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PHARMACOGNOSY AND PHYTOCHEMICAL STUDY OF RHIZOME OF JATAMANSI (*Nardostachys jatamansi* DC.)

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ABSTRACT

Background: *Nardostachys jatamansi* DC. is a small, perennial, dwarf, hairy, rhizomatous, herbaceous, endangered and most primitive species within family Valerianaceae and is commonly known as ‘Spikenard’ in English. It is mainly distributed in the Himalayas from Pakistan, India (Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Sikkim) to Nepal, Tibet and China. *Jatamansi* is very famous plant for mental disorders like as insanity, epilepsy etc. and has several activities including anticonvulsant activity, antiparkinson’s activity, tranquillizing activity, hepatoprotective, neuroprotective, hypotensive, anti-diabetic activity. The aim of present article is to put forward the pharmacognostical analysis of rhizome of *Nardostachys jatamansi* DC. **Methods:** Macroscopic evaluation, microscopic evaluation, physicochemical evaluation, extractive values, phytochemical analysis, T.L.C. study were carried out using rhizome of *Nardostachys jatamansi* DC. and data was obtained. **Results:** Data pertaining to the above cited evaluations was recorded for rhizome of *Nardostachys jatamansi* DC. **Conclusion:** All the values hence obtained were subjected to comparison with

their corresponding standard values as mentioned in API. It was observed that all the values were under their normal range.

INTRODUCTION:

Jatamansi (*Nardostachys jatamansi* DC.) is a very well-known drug used in *Ayurvedic* classics. It is used as *Medhya* (brain tonic), *Balya*, *Keshya* and *Vishaghna* etc. Rhizome is used for medicinal purposes as it is *Bhutaghna* or *Manasa Doshahara* (relieves of psychiatric problems) and *Medhya*.ⁱ The decoction of the drug is also used in neurological disorders, insomnia and disorders of cardiovascular system.ⁱⁱ *Jatamansi* is *medhya* (brain tonic) and *Vatanadishamaka* (sympatholytic) due to these actions it relaxes brain and nervous system and causes vasodilation its chemical constituent Jatamansone is useful in cardiac arrhythmias.ⁱⁱⁱ

Nardostachys jatamansi DC is a small perennial, rhizomatous herb which grows in steep, moist, rocky, undisturbed grassy slopes of India, Nepal, China and Bhutan from 2300 m to 6000 m above sea level.^{iv}

Jatamansi shows marked tranquillizing activity, hypotensive, hypolipidemic, hepatoprotective, neuroprotective, anti-ischemic, antiarrhythmic and anticonvulsant activities. The roots and rhizomes of *Nardostachys jatamansi* have been used to treat hysteria, syncope epilepsy, and mental weakness. It also exhibits cardio protective activity and used in the treatment of neural diseases. The essential oil obtained from the roots shows various pharmacological activities including antimicrobial, antifungal, hypotensive, anti-arrhythmic and anticonvulsant activity. Rhizomes and roots contain a variety of sesquiterpenes and coumarins. The sedative sesquiterpene valeranone, which is also found in valerian, is a major component of the root essential oil.^v Sesquiterpene is the major component of *N. jatamansi* plant, and also include jatamansone, nardostachone.^{vi} Animal studies done on jatamansone have reported antioestrogenic, antiarrhythmic, antihypertensive, anticonvulsant, sedative and tranquilizing activities.^{vii} In India, the rhizomes and roots are being marketed as an anticonvulsant *Ayurvedic* drug known as Ayush 56 and also used as an antistress agent.^{viii}

MATERIALS AND METHODS:

Material:

- **Test sample:** *Nardostachys jatamansi* rhizome was selected for pharmacognostical study.

Fig. No. 1: Showing crude drug sample used in this study



Collection and authentication of *Jatamansi*-

The rhizomes of *N. jatamansi* were collected from Ludhiana (Punjab) market in December 2016 and on spot physical verification of the sample were satisfactory that the samples were rhizomes of *Nardostachys jatamansi*. The procured sample was sent for authentication at herbarium section, Department of Botany, Rajasthan University, Jaipur and it was found to be an authentic one - **Authentication no.** (RUBL 211624)

(1) Macroscopic study: The collected sample i.e rhizomes of *N. jatamansi* were powdered and studied organoleptically, with naked eye and magnifying lens, with the help of Pharmacognostical procedure i.e. Appearance, size, shape, colour, and odour and findings were recorded.

