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Volatile constituents of endangered species *Nardostachys grandiflora* DC. rhizomes from Uttarakhand Himalaya (India)

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The rhizomes of *Nardostachys grandiflora* DC. syn. *N. jatamansi* DC. were collected from two alpine Himalayan locations of Uttarakhand (India). The essential oils were obtained by hydro-distillation and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) to determine the concentration variation in their constituents. A total of 22 compounds were identified in both the oil samples, accounting 97.6–98.4 %. The major constituents of *N. grandiflora* oils were characterised as patchoulol (39.1-46.8 %) and calarene (15.1-21.6 %). Due to the higher relative area quantum of patchoulol in *N. grandiflora* populations growing in Uttarakhand, there is need to develop a propagation protocol for mass multiplication and *in-situ* and *ex-situ* conservation of *N. grandiflora*.

Keywords: *Nardostachys grandiflora* DC., Valerianaceae, Alpine Himalaya, Essential oil, Patchoulol.

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Introduction

Nardostachys grandiflora DC. syn. *N. jatamansi* DC. (Family-Valerianaceae), commonly known as Indian Nard, Spikenard or Balchar, is a 10-60 cm high perennial herb found in alpine Himalayas[1]. A single plant of *N. grandiflora* can have upto 21 ramets in a dense cluster. Each ramet is composed of two to ten linear-lanceolate to oblanceolate leaves and generally produces one, or in rare cases two to three inflorescences in June-July. Flowers are scented and white, sometimes purple-tinged, born in umbellate heads. Flowers have very small gibbae (nectar containers). A cymose head inflorescence can produce upto 25 achenes, they mature in the month of August-September (Plate 1). The species has very long history of use as medicine in Ayurveda, Homoeopathy, Ethno-medicine and Indian System of Medicine (ISM) to modern medicine industry which is distributed in the Himalayas from Pakistan, India (Jammu and Kashmir, Himanchal Pradesh, Uttarakhand and Sikkim) to Nepal, Tibet and China between 3300 to 5000 m asl. It has been reported that the species has become critically endangered depending on its natural habitats[2-6] due to over-exploitation of rhizomes for medicinal use, habitat degradation and other biotic interferences. Rhizome of *N. grandiflora* is used in perfumery products, tonic, stimulant, laxative, diuretic, anti-spasmodic and stomachache. It promotes the growth of hair and imparts blackness[1,7,8]. Traditionally, Jatamansi is used as tonic, stimulant and antiseptic and also used for the treatment of epilepsy, hysteria, convolutions, heart palpitation, intestinal colic and antiarrhythmic activities[1]. It is active components of many Ayurvedic formulations such as Tapaswiniwati, Jestalabangadi, Chandanadi churna and Rachhogna ghrit[9]. Extensive work on the chemical constituents as well as on the composition of the essential oils of *Nardostachys* is reported in literature[10-12]. Studies on chemical profiling of this species have revealed its great pharmaceutical importance for the mankind, e.g. the oil of spikenard possesses antiarrhythmic activity with possible therapeutical usefulness for auricular flutter[1], anxiolytic and sedative effect. Therefore, it is necessary to access the quality of the oil obtained

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Plate 1—*Nardostachys grandiflora* - naturally growing mature plants

from the Jatamansi collected from its natural habitats. In the present study, the variation in chemical composition of essential oils of *N. grandiflora* rhizome, collected from two alpine Himalayan locations of Uttarakhand (India) has been reported for the first time.

Materials and Methods

Plant material

The fresh rhizomes of *N. grandiflora* were collected during the month of October 2012 from two naturally growing alpine Himalayan locations namely, Kedarnath (Rudraprayag; 30°73′27″ N and 79°07′74″ E; altitude 3400 m) and Hansa bugyal, Ghesh (Chamoli; 30°08′51″ N and 079°57′35″ E; altitude 3300 m) of Uttarakhand (India). The specimens were identified by Dr R M Painuli, Taxonomist, Department of Botany, H N B Garhwal University, Srinagar Garhwal. The voucher specimens have been deposited at herbarium of High Altitude Plant Physiology Research Centre (HAPPRC) (Acc. No.: HAPPRC- NJ/G KR-1, GH- 2).

Isolation of essential oils

The shade dried rhizomes (250 g) were chopped into small pieces and subjected to hydro-distillation for 5 hrs using a Clevenger apparatus. The isolated essential oils were dried over anhydrous sodium sulphate and stored carefully in dark vial at low temperature (4 °C) until analysis.

Gas chromatography

GC analyses of the oil samples was carried out using Agilent (HP7890 GC) gas chromatograph equipped with a Flame Ionization detector (FID) and a HP-5 fused silica column (30 m × 0.32 mm, 0.25 µm film thickness). The sample was injected directly into the column. Nitrogen was used as a carrier gas during analysis. The injector and detector temperature were maintained at 210 and 230 °C, respectively. The column oven temperature was programmed from 60 to 220 °C with an increase in rate of 3 °C/min. The injection volume was 0.2 L.

Gas chromatography-mass spectrometry

Analysis of the oil was performed out on Agilent mass spectrometer (Model 5975C) coupled to an Agilent gas chromatograph with a 60 m × 0.32 mm, 0.25 µm film thickness column (DB5). The sample was injected directly in split less mode. Helium was used as the carrier gas (flow rate 1 mL/min). The column oven temperature was programmed from 60 to 220 °C with an increase in rate of 3 °C/min.Other conditions were the same as described under GC. The mass spectrum was taken with a mass range of 40-600 Daltons.

