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Original Article

Physicochemical Analysis of Sumbul-al –Teeb (*Nardostachys jatamansi* D.C.) Rhizome along with its HPLC Profile

Mohammad Rashid, Aziz ur Rahman*, Qazi Zaid Ahmad, Tajuddin, Syed Shariq Mian

ABSTRACT

Introduction: Sumbul-al–Teeb **(***Nardostachys jatamansi*) is a well known traditional medicinal plant used for therapeutic effect in Unani and Ayurvedic System of Medicine. It has been reported to have many therapeutic activities like antifungal, antimicrobial, antioxidant, hepatoprotective and cardioprotective properties. It is also useful in the management of insomnia and CNS disorders. The present study aims towards the evaluation of the parameters involved in the determination of the quality and purity of *Nardostachys jatamansi* rhizome and its standardization. **Methods:** Organoleptic characters, extractive values, ash values, phyto-chemical analysis, TLC, fluorescence analysis and HPLC profile etc. were the parameters used for the standardisation of the test drug. **Result:** Total ash values, water and alcohol soluble extractive values, moisture contents and volatile oil percentage was found to be 7.08%, 3.54%, 2.5%, 8.6% and 02% respectively. TLC profile of *N. jatamansi* shows 04, 08 and 10 spots in UV short and long wavelength and exposure to iodine vapours respectively. The HPLC pattern shows 34 peaks and the peak no. 01 and 08 are major peaks having area concentration and retention time as 25.974% at 2.8 min. and 29.967% at 4.399 min. respectively**. Conclusion:** The study will provide referential information for the good quality, purity and identification for the future batches of *Nardostachys jatamansi*.

Key words: Phyto-chemical analysis, Quality, Standardization, Unani.

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INTRODUCTION

Nowadays the Indian herbal industry is flourishing at an admirable pace with remarkable increase in the introduction of new herbal pharmaceutical and cosmetic products in the market. But due to ignorance and awful supply chain management of herbal medicines, quality and purity of herbs and their products is not assured. As the efficacy and safety of herbal products is strongly based on their good quality therefore, determination of identity, quality and purity of the herbal medicines is unavoidable. The traditional approach towards standardization for obtaining good quality herbal medicines seems to be insufficient for the current herbal market which invites the need for more advanced techniques. Standardization of the crude drugs involves passport data of the drugs viz., botanical identification, and macroscopic, microscopic and molecular examination, identification of Phytochemical constituent by various chromatographic techniques and biological activity of the whole plant.[1] Due to the high commerce, traders have imperilled plants and their products to adulteration and substitution.

As the rhizome of *N. jatamansi* is often contaminated and adulterated with different plant materials such as *Selinium vaginatu*.[2] Therefore, in this study *Nardostachys jatamansi* DC, *family – Caprifoliaceae (Valirenaceae*) was selected and standardized on their physico-chemical characteristics along with HPLC profile. *Nardostachys jatamansi* is native to the Himalayan regions of India and commonly known as muskroot.[3] It is an excellent remedy popular to the Indian medicinal system and is used from centuries for its health benefits; it can also be used for treatment and other skin diseases. Medicinally it possesses anti-inflammatory, sedative/anodyne, detergent, sialagogue, desiccant, carminative, cardiac tonic, brain tonic, diuretic properties and can be used in cephalalgia, flatulence, ascites, jaundice, hepatitis, ureteralgia, cystitis etc.[4] The rhizome is the source of spikenard oil.[5] It has been reported that Spikenard oil contained p-maaline (18%) and calarene (65%).[6] Monoterpenes are α-pinene, β-pinene, Δ^3-carene; alcohols-β-eudesmol, elemol, oroselol; a semisolid long chain hydrocarbon with molecular formula $C_{30}H_{62}$. β-sitosterol; a terpenic coumarin - jatamansin, a polyoxygenated compound-angelicin and jamansinol have been identified by GLC.[7,8] Nardostachnol 9-hydro aristolene, 1(10)-dehydroaristolene, 2β-maaline and 1, 2, 9, 10-tetrahydro aristolene have been isolated from roots.[9] There are number of compounds reported, which have been isolated from its roots and rhizomes, e.g. sesquiterpene ketone –jatamansone.[10] Liquid alcohols - nardol, calarenol and *n*-hexacosanol; a ketonevaleranone and diethenoid

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ketone - nardostachone, *n*-hexacosen, *n*-hexacosanylisovalerate, *n*-hexacosanylarachiedate, isovaleric acid,valeranal and β-sitosterol have been reported from powdered roots.[11]

