Volatile constituents of *Nardostachys jatamansi* DC. rhizomes: from Uttarakhand Himalaya (India)

[*1]Harish Chandra Andola and [2]Vijaykant Purohit

[1]School of Environmental Sciences and Natural Resource (SENR), Doon University, Dehradun-248001

[2]High Altitude Plant Physiology Research Centre (HAPPRC), H.N.B. Garhwal University (A Central University), Srinagar Garhwal-246 174 (Uttarakhand)

[*]Email: harishhrdi@gmail.com

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Abstract- The rhizomes of *Nardostachys 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) in order to determine the variation of concentration in their constituents. A total of 22 compounds were identified in both the oils, accounting 96.41–98.22%. The major constituents of *N. jatamansi* oils were characterised as patchoulol (46.0–54.11%) and calarene (9.10–15.6%). Due to the higher relative area quantum of patchoulol in *N. jatamansi* populations growing in Uttarakhand, there is need to develop propagation protocol for mass multiplication and in-situ and ex-situ conservation.

Key words: *Nardostachys jatamansi* DC.*;* Valerianaceae; essential oil, patchoulol

Introduction

Nardostachys 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] (Anonymous, 1997). The species has very long history of use as medicine in Ayurveda, Homeopathy, 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 the species has become critically endangered depending on habitats [2-4] (Nayer and Sastry, 1988; Airi et al., 2000; Nautiyal et al., 2003) due to over- exploitation of rhizomes for medicinal use, habitat degradation and other biotic interference. Rhizome of *N. jatamansi* is used in Perfumery products, Tonic, Stimulant, Laxative, Diuretic, Anti spasmodic and Stomach ache. It promotes the growth of hair and imparts blackness (Anonymous, 1997; Kirtikar et al 1993; Nadkarni, 1954). 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 (Anonymous, 1985) and also is the active components of many Ayurvedic formulations such as Tapaswiniwati, Jestalabangadi, Chandanadi churna and Rachhogna ghrit (Adhakari, 1998) etc. Extensive work on the chemical constituents as well as on the composition of the essential oils of *Nardostachys* is reported in literature (Mahesh et al., 2012; Vijendra and Ali 2002; Bagchi et al., 1990 and Sun et al., 1985). Chemical profiling studies on 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 (Anonymous 1997), Anxiolytic effect (Razack and Khanum 2012), Sedative effect (Takemoto et al., 2008). Therefore, it is necessary to access the quality of the oil obtained from the Jatamansi from its natural habitats. In this paper we report the variation of chemical composition between two populations collected from different location in Garhwal Himalaya.

Material and Methods

Plant Material

Fresh rhizomes of *N. jatamansi* were collected during the month of October, 2012 from two naturally growing locations of Garhwal Himalaya of Uttarakhand (India); Kedarnath (Rudraprayag); 30°73′27″ N and 79°07′74″ E; altitude 3400 m and Hansabugiyal, Ghesh (Chamoli); 30°08′51″ N and 079°57′35″ E; altitude 3100 m. The specimens were identified by Prof. R.D. Gaur, Department of Botany, H.N.B. Garhwal University, Srinagar Garhwal. The voucher specimens have been deposited at herbarium of HAPPRC (Acc. No.: HAPPRC- AG/G SBB-1, 2).

Isolation of Essential oils

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

Gas Chromatography (GC)

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°C and 230°C, respectively. The column oven temperature was programmed from 60° to 220° with an increase in rate of 3°/min. The injection volume was 0.2μL.

Gas chromatography-mass spectrometry (GC-MS)

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 oven temperature was programmed from 60° to 220° at 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 (Adams, 2009).

Results and discussion

The essential oils obtained from *N. jatamasnsi* rhizomes were slightly viscous and pale yellow in colour with strong spicy odour. The yields were ----% for Kedarnath population and ----% for Hansabugiyal (Ghes) population.

The composition of the essential oils obtained from *N. jatamasnsi* rhizomes is presented in Table 1. Altogether, 22 compounds were identified by GC and GC/MS representing 98.4% (Kedarnath oil) and 97.6% (Ghesh oil). Both the oils were dominated by oxygenated sesquiterpenes, representing 58.7% and 46.0% in Kedarnath and Ghesh oils, respectively. The sesquiterpene hydrocarbons were found to be 35.8% (Ghesh oil) and 31.5% (Kedarnath oil).

The GC and GC/MS analysis of essential oils of *N. jatamansi* allowed the detection of 24 components indentify in accounting 98.22% occurred in Kedarnath and 96.41% in Hansabugiyal (Ghes) of the oil (Table 1). Quality assessment of the essential oils from *Nardostachys jatamansi* obtained from Kathmandu valley market (Paudyal et al., 2012) identified 31 compounds with total 63.41% of essential oil which is quantitavely less in amount as compared with two populations of present study.

The major common compound of *N. Jatamansi* in both the location were characterised as Patchouli alcohol (54.11% in Kedarnath and 46.0 % in Hansabugiyal Ghes) and Calarene () (15.6% in Hansabugiyal Ghes and 9.10% in Kedarnath) with high degree of variation between the location. It yields up to 1.9% of a pale yellow essential oil (spikenard oil) with a pleasant odour, suggestive of patchouli or valerian and oil also possesses antiarrhythmic activity with possible therapeutical usefulness for auricular flutter; it is less effective than quinidine but has the advantage of being less toxic (Anonymous, 1997). Pachouli alcohol (54.11%), Caryophyllene oxide (10.25%), Calarene (9.10%), Elemene (4.56%) and Formic acid (4.4%) were the major compounds in the sample collected from Kedarnath, a totals of 24 compounds detected including other minor compounds like Caryophyllene (3.3%), Gurjunene (2.5%) and H-epi-cubedol (2.20%) found greater than 2% of peak area of FID response. Sample collected from Hansabugiyal (Ghes) showed Pachouli alcohol (46.0%), Calarene (15.6%), Caryophyllene (5.8%), p- Myrecene (5.6%) and H-epi-cubedol (5%) as the major compounds whereas other minor compounds like Calarene oxide (3.5%), p-Myrecene (2.33%), 1,8-Cineole and α-pineene (2.1%) found greater than 2% of peak area of FID response. Study of volatile constituents of the rhizome of *Nardostachys jatamansi* (DC.) by Mahalwal and Ali 2002 showed n-Hexane (0.2%), 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 are the same with our result sample collected from Kedarnath population.

Table 1. Volatile constituents of *Nardostachys jatamasnsi* rhizomes from two locations of Uttarakhand Himalaya (India)

Constituents	RI	Composition (%)	
		Kedarnath	Hansabugiyal
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
γ-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)

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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