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Research Article

STANDARDIZATION, VALIDATION AND ELEMENTARY PHYTOCHEMICAL SCREENING OF *VALERIAN* AND *NARDOSTACHYS*

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ABSTRACT

Indian *Valerian* is a member of the Valerianaceae family that includes up to 250 species. The herb *Valerian* is used for its calming and antispasmodic properties. It is used in anxiety, insomnia, epilepsy, failing reflexes, hysteria, neurosis and sciatica. There is a lot of confusion regarding nomenclature and originality of *Valerian*. Among hundreds of similar species available, very few have real medicinal and hence commercial importance. A close relative called *Nardostachys* is used as a substitute of *Valerian* in Chinese medicines. Keeping in view the immense medicinal importance and critically endangered status of the plant, the present study tries to characterize and validate the real *Valerian* properties using chemical fingerprinting. The root extracts of *Valeriana jatamansi* and *Nardostachys jatamansi* (a substitute) in different solvents are investigated for their phytochemical composition using various qualitative tests. TLC and GC-MS analysis is carried out for both the plant extracts. Essential Oil content is extracted from dried roots of the plants and components are found. The present investigation is maiden attempt to compare and report components and properties *Valerian* and its substitute.

INTRODUCTION

Valerian and *Nardostachys* belong to the Valerianaceae (now, Caprifoliaceae) family. *Valeriana* (Valerianaceae) originated from the Latin word "Valere" meaning 'to be in good health'. It is indigenous to the temperate Himalayas and found in India, Bhutan, Burma, Pakistan and Afghanistan. The herb *Valerian* is used for its calming and antispasmodic properties[1-2]. *Valeriana jatamansi* Jones Syn. *Valeriana wallichi*, popularly known as Indian Valerian (English), Mushkbala (Kashmiri), Sugndhawal or Tagar (Sanskrit)[3], is being labeled as critically endangered due to over-exploitation of rhizomes for its medicinal value, habitat degradation and other biotic interferences in its distribution ranges. *Nardostachys jatamansi,* a substitute, is more commonly used in Chinese medicine. *Nardostachys* was known in ancient times as "nard" and, later, as spikenard. In India, it is used in making many massage oils and is said to be useful for many diseases, especially beneficial as a sedative and to treat disorders of the digestive and respiratory systems[4-5].

Authentication and scientific validation of a medicinal plant is a fundamental requirement of Pharmaceutical industry and other organizations dealing with herbal drugs. Selection of the chemical markers is crucial for the quality control of herbal medicines.[6]

As with all plants that share common names, there is always the possibility that people will become confused and use the incorrect plant, trusting in the country name alone In current study, the chemical fingerprint of *Valerian* is generated which can prove helpful for the standardization and validation purpose. The present work has been carried out along the following lines:

1. The root extracts of *Valeriana jatamansi* and *Nardostachys jatamansi* in various solvents are investigated for their phytochemical composition, using various qualitative tests.
2. The TLC profile is generated for detection of marker compound; valerenic acid.

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3. Individual components in both plant extracts are identified and compared using GC-MS.
4. Essential Oils from dry roots are extracted using hydro-distillation technique and the components are identified by GC-MS analysis.

Experimental Section

Plant material: The authenticated plant material is collected from KUBG, Dept. of Botany, University of Jammu and Kashmir, Srinagar Campus. The roots are cleaned and disinfected with 15%H_2O_2 followed by washing with double distilled water. Shade dried roots are powdered with the help of electronic blender and the powder is stored in air tight bottles at room temperature prior to use. Commercially available samples are collected from local Ayurvedic markets of various areas like Mumbai, Manali and Nepal are processed in the same way.

Chemical requirements: HPLC grade n-Hexane, Ethyl Acetate, Chloroform, Ethanol Methanol and Water are used for extraction as solvents.

Extraction procedure: All the extracts are prepared in a Soxhlet apparatus using continuous hot percolation method at 55^{0}C for at least 8Hrs[7]. The prepared crude extracts are concentrated using rotary evaporator under vacuum at room temperature. The concentrated extracts are stored at 4^{0}C in air tight bottles and used within one week for the experiments. The alcohol(5ml) and aqueous(3ml) extracts are mixed at room temperature with Dichloromethane(25ml for each); kept in ultrasonic bath for 20mins and are isolated by separatory funnel. The combined extracts are filtered and evaporated to dryness and are used for chromatographic analysis.