(2) Microscopic study: All fresh samples were cut in very thin slices with the help of blade and were dipped in water for some time to make them soften. After that staining was done with safranin. After staining, mounting was done on microslides. In this process, sections were transferred on slides & glycerine was added on sections. Then coverslip was put on sections, excess water was wiped out & then the slides were observed in microscope & photos were taken.

(3) Determination of Moisture Content: Moisture content was determined by placing weighed sample of 5gm of drug in oven at 105°C for 5 hours, and weight of sample was calculated for every 30 minutes, until the weight of the sample came out to be constant, no variation of weight was recorded. This sample was allowed to cool at room temperature in a desiccator for 1 hour before weighing.^{ix}

(4) Determination of pH: The pH value of an aqueous liquid may be defined as the common reciprocal of the hydrogen ion concentration expressed in gram per litre. It practically means the quantitative indication of the acidity or basic nature of a solution. pH value was calculated as per the methods mentioned in the Ayurveda Pharmacopeia of India.^x

(5) Determination of Extractive values: Determination of Alcohol Soluble Extractive: Alcohol-soluble extractive value was calculated as per the methods mentioned in the Ayurveda Pharmacopeia of India.^{xi}

Determination of Water Soluble Extractive: Procedure was same as that of alcohol soluble extractive value and it was proceeded using distilled water instead of alcohol.^{xii}

(6) Determination of Total Ash: The total ash method is designed to measure the total amount of material remaining after ignition. This includes both physiological ash which is derived from the plant tissue itself and non-physiological ash which is the residue of the extraneous matter (e.g. sand and soil) adhering to plant surface. Total ash value was calculated as per the methods mentioned in the Ayurveda Pharmacopeia of India.^{xiii}

(7) Determination of Acid Insoluble Ash: Acid insoluble ash value was calculated as per the methods mentioned in the Ayurveda Pharmacopeia of India.^{xiv}

(8) Determination of Water Soluble Ash: Water soluble ash value was calculated as per the methods mentioned in the Ayurveda Pharmacopeia of India.^{xv}

(9) Phytochemical screening^{xvi} : Freshly prepared extracts were subjected to preliminary phytochemical screening. Presence of carbohydrates (Molisch's Test, Benedict's test, Barfoed's test, Fehling solution test), alkaloids (Mayer's test, Dragondroff's test, Wagner's Test, Hager's Test), amino acids (Ninhydrin test), proteins (Biuret test, Xanthoprotic test, Millons test), saponins (Foam test), glycosides (Borntrager's test), Phenolic Compound, flavonoids (Shinod test), steroids (Salkowaski test) and tannins (Ferric chloride solution, Lead acetate test, Pot. Dichromate test) were tested.

(10) Chromatography: Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.

Identification can be effected by observation of spots of identical R_f value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi- quantitative estimation.

Preparation of test sample- The coarsely powdered dried plant materials (1 g) were successively extracted on small scale with methanol (10 ml) at 90°C 1 hrs using Reflux condenser.

Stationary phase- T.L.C. plate coated with 0.25 mm layer of silica gel 60 F254 with fluorescent indicator was used. (Each plate dimension is 10 cm long and 2 cm width).

Activation of pre-coated Silica gel 60 F254: Plates were dried in hot oven at 105°C for one and half hour.

Solvent system- Petroleum ether: Chloroform (8:2)

Procedure- Apply 10µl Methanolic extract of *Nardostachys jatamansi* on a TLC plate as bands of 10mm. develop the plate to a distance of 8cm from the line of application. Dry the plate in air and spray with solution

of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110° for about 5 minutes or till the bands are clearly visible.^{xvii}

Rf Value: Measured and recorded the distance of each spot from the point of its application and calculated Rf value by dividing the distance travelled by the spots with the distance travelled by the front of the mobile phase.

RESULT AND DISCUSSION:

MACROSCOPICAL EXAMINATION OF RHIZOME

N. jatamansi is perennial herb. The plant is about 10-60cm in height and with stout and long woody root stocks.

Leaves- These are rosy, slightly pink, or blue in dense cymose.