Other sesquiterpenes include nardostachone, jatamansinol, jatamansic acid, jatamansinone, nardostachyin, nardosinone, jatamol A and B etc. A new sesquiterpene acid and new pyranocoumarin: 2', 2'-dimethyl-3'-methoxy-3', 4'-dihydropyranocoumarin were reported. Actinidine, an alkaloid was also reported.[5]

The present study was carried out in order to standardise the rhizome of *Nardostachys jatamansi* with a view to develop its quality parameters, and also to deliver referential information for the identification of the crude drug so as to check the substitution and adulteration and to ensure the effectiveness of a drug in treating different body ailments.[12] Parameters include macroscopy, powder analysis, physicochemical parameters and preliminary phyto-chemical screening alongwith HPLC profile.

MATERIAL METHOD

Collection of sample

Dried rhizome of *N. jatamansi* was procured from local market of Aligarh and was properly recognized from the accessible literature and authenticated by Prof. S. H. Afaq. The sample with specimen voucher no. SAMU/NJ-R-0001/17 was deposited in the Department of Saidla, Faculty of Unani medicine, Aligarh Muslim University, Aligarh, for future reference. It was crushed and sieved to coarse powder mechanically and stored in air tight container for study.

Macroscopy and organoleptic characters

The organoleptic characters of the crude drug were observed with sensory organs and was analysed for its colour, odour and taste, size, shape, fracture and surface.

Physicochemical parameters

Ash values, alcohol and water soluble extractive values, volatile oil estimation and loss on drying of the test drug was determined as per the methods recommended by Ayurvedic Pharmacopeia of India (API).[13] and British Pharmacopeia.[14]

The fluorescence analysis of the rhizome powder was done by treating with the different chemical reagents and observed under Ultra violet light and day light.[15,16]

TLC

Thin layer chromatographic analysis of the methanolic extract of *Nardostachys jatamansi* was carried out via chloroform: methanol (24:1) as mobile phase in percolated silica gel $60F_{254}$ TLC plates. Spotted TLC plates were exposed to iodine vapours in iodine chamber and were also visualized in day light and UV short and long wavelength. The Rf value of spots was determined by the given formulae.[14,17]

$$\text{Rf value} = \frac{\text{Distance travelled by the Spot}}{\text{Distance travelled by the solvent}}$$

Preliminary phyto-chemical screening

The extracts were introduced to preliminary phyto-chemical analysis and investigated for the presence of various phyto-constituents like alkaloids, carbohydrates, glycosides, flavonoids, proteins, steroids, saponins, etc. with following parameters.[18,19]

HPLC profile of *N. Jatamansi*

HPLC profile of the methanolic extract of the *N Jatamansi* was done. For this Shimadzu Prominence Isocratic HPLC System equipped with LC-20 AD Solvent delivery unit, Rheodyne Injector, SPD-20A prominence Uv-vis detector system along with C18G120A column, 250x4.6 mm 5U with guard column was used. The methanolic extract of coarsely powdered drug was obtained with the help of soxhlet's extraction method, extract was filtered and allowed to evaporate on water bath. This dried alcoholic extract was dissolved in HPLC grade methanol and used for study. The chromatographic analyses were carried out at room temperature using reversed phase and software driven peaks were obtained (Figure 2). The pressure and flow rate was 127 kgf and 1.0 ml/min, respectively. Detector for HPLC was UV and the wavelength was 254 nm. Mobile phase for HPLC profile of extract consisted of HPLC grade methanol (Merk life science Pvt. Ltd.) only.

RESULTS AND DISCUSSION

Modern system of medicine relies on sound experimental data, toxicity studies and human clinical studies. But there is a lack of pharmacopoeial standards on raw material / finished products. The insufficient quality standards have led to the occurrence of mild to serious adverse effects. Hence, the standardization of herbal ingredients is the basic requirement in order to establish the identity, purity and quality.[20] Herbals are traditionally considered safe and are remarkably consumed by people without prescription. However, it is advocated that some can cause health problems, some are not effective and some may interact with other medicines. Standardization is crucial for the assessment of the quality, purity and authenticity of the drugs, based on the physicochemical parameters, TLC, HPLC and on the presence of active principles.[21] A standardized and good quality drug is the assurance of its therapeutic effectiveness and global acceptance. *Nardostachys jatamansi* is a well-known drug of Unani System of Medicine used to treat various body ailments such as inflammatory conditions. Therefore, for this study *Nardostachys jatamansi* was selected and standardized on their physicochemical parameters such as organoleptic characters, ash values, extractive values, volatile oil estimation, fluorescence analysis, qualitative estimation, TLC along with HPLC profile.