Preliminary Phytochemical Tests

All the plant extracts are subjected to qualitative chemical screening for the identification of various active constituents, like Valepotraites, Saponins, Tannins, Phenolics, Alkaloids, Flavonoids and other Phytoconstituents using Standard Procedures[8].

TLC of extracts for Isolation and Identification of Active Constituents

The TLC profile is generated for detection of marker compound Valerenic acid, in both plant extracts; for validation purpose. TLC plates (10cmX10cm and 1mm thickness of silica gel GF254) are developed in solvent system (Hexane:Ethyl acetate:Glacial Acetic acid 65:35:0.5) with standard reference. The chromatogram is visualized by UV lamp.

GC-MS Analysis

The Methanolic extracts are subjected to GC-MS Analysis. The column type used is capillary (30m length) with 0.25mm dia. The eluted peaks are identified using NIST library by comparing with the mass spectral data and retention indices in the literature.

Essential Oil extraction

The Essential Oil is extracted using hydro-distillation for 6Hrs using a Clevenger-type apparatus. The extracted oil is collected, weighed and stored at 4^{o}C in dark until analyzed.

RESULTS AND DISCUSSION

Table 1 shows the results for various phytochemical tests performed using standard plant root extracts. The market samples from Mumbai, Nepal and Manali, too show the presence of Flavanoides, Terpenoides, Tannins and Phenolic compounds and presence of Valepotraites, indicating validity in comparison with the standard plant extracts. In *Nardostachys*, Valepotraites are not detected in qualitative tests.

Table 1 Preliminary phytochemical screening of *V. jatamansi* and *N. jatamansi*

Sr. No.	Constituents	Test	ChE		EAE		WE		EE		ME	
			Vj	Nj	Vj	Nj	Vj	Nj	Vj	Nj	Vj	Nj
1.	Alkaloids	Dragendroff's test	-	-	-	-	-	+	-	+	-	+
		Hager's test	-	-	-	-	-	+	-	+	-	+
		Wagner's test	-	-	-	-	-	+	-	+	-	+
		Mayer's test	-	-	-	-	-	+	-	+	-	+
2.	Flavonoids	Conc.H_2SO_4 test	+	-	-	-	-	-	+	+	+	+
		10%lead acetate test	+	-	-	-	-	-	+	+	+	+
3.	Glycosides	Fehling's test	-	-	-	-	-	-	+	-	+	-
4.	Reducing sugars	Fehling's test	-	-	-	-	-	+	+	+	+	+
		Benedict's test	-	-	-	-	-	+	+	+	+	+
5.	Steroids	Salkowsky test	-	-	-	-	-	+	-	+	-	+
6.	Sponins	Foam test with D/W	-	-	-	-	-	-	-	+	-	+
7.	Terpenoids	Conc.H_2SO_4 test	+	-	+	-	+	+	+	-	+	-
8.	Tannins and Phenolics	Ferric chloride test	+	+	+	+	+	+	+	+	+	+
		Potassium dichromate test	-	-	-	-	-	-	+	+	+	+
		Lead acetate test	+	+	+	+	+	+	+	+	+	+
9.	Amino acids	Ninhydrin test	-	-	-	-	-	+	-	+	-	+
10.	Quinones	Conc.H_2SO_4 test	+	-	-	-	-	-	+	-	+	-
11.	Valepotraites	Test for valepotraites	+	-	+	-	+	-	+	-	+	-

Note: ChE: Chloroform Extract, EAE: Ethyl Acetate extract, WE: Water Extract, EE: Ethanolic Extract, ME: Methaolic Extract; Vj: *V. jatamansi*, Nj: *N. jatamansi*; + denotes present and – denotes absent.

TLC RESULTS

In TLC profile, four samples among tested seven market samples show the presence of Valerenic acid, as the Rf value of spot matches with the standard Valerenic acid containing sample.