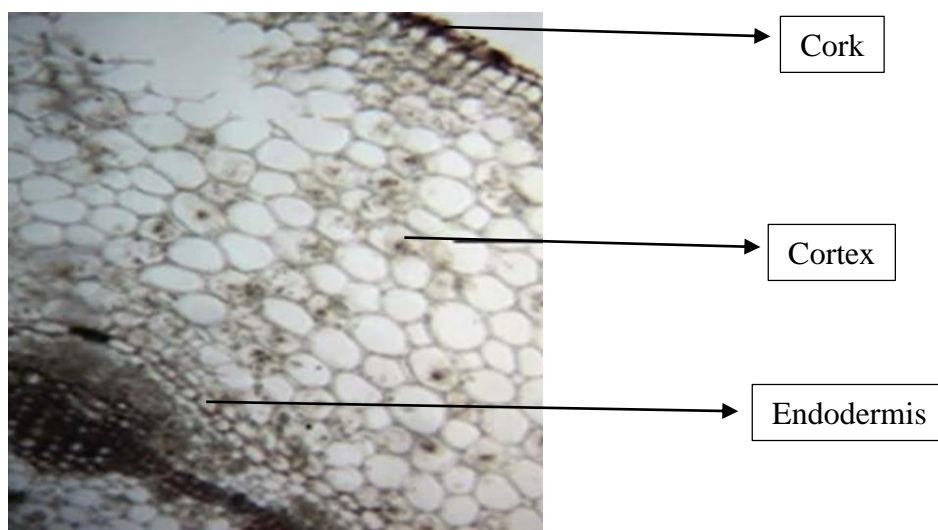
Size and Shape- Rhizomes are 2.5-7.5 cm. in length and elongated and cylindrical.

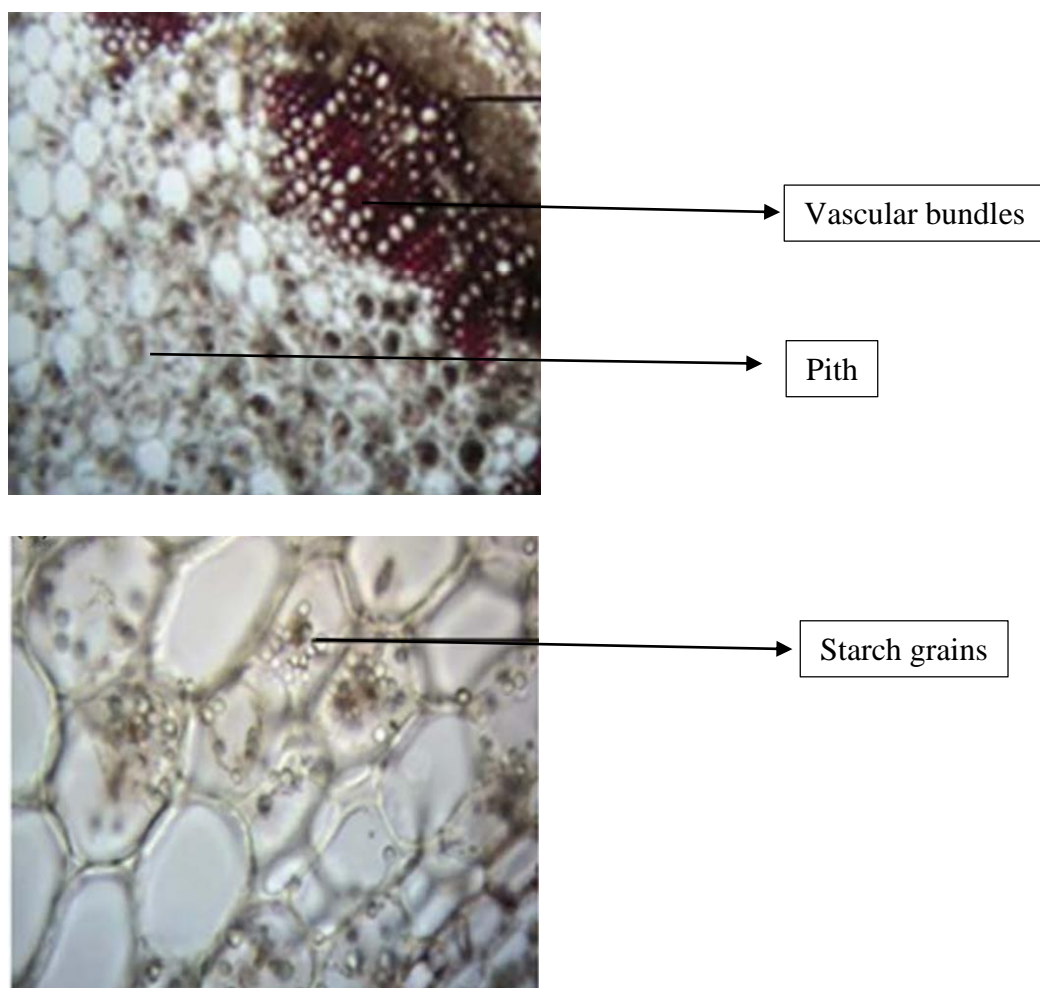
Color- Dark grey rhizomes are crowned with reddish brown tufted fibres. Internally they are reddish brown in color.

