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Rashmi Chandail
M.Phil. Scholar Chemistry,
Apex University Jaipur
Rajasthan, India

Dr. Manjul Mishra
Associate Professor,
Department of Chemistry,
Apex University Jaipur
Rajasthan, India

Corresponding Author:
Rashmi Chandail
M.Phil. Scholar Chemistry,
Apex University Jaipur
Rajasthan, India

Phytochemical analysis and pharmacological study of the plant *Morina longifolia*

Rashmi Chandail and Dr. Manjul Mishra

Abstract
Essential oil from the leaves of *Morina longifolia* was isolated (yield 1.43%) using the hydro-distillation method and analysed for its chemical composition by capillary GC-FID and GC-MS. The oil showed the presence of 47 compounds representing 82.29% area of total chromatogram. The oil was dominated by sesquiterpenes amounting to 53.02% with germacrene D (20.74%), α-cadinol (4.09%), and germacrene D-4-ol (3.73%) as major constituents. The composition and characteristic odor of the oil is indicative of its use in fragrance applications.

Keywords: *Morina longifolia*, essential oils, chemical composition etc.

Introduction
Morina longifolia (Dipsacaceae), commonly known as Whorl flower, Biskandra, or Somrus, is an erect, spinous, perennial herb distributed in the temperate and alpine region of the Himalayas from Kashmir to Bhutan at an altitude of 2,400 to 4,200m. Owing to the strong aromatic properties, the plant is used as incense in the preparation of incense sticks and dhoop sticks. Because of its sweet and astringent taste with a heating potency, the stems, leaves, and flowers are used in Tibetan medicine. The root part and aroma of the flowers are used as poultice on boils and wounds and for unconsciousness, respectively, in Indian traditional medicine. It is also used in veterinary medicines in treatment of maggot wounds. Downloaded by [Arvind Kumar] at 23:30 28 September 2013 Sterols, triterpenes, saponins, alkaloids, and flavonoids were identified in Morina genus. Phenylpropanol derivatives, sesquineolignans, and neolignans were isolated and characterized from the roots of M. chinensis and taraxastane-type triterpenoid saponins and neolignans from M. kokonorica. Five ursane type saponins, monepalosides, and mazusaponin II, and six flavonoid glycosides were characterized from the whole plant of M. nepalensis var. alba. Gas chromatography mass spectroscopy (GC-MS) analysis of essential oil (yield 0.15%) isolated from M. persica flowers using hydro-distillation identified thirty two compounds representing 96% of the total with (2E, 6E) farnesol (58.79%) and germacrene D (10.99%) as major constituents . Phytochemical investigations of *M. longifolia* have shown the presence of morinoursolic acids A and B, n-triacont-3-one, 8-methylditriacont-7-ol, and β-sitosterol, 2,6-dihydroxy-5-methoxy-(3-C-glucopyranosyl) benzoic acid, β-sitosterol, p-hydroxybenzoic acid, caffeic acid and oleanolic acid (6). Very little work has been done on the volatile constituents of *M. longifolia*. The roots have been shown to produce the essential oil that contains a solid hydrocarbon (7.6%), lauraldehyde (3.1%), phenyl acetaldehyde (12.6%), methyl salicylate (2.7%), phenyl propyl cinnamate (7.9%), phenyl propylalcohol (29.9%), and an unidentified ester (11.5%) (26). When the composition of the essential oil isolated from the aerial parts of the *M. longifolia* grown in Gopeshwar Forest Division, Uttarakhand, India was studied for three seasons January, May and September the oil was found to contain 20 compounds belonging to two monoterpene hydrocarbons, four oxygenated monoterpenes, five sesquiterpene hydrocarbons, one oxygenated sesquiterpene, and four oxygenated compounds, all of which showed seasonal variation. The oil was dominated by monoterpene constituting 36.77% to 48.44% of the oil with myrcene (14.48% to 18.65%) and geranyl formate (7.5% to 10.61%) as major constituents. The sesquiterpenes were found to be varied from 24.87% to 29.29% with the germacrene D (1.46% to 5.56%), aromadendrene (2.4% to 5.26%), 2,3-dihydrofarnesol (4.88% to 5.87%), and bicyclogermacrene (2.32% to 6.83%) as

major constituents. In this study, the essential oil from the leaves of *M. longifolia* was isolated using hydro-distillation and analysed by GC-FID and GC-MS.

