



Original article

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Investigation on phytochemical, antimicrobial activity and essential oil constituents of *Nardostachys jatamansi* DC. in different regions of Nepal

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ABSTRACT

Objective: To study chemical constituents of essential oil of the roots of *Nardostachys jatamansi* found in different regions of Nepal and also to investigate phytochemical as well as antimicrobial activity of the sample with high yield of essential oil.

Methods: The essential oils of roots of plant from five different regions were extracted by hydro-distillation and analyzed by gas chromatography-mass spectroscopy for their chemical constituents. The root samples were also subjected to hydro-alcoholic extraction and then fractionated with hexane, chloroform, *n*-butanol and water so as to perform phytochemical screening and antimicrobial activity.

Results: The essential oil yield of sample from Jumla was found to be the highest followed by a sample from Nepalgunj, Surkhet and Kathmandu whereas that of sample from Dharan was found to be the lowest. Gas chromatography-mass spectrometer analysis of essential oil of five samples showed that “2-beta pinene” appeared dominated in three samples, namely, 6VJ Nepalgunj, 9VJA Jumla and 10VJ Surkhet. Similarly “alkohol aus neoclovenoxid” in 8VJ Dharan and “methoxy phenyloxime” in 13VJA Kathmandu was found to be present in the highest amount. Phytochemical screening of different fractions of sample 9VJA Jumla showed the presence of alkaloids, terpenoids, glycosides, proteins and amino acids, and carbohydrates *etc.* Antimicrobial susceptibility test of same fractions showed the *n*-butanol fraction potent against all pathogens and most affected one was *Escherichia coli*.

Conclusions: Our study suggests that the essential oil of *Nardostachys jatamansi* found in Nepal contains more than 80 compounds with their quality and quantity differing from place to place.

1. Introduction

Nardostachys jatamansi (*N. jatamansi*) belonging to the family Valerianaceae has great medicinal value. Many research works have shown that it has antifungal and antibacterial[1], anticonvulsant[2], anti-oxidant, anti-cataleptic[3,4], anticancer[5], anti-depressant[6], anti-Parkinson[4,7], anti-diabetic hepatoprotective[8,9], tranquilizing and anti-inflammatory activities [10-12]. It has also effected on estrogen, hair growth and against androgen[13-15].

Nardostachys is a primitive genus having restricted geographical distribution in India[16], Southwest china[17], Nepal[18], Bhutan

and Sikkim[19,20]. There is conflicting information regarding its occurrence in Myanmar, Afghanistan and Pakistan[14]. The herbs are distributed on undisturbed slopes (2 200–5 000 m) of the alpine region of Himalayas[21]. Nepal is a Himalayan country with great repository of natural products. So, there is a huge scope for the characteristic detailed study of many ethno botanical plants including *N. jatamansi* having significant medicinal values in Nepal[22]. The species is commonly available in the local markets of nearly all over the Nepal.

In Nepal, there is limited information available regarding the essential oil content, antibacterial activity and phytochemical screening of *N. jatamansi*. Thus, the aim of the present study is to know variation of chemical constituents of essential oil, perform phytochemical screening, and evaluate antibacterial activity against human pathogenic bacteria.

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2. Materials and methods

2.1. Plant materials

Dry roots of *N. jatamansi* were collected and verified by the Department of Herbarium, National Ayurveda Research and Training Centre, Ministry of Health and Population, Government of Nepal, Kirtipur, Kathmandu, Nepal in May 2014. The voucher specimens were deposited at the National Ayurveda Research and Training Centre, Kirtipur, Kathmandu, Nepal. The study was conducted in Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal from February to May 2015.

2.2. Bacterial strains

The strains of *Escherichia coli* (ATCC 25922) (*E. coli*), *Klebsiella pneumoniae* (ATCC 700603) (*K. pneumoniae*), *Pseudomonas aeruginosa* (ATCC 27853) (*P. aeruginosa*), *Salmonella typhimurium* (ATCC 14028) (*S. typhimurium*), and *Staphylococcus aureus* (ATCC 25992) (*S. aureus*) were available in Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal.

2.3. Preparation of extracts

A weighed quantity of dried roots was subjected to continuous hot percolation in a Soxhlet apparatus with 50% alcohol at 50 °C[23]. The extract was evaporated under reduced pressure by using Rota-flash evaporator until all the solvent had been removed. The crude extract was then fractionated successively by *n*-hexane, chloroform and *n*-butanol remaining being the aqueous one. These fractions were then used for phytochemical screening and antimicrobial sensitivity testing.

2.4. Moisture content determination

The amount of moisture present in each sample was determined by using Dean Stark apparatus[24].