Organoleptic characters of *N. jatamansi*

Organoleptic properties are the critical parameter for the rapid identification and consumer acceptance. Sensory evaluation-visual macroscopy, colour, odour, taste, fracture are the common features helped in identification of the crude drug. The organoleptic properties of rhizome of *N. jatamansi* have been mentioned in Table 1.

Physicochemical analysis of *N. jatamansi*

Ash values, alcohol and water soluble extractive values, loss of weight in powdered drug after drying at 105^{0}C and moisture contents are the indicators of the purity, quality and authenticity of any crude drug. Therefore, to standardise a herbal drug these parameters have basic importance and unavoidable. Total ash values, acid insoluble and water soluble ash values reveals the information related to the adulteration of crude drug with inorganic matter. The water and alcohol soluble extractive values indicate the amount of the extract that the drug yields in a solvent.[16] Less or more extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying, or storage of plant products.[22] Low or high moisture contents may affect the quality of the drug and hence, its efficacy. The excessive moisture is an ideal medium for the growth of the different types of microorganisms which subsequently damages the drug.[23] This drug is also well known for its oil contents. The inappropriate method of extraction of oil or distillation

Table 1: Organoleptic characters of *N. jatamansi*

Rhizome of N. jatamansi	Characters
Shape	Elongated and cylindrical
Size	Rhizomes are 2.5 to 7.5 cm in length
Colour	Dark grey rhizomes are crowned with reddish brown tufted fibers. Internally they are reddish brown in colour
Fracture	Easy and splintery
Surface	Hairy
Odour	Highly agreeable, aromatic
Taste	Acrid, slightly bitter and aromatic

Table 2: Physicochemical values of *N. jatamansi*

Parameters	Observed Valuesin % (Mean ± SD)
Total ash	7.08 ± 0.03
Acid insoluble ash	3.71 ± 0.18
Water soluble ash	0.58 ± 0.07
Alcohol soluble extract	3.54 ± 0.72
Water soluble extract	2.5 ± 0.06
Volatile oil	02 ± 0.04
Loss on drying	9.12 ± 0.12
Moisture content	8.6 ± 0.2517

Table 3: Fluorescence analysis of *N. jatamansi*

Reagents	Visible light	UV light	
		Short 254 nm	Long 366 nm
Powder as such	Brown	Green	Dark Brown
Powder+1N HCl	Light Brown	Green	Light Brown
Powder+50% H_2SO_4	Black	Black	Indigo
Powder+50% HNO_3	Yellow	Light green	Black
Powder+Glacial acetic acid (GAA)	Brown	Light green	Grey
Powder+1N NaOH in water	Dark Brown	Black	Black
Powder+1N NaOH in methanol	Yellow	Dark Brown	Indigo
Powder+Wagner's reagent	Brown	Brown	Indigo
Powder+Drangendorff reagent	Yellow	Green	Indigo
Powder+Benedict's reagent	Brown	Brown	Indigo
Powder+Fehling reagent	Brown	Brown	Indigo
Powder+Lead Acetate (1%)	Brown	Brown	Indigo

and storage may spoil the quality of the drug and hence the oil.[23] Therefore, to assess the quality of *N. jatamansi* it is also necessary to determine volatile oil percentage of the drug. All the values were determined in triplet and the results are depicted in Table 2.

Fluorescence analysis of *N. jatamansi*

Some constituents in many natural products exhibit fluorescence in the daylight ultra violet light and if the substance itself is not fluorescent, it may often be converted into fluorescent through the application of different reagents. Hence, the qualitative assessment of the test drug is carried out in this manner also which serves as an important parameter for pharmacognostic evaluation of crude drugs.[24] (Table 3).

Phytochemical analysis of *N. jatamansi*

The efficacy and pharmacological therapeutic effects of any herbal medicine is depends on their secondary metabolites i.e. phytoconstituents such as alkaloids and glycosides etc. The presence or absence of these phytoconstituents also indicates the quality of the crude drug.[24] Therefore, it is also necessary to determine the presence of active secondary metabolites in the test drug, the results are shown in Table 4.