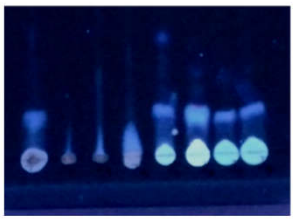

Figure Legends:
Spot 1: Standard Valerenic acid (Rf = 0.49)
Spot 2: *N. jatamansi* market sample from Mumbai
Spot 3: *N. jatamansi* market samples from Manali
Spot 4: *N. jatamansi* market samples from Nepal
Spot 5: *V. jatamansi* market sample from Mumbai
Spot 6: *V. jatamansi* dry root samples from Kashmir
Spot 7: *V. jatamansi* market samples from Manali
Spot 8: *V. jatamansi* market samples from Nepal

GC-MS Results for Dry Root Extracts

Results for GC-MS analysis of standard plant root extract are discussed in following tables.

Table 2 Components identified by GC-MS Analysis of *Valeriana jatamansi* (Methanolic)

Sr.No.	Compound	Mol. Formula	CAS No.	Mol.Wt.
1	Butanoic acid,3-methyl	$C_5H_{10}O_2$	503-74-2	102
2	Pentanoic acid,3-methyl	$C_6H_{12}O_2$	105-43-1	116
3	4H Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	28564-83-2	144
4	Isosorbide Dinitrate	$C_6H_8N_2O_8$	87-33-2	236
5	6-Acetyl-β-d-mannose	$C_8H_{14}O_7$	-	222
6	2(3H)-Furanone-5-heptyldidro-	$C_{11}H_{20}O_2$	104-67-6	184
7	Cyclopropane,1-	$C_{11}H_{16}O$	-	164

	cyclopropylethynyl-2-methoxy-3,3-dimethyl			
8	2-Adamantanol,2-(bromomethyl)	$C_{11}H_{17}Bro$	-	244
9	2(3H)-Napthalenone,4,4a,5,6,7,8-hexahydro-1-methoxy	$C_{11}H_{16}O_2$	70703-08	180
10	2-Isopropyl-5methoxy-6-oxabicyclo(3,1.0)hexane-1-carbohaldehyde	$C_{10}H_{16}O_2$	-	168
11	Dodecanoic acid,3-hydroxy	$C_{12}H_{24}O_3$	1883-13-2	216
12	7-Methyl-7-tetradecan-1-ol acetate	$C_{17}H_{32}O_2$	130996	268
13	Benzoic acid,2,4,6-trimethyl-2,4,6-trimethylphenyl ester	$C_{19}H_{22}O_2$	217323	282

Table 3 Components identified by GC-MS Analysis of *Nardostachys jatamansi* (Methanolic)

Sr. No.	Compound	Mol. Formula	CAS No.	Mol. Wt.
1	Boranamine,N,N,1-trimethyl-1-phenyl	$C_9H_{14}BN$	3519-71-9	147
2	Imidazole,2-amino-5-[(2-carboxy)vinyl]	$C_6H_7N_3O_2$	-	153
3	Falcorinol	$C_{17}H_{24}O$	-	244
4	1-Heptatriacotanol	$C_{37}H_{76}O$	105794-58-9	536
5	Cholestan-3-ol,2-methylene-(3β,5α)-	$C_{28}H_{48}O$	22599-96-8	400
6	Spiro[tricyclo(4.4.0.0(5,9))decane-10,2'oxirane]	$C_{15}H_{24}O_3$	-	252
7	1-Heptatriacotanol	$C_{15}H_{24}O_3$	-	252
8	Pregn-5-en-20-one,12(acetyloxy)-3,8,14,17tetrahydroxy(3β,12β,14β,17α)-	$C_{23}H_{34}O_7$	3513-02-8	422
9	10,13-Octadecadynoic acid, methyl ester	$C_{19}H_{30}O_2$	18202-24-9	290
10	11,13-Dihydroxy tetradec-5-ynoic acid, methyl ester	$C_{15}H_{26}O_4$	-	270
11	Corymbolone	$C_{15}H_{24}O_2$	97094-19-4	236

Methanolic extracts of both the plant roots are subjected to GC-MS and individual components are found out with its Formula, CAS No. and Molecular weight. The bioactivity of every component, when found out individually, shows properties like, anti-pain, anticonvulsant, antioxidant, antimicrobial, antifungal, anticancer and antitumor properties. Also some of the components are useful in flavor and fragrance industry.

GC-MS Results for Essential Oils

According to the European Pharmacopoeia, the crude drug/root sample must contain not less than 0.5% (v/w) of essential oil. The plant root samples yield 0.3-2.1% (v/w) essential oil that gives a warm, musty, woody, heavy aroma and are medium in viscosity. The *V. jatamansi* oil is brownish in colour and gave unpleasant aroma because of presence of isovaleric acid whereas *N. jatamansi* oil which is lighter and greenish in colour, gave comparatively pleasant, musty aroma.