2.5. Essential oil extraction and gas chromatography-mass spectrometry (GC-MS) analysis

Hydro-distillation procedures were done according to the European Pharmacopoeia[25]. Each sample was subjected to Hydro-distillation by using modified Clevenger type apparatus for 4–5 h. The oil was then extracted down the graduated tube by using hexane. The amount of oil in dry sample is calculated by subtracting the moisture content from the sample weight taken.

Analytical gas chromatography (GC) was recorded on a gas chromatograph with a flame ionization detector by using a DB-5 column capillary (30 m × 0.25 mm inside diameter, J and W Scientific, USA) and 0.1 mm film thickness. The temperature program was 50 °C for 2 min and increased at 10 °C/min up to 300 °C for 3 min. The carrier gas was helium at a flow rate 1 mL/min. Mass spectrometer was operated in the electron impact mode with ionization energy of 70 eV on a JEOL AX505 mass spectrometer connected to HP-9000 computer system. The detected compounds

were identified by processing the raw GC-MS data and comparing with the National Institute of Standard and Technology, USA mass spectral database and from retention times and mass spectra of standard compounds[22,26]. Relative amounts of detected compounds were calculated based on GC peak areas.

2.6. Phytochemical screening

The various fractions were analyzed for the presence of various phytoconstituents by using standard phytochemical procedures[27].

2.7. Antibacterial assay

The antibacterial activity of the extract was performed by agar well diffusion method. The strains of *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC 700603), *P. aeruginosa* (ATCC 27853), *S. typhimurium* (ATCC 14028) and *S. aureus* (ATCC 25992) were grown in nutrient broth to get required turbidity and swabbed uniformly on Muller Hinton agar (MHA) plates with the help of sterile cotton swab to get uniform growth. Wells in the MHA plates were made with the help of a sterile cork borer with the internal diameter of 6 mm. A total of 50 µL solution (50 mg of extract in 1 mL of dimethyl sulfoxide) of each extract were loaded into separate wells with the help of micropipette for each bacterial strain. The MHA plates were made in duplicate and incubated at 37 °C for 24 h and the diameter of the zone of inhibition was measured with the help of a ruler in mm.

3. Results

3.1. Moisture content

In order to determine the essential oil content of various samples in dry basis, the moisture content was determined by using Dean stark apparatus. Table 1 shows that moisture content of sample from Kathmandu (13VJA) was greatest *i.e.* 7.4% than that of sample from Surkhet (10VJ) which contained 6.8% followed by Jumla (9VJA) with 5.3%, Dharan (8VJ) with 5.3% and Nepalgunj (6VJ) with 5.2%.

Table 1

Moisture content of different samples of *N. jatamansi*. %.

Sample	Place	Moisture (w/w)
6VJ	Nepalgunj	5.2
8VJ	Dharan	5.3
9VJA	Jumla	5.3
10VJ	Surkhet	6.8
13VJA	Kathmandu	7.4

3.2. Essential oil content

The essential oil content of different root samples of *Nardostachys jatamansi* was determined by hydro-distillation method. The observation in Table 2 shows high content of essential oil in sample from Jumla (9VJA) which was 1.17% in wet basis and 1.23% in dry basis. The essential oil content of sample from Dharan (8VJ) was found to be minimum which was 0.53%. The sample from Nepalgunj (6VJ), Surkhet (10VJ) and Kathmandu (13VJA) was found to contain 1.10%, 1.07% and 0.93% respectively of essential oil.

Table 2Essential oil content in different samples of *N. jatamansi*. %.

Sample	Place	Oil (wet basis) (v/w)	Oil (dry basis) (v/w)
6VJ	Nepalgunj	1.05	1.10
8VJ	Dharan	0.50	0.53
9VJA	Jumla	1.17	1.23
10VJ	Surkhet	1.00	1.07
13VJA	Kathmandu	0.57	0.93
Average		0.86	0.97

3.3. GC-MS analysis

Five essential oil samples were subjected to GC-MS for analyzing their compositions. Every sample was found to contain about 27–37 compounds of which some were identified and few are still unidentified. Some compounds were common in all samples and others were totally different from each other so that the total identified compounds from all samples were found to be more than 80. Table 3 shows the qualitative and quantitative composition of all five essential oil samples. 2-Beta pinene was found to be present in most samples like 6VJ, 8VJ, 9VJA and 10VJ. Alpha pinene was also found to be present in three samples including 6VJ, 9VJA and 10VJ. Some compounds were found to be present in maximum amount in particular sample such as methoxy phenyloxime in 13VJA, alcohol aus neoclovenoxid in 8VJ. Table 4 shows relative amount of major compounds in different samples.