TLC of *N jatamansi*

TLC is one of the important parameter equips with the qualitative and semi-quantitative information of the drug. If the drug is adulterated or exhausted which in turn may increase or decreases the number of spots

Table 4: Phytochemical analysis of *N. jatamansi*

Chemical Constituents	Test / Reagents	Inference
Alkaloids	Dragendroff's reagent	Present
	Hager's test	Present
	Wagner's reagent	Present
	Tannic acid test	Present
	Mayer's reagent	Present
Carbohydrates	Fehling's test	Present
	Molish's test	Present
	Barfoed's test	Present
	Benedict's test	Present
Flavonoids	Shinoda test	Present
	Zinc hydrochloride test	Present
	Alkaline reagent test	Present
Tannins	Ferric chloride test	Present
	Gelatin test	Present
	Lead acetate test	Present
Proteins	Warming test	Present
	Biuret test	Present
Steroids	Salkowski test	Present
	Sulphur powder test	Present
	Hosse's reaction	Present
	Moleschott's reaction	Present
Glycosides	NaOH Test	Present
Saponins	Frothing with NaHCO3	Present
Fats and fixed oils	Copper sulphate / Sodium hydroxide	Present

Table 5: TLC Profile of *N. jatamansi*

Drug	Extract	Mobile Phase	Observation					
			In long wavelength		In short wavelength		After exposure to iodine vapours	
			Rf value	Colour of Bands	Rf value	Colour of Bands	Rf value	Colour of Bands
Sumbul-ut-Teeb	Methanolic	Chloroform:Methanol 24:1	0.74	Light blue	0.10	Light Violet	0.03	Brown
			0.81	Light blue	0.18	Ash Colour	0.10	Light Brown
			0.92	Light blue	0.21	Ash Colour	0.34	Yellowish
			0.95	Pink	0.46	Light Violet	0.42	Yellowish
					0.54	Light Violet	0.50	Yellowish
					0.71	Ash Colour	0.71	Yellowish
					0.91	Violet	0.80	Yellowish
					0.95	Light Violet	0.85	Yellowish
							0.91	Light Brown
							0.95	Brown

Table 6: HPLC peak table of *N. Jatamansi*

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.800	51022490	3999862	25.974	27.040
2	2.958	17498737	2035176	8.908	13.758
3	3.112	17133712	1448847	8.722	9.795
4	3.358	22115659	1398390	11.258	9.453
5	3.631	5404008	912771	2.751	6.171
6	3.760	12680670	967824	6.455	6.543
7	4.059	8507107	599396	4.331	4.052
8	4.399	58866076	3259988	29.967	22.038
9	4.714	41757	9169	0.021	0.062
10	4.861	94619	6792	0.048	0.046
11	6.520	42582	3473	0.022	0.023
12	7.137	191120	21866	0.097	0.148
13	7.458	41893	1733	0.021	0.012
14	7.967	4840	709	0.002	0.005
15	8.883	3938	486	0.002	0.003
16	9.615	6029	2355	0.003	0.016
17	10.715	48930	3440	0.025	0.023
18	11.314	126621	9723	0.064	0.066
19	11.541	93439	7904	0.048	0.053
20	11.843	159512	11417	0.081	0.077
21	12.480	243216	14527	0.124	0.098
22	13.061	98650	5827	0.050	0.039
23	16.800	21224	1274	0.011	0.009
24	18.508	9267	442	0.005	0.003
25	19.205	98329	4279	0.050	0.029
26	20.984	13585	731	0.007	0.005
27	21.291	21532	920	0.011	0.006
28	21.961	16937	496	0.009	0.003
29	23.315	25369	952	0.013	0.006
30	23.933	247112	9334	0.126	0.063
31	25.328	74338	2435	0.038	0.016
32	26.661	1442445	49067	0.734	0.332
33	35.632	10564	249	0.005	0.002
34	37.411	29702	523	0.015	0.004
Total		**196436011**	**14792377**	**100.000**	**100.000**

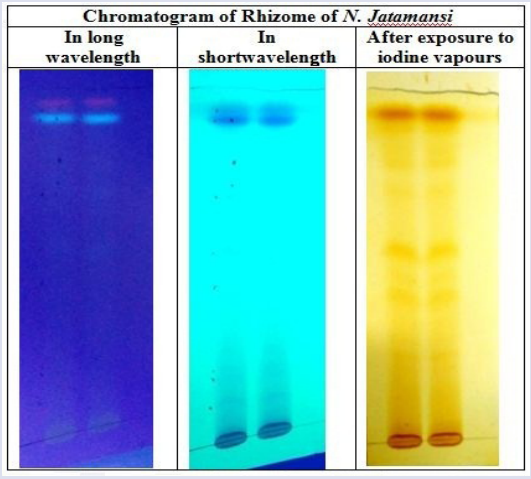

Figure 1: TLC Profile of *N. jatamansi* rhizome.

and change in the Rf values.[16] The TLC profile alongwith images of TLC are illustrated in Table 5 and Figure 1 respectively.

