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Essential oil composition of *Morina longifolia* Wall. ex DC. from the Himalayan region

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Morina longifolia Wall. ex DC. is an important aromatic plant that is also well-known in ethnomedicine in the Himalayan region. The aerial parts of the plant were collected during the flowering stage and steam distilled in order to characterize the essential oil composition. Thirty-three compounds constituting 94.2% of the total volatiles were identified using gas chromatography–flame ionization detection (GC–FID) and GC–mass spectrometry (GC–MS). The major constituent of the volatile fraction was β-myrcene (42.5%), whereas other important constituents were bicyclogermacrene (8.9%), germacrene D (6.7%) and limonene (6.3%). The characterization of the essential oil might help out to establish new potential applications for this species, both as herbal supplement and as fragrance agent.

Keywords: bicyclogermacrene; essential oil composition; germacrene D; limonene; *Morina longifolia*; Morinaceae; myrcene; terpenes

Introduction

The Morinaceae family is represented by fifteen perennial herb species, scattered from Eastern Europe to Central Asia (1, 2). However the taxonomy of this family, possibly linked to Valerianaceae, Dipsacaceae and Linnaceae (3) is still being disputed (4, 5). In addition, other authors have attributed to the Morinaceae thirteen species only, those constituting the genera *Acanthocalyx*, *Morina* and *Cryptothladia* (6). All authors agree on the number of species from these three genera growing spontaneous in India, six in total, namely *Acanthocalyx alba*, *A. nepalensis*, *Cryptothladia ludlowii*, *Morina coulteriana*, *M. persica* and *M. longifolia*.

Morina longifolia Wall. ex DC. is known also as Himalayan whorlflower, one of the most important aromatic species of Himalaya growing in the high meadows between 2400–4200 m above sea level (a.s.l.). It is a rosette-forming, 60 cm tall, evergreen perennial plant with fragrant, spiny and linear, dark-green basal leaves and whorled clusters of white flowers borne on spikes in midsummer. The blooming period spans from June to September; flowers are initially white, later turn pink, then red after fertilization. Roots and floral parts are highly aromatic, as well as leaves, which have a citrus-like scent once crushed, similar to *Calamus* (2).

Roots are applied as poultice on boils in ethnomedicinal practice (2), whereas the whole plant is used in the preparation of 'Dhoop and Agarbatties' due to its strong flavor (7) while aerial parts are an ingredient of incense; other applications as a herbal remedy are in the cure of worm-infected wounds in animals (8) as well as in the treatment of gastroenteric disorders (9, 10).

In general, wild plants have been regarded as a natural reservoir of novel and more exotic fragrances (2), however even after the assessment of *Morina longifolia* properties and its use in traditional medicine, the attention has been limited because of the lack of information about its chemical composition. Therefore, the aim of this work was to carry out a complete characterization on the essential oil of this herb as part of our phytochemical investigation on medicinal and aromatic plant species (MAPs) of northwest Himalaya, for this region is a well known source of a variety of MAPs and hosted extensive field visits in the past aimed at bioprospecting its natural resources.

Experimental

Plant material

Morina longifolia wild samples were taken at Tungnath (3600 m a.s.l., 30°14'N lat. and 79°13'E long.), in Rudraprayag district, Garhwal Himalaya, Uttarakhand, India. The aerial parts (top two-thirds) of the plant were collected at full flowering stage, during the second week of August 2009, taken to laboratory and washed properly. A voucher specimen was authenticated by Dr P. Prasad of Botanical Survey of India, Dehradun, and deposited in the same herbarium (BSD 055/2009). The chopped plant material was used for the extraction of essential oil by using a hydrodistillation method in a

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Clevenger-type apparatus for 3 hours. After decanting, the oil was dried over anhydrous sodium sulfate and stored at 4°C prior to gas chromatography–mass spectrometry (GC–MS) and GC–flame ionization detection (GC–FID) analyses. The essential oil content was determined as percentage on fresh weight basis as an average of three independent extractions. The combined essential oils were then used for further analysis.

Analysis of the essential oils

GC–FID analysis was carried out using a Perkin–Elmer Clarus 500 GC equipped with a 30 m × 0.32 mm Elite-5MS capillary column (0.32 μm film thickness). For each sample 1 μL was diluted with 300 μL of diethyl ether (Et_2O) and injected (0.5 μL) in the 'split' mode (1:30) with a column temperature program of 40°C for 5 minutes, then increased to 280°C at 4°C/minute and finally held at this last temperature for 10 minutes. Injector and detector were set at 250 and 300°C, respectively, and the carrier gas was helium (He) with a head pressure of 12.0 psi. GC–MS analyses were carried out using a Perkin–Elmer Clarus 500 GC equipped with a Clarus 500 mass spectrometer using the same capillary column and chromatographic conditions as for the GC–FID analysis. Mass spectra were acquired over 40–500 amu range at 1 scan/second with ionizing electron energy 70 eV, ion source 200°C. Transfer line was set at 300°C, carrier gas was He at 1.0 mL/minute. The identification of the oil components was performed by their retention index (RI) (11, 12), authentic reference compounds, peak matching library search, as well as published mass spectra (12, 13). Retention indices were calculated using the *n*-alkane series (C_6–C_{32}) under the same GC conditions as for the samples. The relative amount (%) of individual components of the oil is expressed as percent peak area relative to total peak area from the GC–FID analyses of the whole extracts.