Table 3Various compounds identified in various oil samples of *N. jatamansi* by GC-MS. %.

S.N.	Retention time	Name of compounds	Area of peak				
			6VJ	8VJ	9VJA	10VJ	13VJA
1	7.08	Octane (CAS) <i>n</i> -octane	-	-	-	-	0.75
2	7.78	Alpha thujene	0.22	-	0.15	-	-
3	7.94	Alpha pinene	7.37	-	8.53	6.00	-
4	8.45	Isooctyl chloride	-	-	-	-	0.95
5	8.81	2-Beta pinene	13.00	0.51	19.26	18.82	-
6	9.04	Methoxy phenyl oxime	-	-	-	-	21.22
7	9.06	Decane (CAS) <i>n</i> -decane	-	0.34	-	-	-
8	9.59	Alpha lonone	-	-	0.28	-	-
9	9.65	1,8-Cineole	-	-	-	5.73	-
10	9.74	Alpha pinene	-	-	-	-	0.67
11	9.98	Octane, 5 ethyl-2 methyl (CAS)	-	0.08	-	-	-
12	10.09	Gamma terpinene	0.23	-	-	0.61	-
13	10.21	Sec-amyl velarate	0.51	-	-	-	-
14	10.27	Bicyclo germacrene	-	-	1.31	-	-
15	10.43	2-Beta pinene	-	-	-	-	4.69
16	10.50	Decane (CAS) <i>n</i> -decane	2.02	-	-	1.46	-
17	10.55	Undacane (CAS) <i>n</i> -undecane	-	1.94	-	-	-
18	10.50	Linalool	1.29	-	-	2.22	-
19	10.65	Decane (CAS) <i>n</i> -decane	-	-	1.42	-	-
20	10.77	Alpha-terpinolene	-	-	1.32	-	2.73
21	10.78	Trimethyl-tetrahydronaphthalene	-	2.97	-	-	-
22	10.84	2-Octenal	-	-	-	-	1.59
23	10.90	D-fenchyl alcohol	0.59	-	0.10	0.53	-
24	11.03	Alpha-campholenol aldehyde	0.91	-	-	-	-
25	11.14	Zierone	-	3.97	-	-	-
26	11.25	Trans-pinocarveol	2.25	-	-	4.61	-
27	11.38	Beta-neoclovene	-	5.24	-	-	-
28	11.40	Isopinocarveol	-	-	2.03	-	-
29	11.50	Tetradecane (CAS) <i>n</i> -tetradecane	-	-	-	-	2.24
30	11.51	Pinocarvone	1.42	-	-	1.43	-
31	11.58	Juniper camphor	-	-	-	-	-
32	11.58	1-Borneol	-	-	-	0.83	-
33	11.60	Beta-neoclovene	-	8.93	-	-	-
34	11.92	Undecane (CAS) <i>n</i> -undecane	-	-	-	-	3.87
35	11.94	Myrtenol	-	-	7.22	4.78	-
36	12.00	Alkoholaus neoclovenoxid	-	8.23	-	-	-

Table 3 (continued)