Identification of components

The identification of constituents was performed on the basis of retention index (RI), determined with reference to the homologous series of n-alkanes, C_8-C_{24} with co-injection of standards (Sigma Aldrich, USA) under same analytical conditions and by matching their recorded mass spectra with the MS library (NIST/Pfleger/Wiley) and available literature[13].

Results and Discussion

The essential oils obtained from *N. grandiflora* rhizomes were slightly viscous and pale yellow in colour with strong odour. The oil yields were recorded 0.4 and 0.6% in Kedarnath and Hansa bugyal populations, respectively. The composition of the essential oils obtained from *N. grandiflora* rhizomes is presented in Table 1. Altogether, 22 compounds were identified by GC and GC/MS representing 97.6 % (Hansa bugyal oil) and 98.4 % (Kedarnath oil). Both the oils were dominated by oxygenated sesquiterpenes, representing 46.0 and 59.7 % in Hansa bugyal and Kedarnath oils, respectively. The sesquiterpene hydrocarbons were found to be 35.8 % (Hansa bugyal oil) and 31.5 % (Kedarnath oil).

Table 1—Estimation of volatile constituents of *N. grandiflora* rhizomes collected from two populations of Uttarakhand Himalaya (India)

Constituents	RI	Composition (%)	
		Kedarnath	Hansa bugyal
Formic acid	406	4.4	1.6
Propionic acid	446	1.4	0.8
α-Pinene	939	0.1	2.1
β-Pinene	981	0.4	0.9
β-Myrcene	992	0.2	2.3
p-Cymene	1027	0.4	5.6
1,8-Cineole	1031	0.2	2.3
Terpinen-4-ol	1179	0.1	tr
-Terpineol	1199	Tr	0.2
α-Copaene	1377	0.5	0.9
β-Elemene	1393	4.6	1.9
α-Gurjunene	1412	4.1	tr
β-Caryophyllene	1418	3.3	5.8
Calarene	1435	15.1	21.6
α-Patchoulene	1456	1.9	0.7
α-Humulene	1459	0.3	3.5
β-Guaiene	1495	1.4	0.9
α-Selinene	1498	0.3	0.5
Cubebol	1516	2.2	5.0
Caryophyllene oxide	1586	10.3	1.3
Patchoulol	1656	46.8	39.1
Valeranone	1676	0.4	0.6
MONOTERPENE HYDROCARBONS		1.1	10.9
OXYGENATED MONOTERPENES		0.3	2.5
SESQUITERPENE HYDROCARBONS		31.5	35.8
OXYGENATED SESQUITERPENES		59.7	46
Others		5.8	2.4
Total identified (%)		98.4	97.6

RI: Retention index relative to n-alkanes (C_8-C_{24}) calculated on a non-polar HP-5 capillary column; tr: trace (<0.05)

The major compounds of *N. grandiflora* in both the locations were characterised as patchoulol (46.8 % in Kedarnath and 39.1 % in Hansa bugyal) and calarene (15.1 % in Kedarnath and 21.6 % in Hansa bugyal) with high degree of variation between the locations. Further, some other notable constituents such as caryophyllene oxide (10.3 %), β-elemene (4.6 %), α-gurjunene (4.1 %), β-caryophyllene (3.3 %) and cubebol (2.2 %) were the major compounds in the sample collected from Kedarnath, while sample collected from Hansabugyal showed β-caryophyllene (5.8 %), *p*-cymene (5.6 %), cubebol (5.0 %), α-humulene (3.5 %), β-myrcene (2.3 %), 1,8-cineole (2.3 %) and α-pinene (2.1 %) in noticeable amounts. A previous study on the volatiles of the *N. jatamansi* rhizomes has shown α-pinene (0.1 %), β-pinene (0.4 %), p-cymene (0.4 %), 1, 8-cineole (0.2 %), terpinene-4-ol (0.1 %), copaene (0.30 %) and caryophyllene (3.3 %) compounds[10] which are the same with results obtained in sample collected from Kedarnath population in present study. The essential oils of *N. grandiflora* obtained from Kathmandu valley market[5] identified by 15 compounds with total 63.4 % of essential oil having distinct composition (β-gurjunene 29.1 %, jatamansone 9.7 % and aristolenone 6.5 % in major amounts) as compared with two populations of present study.

Conclusion

There is a need to identify the active compounds in the extracted oils, responsible for the various pharmacologic activities exhibited. This will help in specific therapeutic application of the oil. There is also a need for exhaustive characterization of all the compounds in a given oil extract as this will help in a more accurate chemotyping. Chemotyping could be more meaningful and accurate, if the sample size and geographical coverage are large while also noting the data related to soil type, climate/weather condition, season, etc. and time of collection of cultivated or wild growth. There is also the need to match the various chemotypes with the level and type of pharmacologic activities displayed. For instance, oil richer in calarene may display more antimicrobial activity than in low oil calarene. *N. grandiflora* oil is indeed an under-exploited economic resource that researchers and industrialists could take advantage of as alternative source of raw material. The results of present study indicate that the species can be explored for patchoulol and its allied compounds, which are already established in conservation and mass multiplication of the elite source for sustainable utilization.

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