Materials and Methods

Plant Materials

M. longifolia leaves were collected from Deoband block, Kanasar range, Chakrata forest division, Uttarakhand, India in August 2009. The plant was authenticated at Systematic Botany Branch, Botany Division, Forest Research Institute, Dehradun, India.

Isolation of Essential Oil

Downloaded by [Arvind Kumar] at 23:30 28 September 2013 the fresh leaves were cut into small pieces and hydro-distilled in a Clevenger-type apparatus for 4 h. The distillate was extracted with diethyl ether (Merck), the ethereal layer dried over anhydrous sodium sulphate, and ether removed by gently heated water bath (35◦C) yielding a brownish yellow oil. This was stored in a sealed glass amber vial at 4◦C until analysed.

Chromatographic analysis

For chromatographic analysis, essential oil (50 µL) and tetradecane (99%, Sigma-Aldrich, St. Louis, MO, USA, used as chromatographic internal standard) (2 µL), were dissolved in dichloromethane (Sigma-Aldrich) to make up 1.0 mL of final volume. Constituents of the essential oil were characterized using chromatographic (retention times and indices, comparison with published data) and spectroscopic (mass spectra interpretation, comparison with libraries such as Adams, Wiley, and NIST) data. The relative amounts of individual components were calculated based on GC integrator peak areas without using correction factors.

GC-MS analysis

GC-MS analysis of the essential oil was performed on gas chromatograph GC 6890N Series (Agilent Technologies, Palo Alto, CA, USA), equipped with a mass spectrometer 5975 (electron impact ionization, EI, 70 eV; Agilent Technologies), split/split-less injector (1:30 split ratio), a 7863 automatic injector, and an MSD ChemStation E.01.00.237 data system, which included the spectral libraries Adams, Wiley, and NIST. A fused-silica capillary column DB-5MS (J&W Scientific, Folsom, CA, USA) of 60 m × 0.25 mm id, coated with 5%-phenyl poly(methylsiloxane) and 0.25-µm film thickness, and a DB-WAX (J&W Scientific) of 60 m × 0.25 mm id × 0.25 µm film thickness, coated with poly(ethyleneglycol) were used. With the DB5MS column, the GC oven temperature was set to rise from 45◦C(5min) to 150◦C(2min)at4◦C.min−1, then to 250◦C(5min)at5◦C.min−1,and finally to 275◦C (15min), at 10◦C.min−1. For DB-WAX column, the oven temperature program was similar to that of DB-5MS column. The temperatures of the injector port, ionization chamber, and detector were set at 250◦, 230◦, and 280◦C, respectively. Helium (99.995%, Linde, Bucaramanga, Colombia) was used as a carrier gas, with 170 kPa column head pressure and 22.95 cm.s−1 linear velocity (1 mL.min−1 at constant flow). Mass spectra were acquired by automatic scanning in the mass range m/z 30 to 300 at 5.1 scan.s−1. Homogeneity of peaks were checked with the aid of mass chromatograms of characteristic fragment ions and with the help of peak purity function of MSD ChemStation software. Extracted ion chromatograms were used to simplify the chromatographic profile during the peak assignment process. Downloaded by [Arvind Kumar] at 23:30 28 September 2013 GC-FID Analysis The GC analysis and essential oil constituents quantification were performed on a gas chromatograph GC 6890N Series (Agilent Technology), equipped with FID, split/splitless injector (1:30 split ratio) and a data system (GC ChemStation Rev. B.01.03(204).ADB-5 (J&W Scientific, Folsom, CA, USA) 60 m × 0.25 mm id capillary column coated with 5%-phenyl poly(methylsiloxane) (0.25-µm film thickness). The oven temperature for the column was programmed identically as for the GC-MS analysis. Helium (99.995%, Linde, Bucaramanga, Colombia) was used as a carrier gas, with 170 kPa column head pressure and 22.95 cm/s linear velocity (1.0 mL/min at constant flow). Hydrogen and air at 30 and 300 mL/min, respectively, were used in the FID, with nitrogen (30 ml/min) as a makeup gas. The retention indices were determined from a set of n-alkanes (C6-C25), Polyscience Corp., Niles, IL, USA) using a linear scale on DB-5 capillary column under identical experimental condition.