Odour- Aromatic, taste- Pungent, slightly bitter.

MICROSCOPICAL EVALUATION OF RHIZOME

Fig 2: Microscopy of Rhizome of *N. jatamansi*





Transverse section of rhizome shows cork consisting of 2-5 layers of cells. Cortex characterised by the presence of schizogenous canals, phloem in form of patches of small cells, cambium ring distinct and continuous. Xylem consists of vessels, scattered individually or in rows of two or three vessels, with scalariform thickening. Older rhizomes show one or more stellate shaped rings of interxylary and medullary cork, completely or incompletely separating the rhizome into four to nine vascular strands by joining outer cork, each separated strand encircled by a few layers of cork cell consisting of an outer cortex zone followed by vascular bundles.

TABLE 1: PHYSICO-CHEMICAL ANALYSIS

Sr. No.	Physicochemical standards	Results % w/w	API standard value (API part-1, Vol.I)
1	Moisture content	8.13%	Not mentioned
2	pH Value	4.53	Not mentioned
3	Water soluble extractive value	7.64%	NLT 5%
4	Alcohol soluble extractive value	9.16%	NLT 2%
5	Total ash	7.71%	NMT 9%

6	Acid insoluble ash	3.17%	NMT 5%
7	Water soluble ash	0.56%	Not mentioned

Moisture content is water holding capacity of a sample, high moisture content in a sample indicates that it may decrease the stability of the sample. Moisture content in rhizome was 8.13%.

pH is a method of quantity analysis of acidic and basic nature of drug. pH of rhizome was 4.53 which is acidic in nature.

Extractive value show soluble content present in sample. Water soluble content present in rhizome was 7.64%.

Alcohol soluble content present in rhizome was 9.16% .

Total Ash is a quantity analysis technique to determine siliceous material and inorganic substance in a sample.

Acid Insoluble Ash shows siliceous material and heavy metals. Water Soluble Ash shows quantity of inorganic substance in Ash.

Rhizome had Total Ash 7.71%, Acid Insoluble Ash 3.17% and Water Soluble Ash 0.56%.