Results and discussion

Upon distillation, a yellow-colored essential oil was collected with a yield of 0.16 ± 0.01% on fresh weight basis (w/v). Thirty-three compounds constituting 94.2% of the total volatile fraction were identified by GC–FID and GC–MS analysis. The composition of the oil is reported in Table 1, in which the percentage composition and the RI of each constituent were reported. GC–FID trace of the volatile oil from *Morina longifolia* is presented in Figure 1.

The major component of the essential oil turned out to be β-myrcene, amounting to 42.5% of the total volatiles. This compound is an open-chained olefinic monoterpene used in the perfume industry, highly valued as a key intermediate for the preparation of flavor and fragrance ingredients such as menthol, citral, citronellol, citronellal, geraniol, nerol and linalool (14). For this purpose, β-myrcene has been also the subject of a number of biotransformation studies (15). Other impor-

Table 1. Composition of the essential oil of *Morina longifolia* Wall. ex DC.

S.N.	Compound[a]	RI[b]	RI[c]	Percentage
1	β-pinene	966	974	0.7
2	β-myrcene	987	988	42.5
3	limonene	1021	1024	6.3
4	*Z*-β-ocimene	1034	1032	0.9
5	*E*-β-ocimene	1043	1044	0.2
6	undecane	1099	1100	0.9
7	decanal	1203	1201	2.1
8	octyl acetate	1222	1211	0.1
9	tridecane	1299	1300	0.5
10	citronellyl acetate	1348	1350	0.1
11	*E*-2-undecenol	1369	1365	1.0
12	geranyl acetate	1377	1379	0.3
13	Z-5-dodecen-1-al	1383	1389[d]	4.6
14	*Z*-caryophyllene	1403	1408	0.4
15	dodecanal	1405	1408	1.5
16	β-copaene	1427	1430	0.3
17	α-humulene	1451	1452	0.5
18	9-epi-*E*-caryophyllene	1461	1464	1.6
19	germacrene D	1483	1484	6.7
20	bicyclogermacrene	1497	1500	8.9
21	pentadecane	1498	1500	1.7
22	δ-cadinene	1523	1522	0.5
23	caryophyllene oxide	1585	1582	0.6
24	tetradecanal	1609	1611	0.5
25	α-muurolol	1647	1644	2.9
26	α-cadinol	1655	1652	1.0
27	tetradecanol	1672	1671	1.8
28	*Z*-9-hexadecen-1-al	1796	1800[d]	1.1
29	hexadecanal	1812	1813	0.7
30	hexadecanoic acid	1964	1968	0.2
31	tricosane	2298	2300	1.4
32	pentacosane	2499	2500	1.3
33	heptacosane	2700	2700	0.4
	Total			94.2

Notes: [a]Compounds listed in order of elution of Elite-5MS capillary column; [b]Retention Index (RI) on Elite-5MS calculated by using *n*-alkane series from C_8 to C_{32}; [c]Retention Index (RI) from literature data (11); [d]Retention Index (RI) from literature data (12).

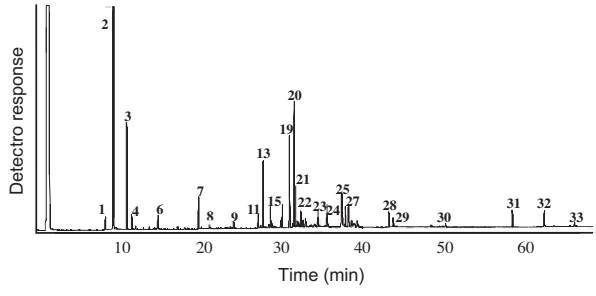

Figure 1. GC–FID trace of the volatile oil of *Morina longifolia* Wall. ex DC. The main components are indicated by numbers; for peak identification (see Table 1).

tant constituents present in appreciable amounts in the essential oil were: bicyclogermacrene (8.9%), germacrene D (6.7%) and limonene (6.3%). These terpenoids are ubiquitous components of flavor and fragrance materials and they are often used in industrial production and transformation. Another abundant group of compounds present in *Morina longifolia* volatile oil is represented by even linear-chain aldehydes both saturated and unsaturated, of which *Z*-5-dodecen-1-al (4.6%), decanal (2.1%), dodecanal (1.5%) and *Z*-9-hexadecen-1-al (1.1%) are the most abundant. These compounds, together with other linear-chain alcohols and alkanes derived from the acetate biosynthetic pathway (16), account for an aggregate 20% of the total oil. Few of these compounds, for example, myrcene, limonene, decanal, germacrene D, bicyclogermacrene, tetradecanal, tricosane and pentacosane are earlier reported from various species of genus *Morina* (17, 18).

From a chemical point of view, *Morina longifolia* essential oil resulted particularly rich in terpenes, as previously discussed, representing about 80% of the total volatiles. Interestingly, no metabolites belonging to the classes of benzenoids and phenylpropanoids could be detected; this feature is not uncommon, since it has been already reported in several different plant families (19–22). Within the order Dipsacales this feature seems to strengthen the taxonomic link between *M. longifolia* and some species from the Valerianaceae family (3, 19, 21, 22).

In conclusion, due to the finding of such a high content of terpenoids, the chemical characterization of *Morina longifolia* might open new perspectives for the cultivation and utilization of this plant species as an ingredient for food supplements, as well as a source of chemicals and flavors for industry.

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