Hydro-distillation of *M. longifolia* fresh leaves produced a brownish-yellow essential oil (yield, 1.43% on moisture-free basis) of characteristic odor and composition. Peak numbers in Table 1 correspond to those appearing in Figure 1 which shows the typical chromatographic profile of the *M. longifolia* essential oil studied. Kovats retention indices, used as complimentary identification criteria, obtained on nonpolar (DB-5) and polar (DB-WAX) stationary phases. Forty-seven compounds (> 0.01%) representing 82.29% of the relative peak area to total chromatogram were identified. The oil was dominated by sesquiterpenes (53.02%) represented by 15 hydrocarbons (34.25%) and 13 oxygenated derivatives (18.77%) with germacrene D (20.74%), α-cadinol (4.09%), and germacrene D-4-ol (3.73%) as major constituents. The oil was poor in monoterpenes (3.08%) comprising two hydrocarbons—myrcene (1.31%) and limonene (0.54%)—and four oxygenated derivatives: cis-p-menth-2-en-1-ol (0.35%), ipsdienol (0.22%), trans-p-menth-2-en-1-ol (0.48%), and trans-piperitol (0.18%). The oil contained five aliphatic hydrocarbon (9.11%), seven oxygenated compounds (16.42%), and one diterpene (0.66%).

Conclusion

The characteristic odor of the leaves of *M. longifolia* prompted the examination of the chemical constituents responsible for their aroma.

The fresh leaves from *M. longifolia* Chakrata forest division, Uttarakhand were hydro-distilled to yield the essential oil and compared with those reported earlier for plants of the same species from Gopeshwar Forest Division, Uttarakhand (15). The results revealed that the *M. longifolia* from these two ecological origins can be distinguished from each other in their essential oil composition, as the former was rich in sesquiterpenes while the latter was dominated by monoterpenes. Comparative study further indicated the presence of four monoterpenes—cis-pmenth-2-en-1-ol, ipsdienol, trans-p-menth-2-en-1-ol, and trans-piperitol—and 15 sesquiterpenes (β-bourbonene, β-elemene, β-ylangene, βcaryophyllene, β-copaene, α-humulene, γ-

muurolene, α-E,E-farnesene, γcadinene, δ-cadinene, α-cadinene, germacrene D-4-ol, 1-epi-cubenol, epiα-muurolol, and α-cadinol). The oil was found to be a main source of germacrene-D. Germacrene D, as major constituent, has also been identified in the essential oils from the resin (29.57%) and heartwood (31.79%) of Shorea robusta (14), the leaves (40%–19%), and the roots (24.85%) of Senecio rufinervis (17), the aerial parts from *Leonurus cardiaca* L. (26.6%–35.1%) (18), and Hypericum perforatum (12.0%–29.5%) (19), the leaves from Desmopsis bibracteata (29.9%), D. microcarpa (28.3%), Guatteria diospyroides (46.4%), G. oliviformis (73.3%), and Unonopsis costaricensis (62.9%) (20), the leaves from Juniperus oxycedrus ssp. macrocarpa (21%) (9), and the fruits of Piper chaba (21.5%) (21). Germacrene-D is a highly revered sesquiterpene of woody spicy odor for perfumery value, besides a key intermediate for biosynthesis of cadinene group compounds. The composition and characteristic odor of the oil indicated its use in fragrance applications.

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