HPLC profile of methanolic extract of *N jatamansi*

The preparative and analytical HPLC has been widely employed for the analysis of herbal medicines in lieu of its high separation capacity. It can also be utilized to analyse almost all constituents of herbal products provided that an optimized procedure is developed which involves optimization of mobile phase and stationary phase along with other chromatographic parameters.[25] The adulteration and impurities can also be determined by this technique. If there is any change in number of peaks or retention time or area of peaks from standard it indicates adulteration or deterioration in the drug. The HPLC pattern shows 34 peaks and the peak no. 01 and 08 are major peaks having area concentration and retention time as 25.974% at 2.8 min. and 29.967% at 4.399 min. respectively followed by peak no. 04, 02, 03, 06 and 07 with concentration of 11.258%, 8.908%, 8.722%, 6.455% and 4.331% respectively. The HPLC profile of the test drug was obtained and recorded for future reference. The details are depicted in Figure 2 and Table 6.

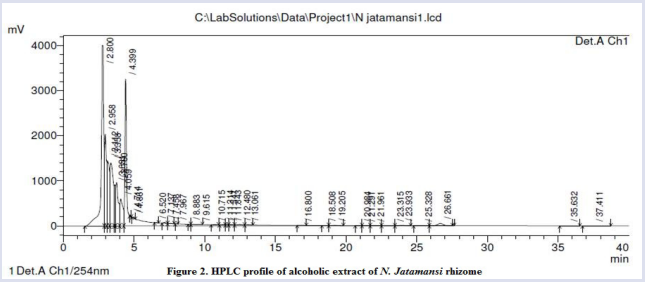

Figure 2: HPLC profile of alcoholic extract of *N. Jatamansi* rhizome.

CONCLUSION

Present study shows that the methods of standardization and identification of *N jatamansi i.e.* organoleptic characters along with physicochemical analysis are the basic and useful parameters to analyse the originality of the test drug. A good quality of drug is the assurance of its efficacy. The results of phytochemical analysis and HPLC fingerprinting also play a key role in identification and authentication of *N jatamansi*. Further these analytical parameters for quality assurance also indicating effectiveness of *N jatamansi* for treating various body ailments. The data obtained in the present work will be useful in identification, standardisation and quality assurance of different samples of *N. jatamansi* and will also be useful in the preparation of the drug's monograph for inclusion in various pharmacopoeias.

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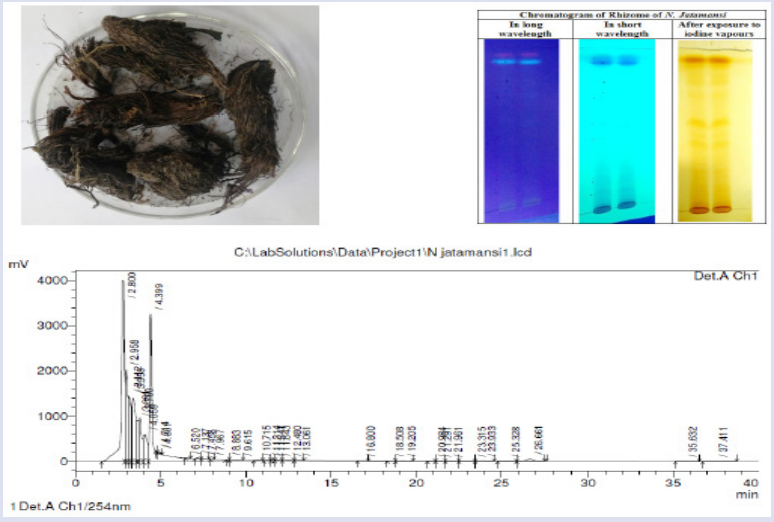
GRAPHICAL ABSTRACT



SUMMARY

- The present study aims towards the evaluation of the parameters involved in the determination of the quality and purity of *Nardostachys jatamansi* rhizome and its standardization. The parameters used for the standardisation of the test drug includes organoleptic characters, extractive values, ash values, phyto-chemical analysis, TLC, fluorescence analysis and HPLC profile etc. The study will provide referential information for the good quality, purity and identification for the future batches of *Nardostachys jatamansi*.

ABOUT AUTHORS

Dr. Aziz ur Rahman is working as Assistant Professor in Department of Saidla (Unani Pharmacy), Faculty of Unani Medicine, Aligarh Muslim University, Aligarh. His research area includes standardization, drug design & discovery, formulation development and experimental research.

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