S.N.	Retention time	Name of compounds	Area of peak				
			6VJ	8VJ	9VJA	10VJ	13VJA
37	12.04	Alpha-terpinolene	-	-	-	-	0.79
38	12.13	Beta-citronellol	-	-	-	0.43	-
39	12.16	Patchouli alcohol	4.98	-	-	-	-
40	12.21	Methyl thymylether	-	-	5.16	1.23	-
41	12.32	2-Isopropyl-1-methoxy-4 methyl benzene	-	-	-	0.22	-
42	12.36	9 Aristolen-1-alpha-ol	2.35	-	-	-	-
43	12.30	Alkohol aus neoclovenoxid	-	10.77	-	-	-
44	12.42	2-Nonenal(E) (CAS) trans-2-nonenal	-	-	-	-	1.72
45	12.44	Patchouli alcohol	-	-	1.90	-	-
46	12.43	Valeriansaeure oct-4-yl ester	2.93	-	-	2.24	-
47	12.50	Cyclododecan (CAS) cyclododecane	-	5.04	-	-	-
48	12.54	Octadecane,1-chloro (CAS) 1-chlorooctadecane	-	-	-	-	1.32
49	12.58	Alkohol aus neoclovenoxid	-	12.16	-	-	-
50	12.58	<i>Cis</i> -thujan-10-oic acid methylester	3.32	-	-	0.33	-
51	12.68	Cyclooctanol acetate (CAS) cyclooctyl acetate	-	-	-	-	1.48
52	12.70	Tetradecane (CAS) <i>n</i> -tetradecane	-	-	-	-	0.72
53	12.81	Methylcomate	-	0.66	-	-	-
54	12.82	Nepatalactol	-	-	-	-	0.91
55	12.86	Tridecane (CAS) <i>n</i> -tridecane	-	0.77	-	-	-
56	12.89	2-Undecanone (CAS)-2-hendecanone	2.27	-	-	2.55	-
57	12.97	Valeranone (+)-	-	-	8.2	-	-
58	13.01	Dodecane (CAS) <i>n</i> -dodecane	-	-	-	-	4.73
59	13.04	Benzene methanol,4-(1-methylethyl)-(CAS)	-	1.88	2.03	-	-
60	13.08	Alpha terpinene	5.64	-	-	10.97	-
61	13.16	Hexadecane,2,6,10,14-tetramethyl-(CAS)	-	0.76	-	-	-
62	13.22	Alpha- terpinene	-	-	6.62	-	-
63	13.23	Myrtenol	-	-	-	-	1.27
64	13.34	Myrtenylacetate	8.89	0.54	5.20	4.97	-
65	13.50	Citronenyl acetate	-	-	-	1.22	-
66	13.61	Delta-3-carene	-	-	-	0.39	-
67	13.63	2,2-Trimethylenedithiocyclooctanol	0.14	-	-	-	-
68	13.66	2-Decanal, (E)-(CAS) trans 2-decanal	-	-	-	-	4.83
69	13.68	Bicyclo elemene	-	-	0.76	-	-
70	13.71	Octahydro-pentalen-1-ol	-	-	-	1.72	-
71	13.74	1,3-Cyclopentanedimethanol	2.26	-	-	-	-
72	13.74	Valeranal	-	0.16	-	-	-
73	13.90	Carota-1,4-dienemethoxycarbonyl	0.70	-	-	-	-
74	14.07	Tetradecane (CAS) <i>n</i> -tetradecane	-	-	-	-	5.79
75	14.10	Gamma cadinene	-	-	-	2.26	-
76	14.11	Ethanol, 2-(dodecyloxy)-(CAS) dodecox	-	2.82	-	-	-
77	14.24	Beta patchoulene	-	-	-	6.68	-
78	14.26	Alpha-terpinene	-	-	-	-	7.93
79	14.34	Valerenic acid	-	3.54	-	-	-
80	14.50	Benzenemethanol,alphamethyl-acetate	6.39	-	-	-	-
81	14.52	Alpha guaiene	-	-	-	1.37	-
82	14.55	Myrtenylacetate	-	-	-	-	1.13
83	14.66	Alpha gurjunene	1.01	-	-	-	-
84	14.68	Valerenic acid	-	2.39	-	-	-
85	14.67	Cadinene	-	-	-	2.01	-
86	14.70	Benzenemethanol,alphamethyl-acetate	-	-	2.08	-	-
87	14.83	(-)-Aristolene	0.35	-	-	0.95	-
88	14.89	2-Undecenal (CAS) undece-2- enal	-	-	-	-	4.55

S.N.: Sample number.

Table 4

Relative amount of compound in different samples.

Compounds	6VJ	8VJ	9VJA	10VJ	13VJA
	Nepalgunj	Dharan	Jumla	Surkhet	Kathmandu
2-Beta pinene	+++	+	+++	+++	-
Methoxy phenyloxime	-	-	-	-	+++
Alkohol aus neoclovenoxid	-	+++	-	-	-

3.4. Phytochemical screening

Table 5 shows the observations of phytochemical screening of the various extracts of sample from Jumla 9VJA. Hexane fraction was found to be contained with fixed oils only. Chloroform and *n*-butanol fractions were rich in terpenoids and lactones. Aqueous fraction was found to contain most of the phytochemical such as alkaloids, flavonoids tannins, saponin, proteins and amino acids, carbohydrates, terpenoids, glycosides and lactones.

Table 5Phytochemical screening of ethanol extract of *N. jatamansi* sample 9VJA.

