environmental conditions and the occurrence of stress factors affecting the plants on a cellular level, which led to profound changes in the terpene biogenesis of the plants.

Previously, a study of *Rh. aureum* essential oil was conducted on specimens growing in the Tunkin Valley (Zabaikal'e, Buryatia, Russian Federation). It was found that in October samples of *Rh. aureum*, the essential oil was dominated by the compounds calarene (5.6 %), *α*-terpineol (1.2 %) and *β*-selinene (0.7 %) and in the November samples by hexanoic acid (10.0 %), carvacrol (7.8 %) and *α*-pinene (5.4 %).[6] These data show that despite the geographical proximity to Zabaikal'e, the *Rh. aureum* growing in Pribaikal'e refers to another chemotype.

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## ХЕМИЈСКИ САСТАВ ЕТАРСКОГ УЉА БИЉКЕ *Rhododendron aureum* ИЗ ПРИБАЈКАЛСКЕ ОБЛАСТИ (РУСКА ФЕДЕРАЦИЈА)

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Дестилацијом воденом паром је изоловано етарско уље из пет узорака лишћа биљке *Rhododendron aureum*, која расте у области Иркутска, Прибајкал, Руска федерација. Уље је анализирано методама GC и GC/MS. Идентификована су једињења која чине 70,5–78,2 % укупног садржаја уља. Идентификовано је 27 једињења на основу њихових хроматографских ретенционих времена и масених спектара. Главни састојци уља су каларен (10,3–66,4 %), *β*-бурбонен (0,5–27,4 %), *α*-селинен (2,1–8,0 %) и каур-16-ен (2,0–6,3 %). Хемијски састав етарског уља *Rh. aureum* зависи од надморске висине на којој биљка расте.

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