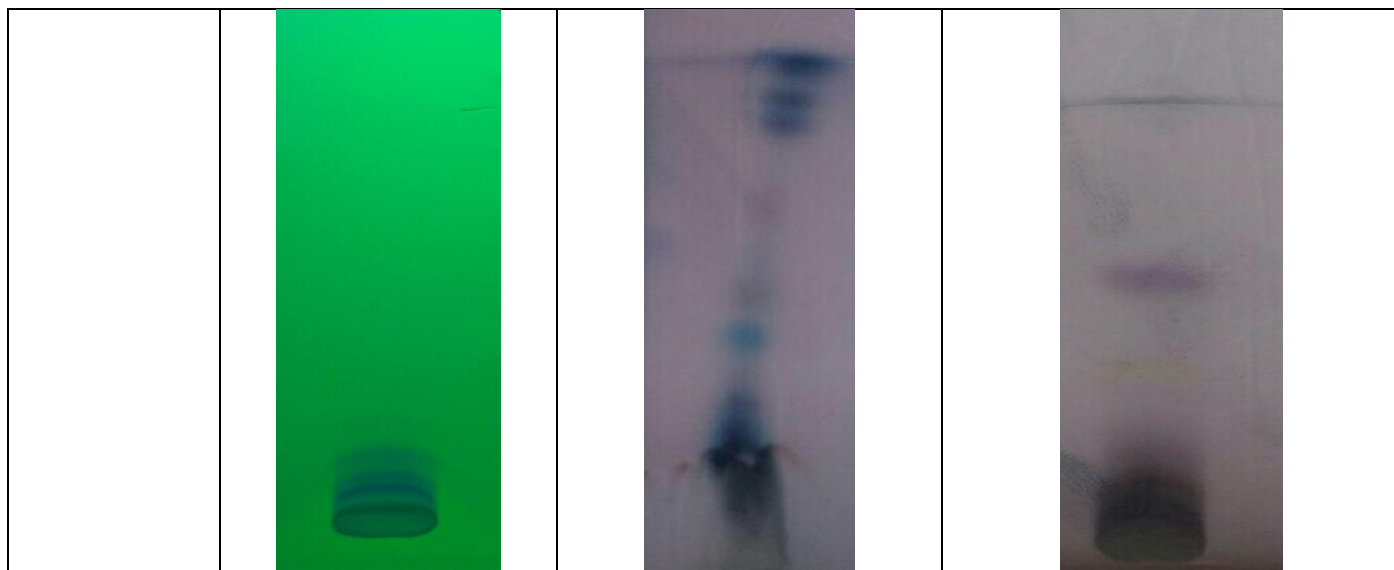
TABLE 2: QUALITATIVE PHYTOCHEMICAL TESTS OF EXTRACTS OF RHIZOME OF *JATAMANSI*

1. Carbohydrate test			
Sr. no.	Name of test	Aqueous extract	Alcohol extract
A.	Molisch test	+ve	+ve
B.	Benedict test	+ve	+ve
C.	Barfoed's test	-ve	-ve
D.	Fehling test	-ve	+ve
2. Alkaloids			
A.	Dragondrof test	+ve	+ve
B.	Wagner's test	+ve	-ve
C.	Hager's test	-ve	+ve
3. Amino acids			
A.	Ninhydrin test	-ve	+ve
4. Proteins			
A.	Biuret test	-ve	-ve

B.	Xanthoprotic test	+ve	+ve
C.	Millon's test	+ve	+ve
5. Saponin			
A.	Foam test	-ve	-ve
6. Glycosides			
A.	Borntragar's test	-ve	-ve
7. Phenolic compound			
A.	Phenolic test	-ve	-ve
8. Steroids			
A.	Salkowaski reaction	-ve	+ve
9. Tannin			
A.	FeCl ₃ test	-ve	+ve
B.	Lead acetate test	+ve	+ve
C.	Potassium dichromate test	-ve	-ve
10. Flavanoids			
A.	Shinod test	-ve	-ve

TABLE 3: CHROMATOGRAPHY OF METHANOLIC EXTRACT

Sample	<i>Nardostachys jatamansi</i>		
Mobile Phase	Petroleum ether: Chloroform (8:2)		
Derivatization	Anisaldehyde sulphuric acid reagent		
	Hot methanolic extract		Cold methanolic extract
	UV 254nm	After derivatization	After derivatization
Rf value	0.25, 0.46, 0.85, 0.89, 0.98	0.27, 0.28, 0.47, 0.55, 0.88, 0.92	0.071, 0.114, 0.128, 0.14, 0.17, 0.42, 0.7



CONCLUSION:

T.S. of rhizome of *Jatamansi* shows presence of cork, cortex, xylem parenchyma, pith, and starch grains. Moisture content, total Ash values of rhizome powder, alcohol soluble extractive value, water soluble extractive values were recorded. All the values were found similar to the standard values mentioned in API. Qualitative analysis of extracts is performed to evaluate general phytochemical profile. Positive mollisch test in both extract (water and alcohol) shows the presence of carbohydrates. Reducing sugar or some reducing substance are present in both extract of *N. jatamansi* because of positive Benedict's test. Ketone functional groups are present in alc. extract of *N. jatamansi* of because of positive fehling's solution test. Dragondroff's test positive in both extract, Wagner's test positive in aqueous extract and Hager's tests positive in alcoholic extract show the presence of alkaloids. Positive ninhydrin test in alcoholic extract shows presence of proteins with secondary & primary amines. Xanthoprotic & millon's tests positive in both extract shows the presence of proteins with aromatic amino acids. Alcoholic extract shows positive Salkowaski reactions which indicates presence of steroids. Fecl₃ test is positive in alcoholic extract, lead acetate test is positive in both extract which means tannins are present in the sample.

CONFLICTS OF INTEREST STATEMENT:

There are no conflicts of interest.

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