PC	Text used	Hexane	Chloroform	<i>n</i> -Butanol	Aqueous	Crude
Alkaloids	Wagner's test	-	-	-	-	-
	Dragendorff test	-	-	-	+	+
	Mayer's test	-	-	-	++	++
Flavonoids		-	-	-	+	+
Tannins	Ferric chloride test	-	-	-	++	+
Saponin	Froth test	-	-	-	+	+
	Foam test	-	-	-	+	+
Protein and amino acid	Xanthoproteic test	-	-	-	+	+
	Ninhydrin test	-	-	-	+	+
Terpenoids	Salkowski test	-	++	++	++	+
Fixed oil	Liebermann	-	+	+	+	+
	Burchard's test	+	-	-	-	-
Carbohydrate	Molisch's test	-	-	+	+	+
	Benedict's test	-	-	+	+	+
	Fehling's test	-	-	+	+	+
Glycosides	Keller-killani test	-	-	-	+	+
	Borntrager's test	-	-	-	+	+
	Legal's test	-	-	-	-	-
Lactones	Salkowski test	-	++	++	++	+
	LB's test	-	+	+	+	+

PC: Plant constituent. LB's test: Liebermann Burchard's test.

3.5. Antimicrobial assay

Antimicrobial susceptibility test for various fractions was performed using Agar well diffusion method. The strains of *E. coli* (ATCC 25922), *K. pneumonia* (ATCC 700603), *P. aeruginosa* (ATCC 27853), *S. typhimurium* (ATCC 14028), *S. aureus* (ATCC 25992) and *M. luteus* were used for susceptibility test. Various fractions of *N. jatamansi* root extract were loaded in separate wells of 6 mm diameter each. Table 6 shows the diameter in mm of zone of inhibition by different fractions. Zone of inhibition value against *E. coli* was found to be highest by all fractions representing this species most susceptible. On the other hand *n*-butanol and hexane fractions showed significant inhibition to all the species taken representing these fractions most active. Aqueous fraction was found to be least active against most of the species taken.

Table 6

Antimicrobial susceptibility of various fractions against various organisms. mm.

Organism/ Fraction	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. typhimurium</i>	<i>K. pneumonia</i>	<i>E. coli</i>
Crude	9	6	8	11	14
Hexane	13	13	12	14	15
Chloroform	8	6	10	11	11
<i>n</i> -Butanol	12	14	11	14	21
Aqueous	6	6	6	6	13
Dimethylsulfoxide	6	6	6	6	6

4. Discussion

From the moisture content result of five samples we can conclude that the average water content of roots of *N. jatamansi* found in Nepal is about 6%. The different values of the moisture contents were used to determine the essential oil content in dry basis for each sample.

By analyzing the essential oil content result, it can easily be concluded that the sample from Jumla is of high value for essential oil manufacturers followed by that from Nepalgunj and Surkhet. But it is difficult to correlate it with climatic regions because climate from Jumla is totally different with that from Nepalgunj, former being alpine and later tropic. However, one can easily conclude that the average yield of essential oil in Nepal must be around 0.85% in wet basis and 0.97% in dry basis.

The GC-MS analysis of five oil samples collected from five different regions of Nepal enabled us to study how the chemical constituents of the *N. jatamansi* vary with region of sample collection. More than 80 compounds were identified from different samples collected from nearly all regions of Nepal. Of the identified ones the major compounds presents in essential oil found in various regions of Nepal includes 2-beta pinene, alkohol aus neoclovenoxid, methoxy phenyl oxime *etc.* The study suggested that 2-beta pinene, a mono terpene, appeared dominated in three samples, namely, 6VJ Nepalgunj, 9VJA Jumla and 10VJ Surkhet, and also presented in 8VJ Dharan. It was absent in sample 13VJA Kathmandu which was reach in methoxy phenyloxime. 8VJ Dharan was contained with alkohol aus neoclovenoxid. The absence of mono terpene–2 beta pinene, in sample from Kathmandu was exactly aligned with the result by previous reporting for same place[28]. However, there appeared presence of certain compounds in significant amount in other samples which were not previously considered dominated *i.e.* mono terpene was not identified as dominated in previous studies[28].

Conclusively, we can say that the significant variation of compounds present in essential oil of *jatamansi* may be due to diverse geography of Nepal from east to west and north to south. However, the further efforts may be needed to find the exact factor for diverse presence of compounds in same species.

Sample 9VJA Jumla was selected for phytochemical investigation due to being contained with high amount of essential oil. The similar results as reported by previous studies were observed[14,29]. The result showed the presence of alkaloids, flavonoids tannins, saponin, proteins and amino acids, carbohydrates, terpenoids, glycosides and lactones.

The maximum activity was found to be that of *n*-butanol fraction and least one was that of aqueous fraction. *E. coli* were the most susceptible of all species taken under study. As a whole the extract is found to be effective for all the bacteria in more or less extent. From this study, one can conclude that *n*-butanol fraction root of *N.*

jatamansi shows significant antibacterial activity. This result also corresponds to the various studies upon antifungal and antibacterial activity of hydro-alcoholic extract of *N. jatamansi*[5].

Conflict of interest statement

We declare that we have no conflict of interest.

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