Table 4 *Valeriana jatamansi* Essential Oil composition by GC-MS

Name of compound	Formula	MW	Property
3-methyl butanoic acid	$C_5H_{10}O_2$	102	**Anticonvulsant**
Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, acetate	$C_{12}H_{20}O_2$	196	Fragrance and flavouring material
3-methyl-pentanoic acid	$C_6H_{12}O_2$	116	Fragrance and flavouring material
Borneol	$C_{10}H_{18}O$	154	Traditional Chinese medicine, insect repellent
4-Acetyl-1-methylcyclohexane	$C_9H_{14}O$	138	Fragrance and flavouring material
Acetic acid,1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	$C_{12}H_{20}O_2$	196	-
4,7-methanoazulene(ie Isocaryophilline)	$C_{15}H_{24}$	204	Antiinflammatory, antipain, neuroprotective, antidepressant, anxiolytic, antibacterial and antifungal
Benzene,1-[1,5-dimethyl-4-hexenyl]-4-methyl ie Curcumene	$C_{15}H_{22}$	202	Flavouring agent in food
3-methyl Butanoic acid, 2-phynylethylester	$C_{13}H_{18}O_2$	206	Fragrance and flavouring material
Butanoic acid, 2-methyl-,1,7,7-trimethylbicyclo[2.2.1]hept-2yl-ester	$C_{15}H_{26}O_2$	238	Flavouring agent in food
Trans-Nerolidol	$C_{15}H_{26}O$	222	Fragrance and flavouring material, skin penetration enhancer for better drug delivary
Isoaromadendrene epoxide	$C_{15}H_{24}O$	220	Fragrance and flavouring material
Patchouli alcohol	$C_{15}H_{26}O$	222	Fragrance material, anticancer peroperties
Phenol, 2-methyl-5-[1,2,2-trimethylcyclopentyl-,[S]-,	$C_{15}H_{22}O$	218	traditional Chinese medicine

Table 5 *Nardostachys jatamansi* Essential Oil composition by GC-MS

Name of compound	Formula	MW	Property
Isoleden	$C_{15}H_{24}$	204	fragrance, **anticancer**
Alloaromadendrene oxide-[2]	$C_{15}H_{24}O$	220	good fragrance, larvicidal activity
[-]-Spathullenol	$C_{15}H_{24}O$	220	fragrance
Cyclobutane-tetrakis-[1-methylethylidene]-	$C_{16}H_{24}$	216	-
2[1H]Naphtalenone	$C_{15}H_{22}O$	218	Anti-inflammatory
Eudesma-5,11-[13]-diene-8,12-olide	$C_{15}H_{20}O_2$	232	traditional Chinese medicine
Jatamasone	$C_{15}H_{26}O$	222	Anti - inflammatory, antipyretic, bactericidal, deodorant, fungicidal, laxative, sedative, tonic.
Gamma-Elemene	$C_{15}H_{24}$	204	fragrance, anticancer
Cyclolongifoline oxide, dehydro	$C_{15}H_{22}O$	218	Antioxidant
Ledol	$C_{15}H_{26}O$	222	Antioxidant

CONCLUSION

Selection of chemical markers is crucial for the quality control of herbal medicines. Due to the fact that plant extracts usually occur as a combination of various types of bioactive compounds or phyto-chemicals with different polarities, their separation still remains a big challenge for the process of identification and characterization of bioactive compounds. In the current study, TLC and GC-MS techniques are used to isolate identify the components of the plant extracts. The authenticated plant material collected from Kashmir University is analyzed for generating a Chemical fingerprint of both the plants, *Valeriana jatamansi* and *Nardostachys jatamansi* (a substitute). Commercial root samples collected from various sites are compared with the standard samples for presence of Valerenic acid, Essential Oil content and other components.

This is the maiden attempt to validate and compare *Valerian* roots and its substitute, using Chemical fingerprinting. The data generated in present study will be helpful for validation purpose in pharmaceutical industry, and comparative study of both plants.

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Compliance with Ethical Standards

The manuscript is an original article which has not been submitted for publication elsewhere. This article does not contain any studies with human participants or animals performed by any of the authors. All the authors declare no conflict of interest.

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