



Phytopharmacognostical study of root of *Leptadenia reticulata*

Khushboo Jethva^{1*}, Dhara Bhatt², Maitreyi Zaveri³

¹⁻³ KB Institute of Pharmaceutical Education and Research, Nr. Gh-6 Circle, Sector-23, Gandhinagar, Gujarat, India

Abstract

Leptadenia reticulata or *Jivanti*, an important rasayana drug of India. *Leptadenia reticulata* have different indications in Ayurveda and are used traditionally for several ailments. It is considerable one of the important drug in Ayurveda since 4500 BC. A cooling, demulcent with light strengthening properties of root of *Leptadenia reticulata* is used to make a tonic in traditionally treatment such as seminal discharges snake bite, asthma and tuberculosis. To facilitate correct and easy identification of the root powder of *Leptadenia reticulata*, pharmacognostical studies covering macro and microscopical evaluation, physicochemical constant and phytochemical evaluations were performed. The morphological and microscopical characters were compared with the available literature. The ash of the whole plant powered is 6.50% and an extractive value in water is more than in alcohol. The phytochemical investigation of root powder revealed the presence of alkaloids, glycosides, flavonoids, sugar, saponins and tannins compounds. These specific identities will be useful in the standardization of the drug.

Keywords: *Leptadenia reticulata*, root powder, pharmacognostical study, physico-chemical parameters, phytochemical screening

1. Introduction

Herbs are concentrated foods that provide vitamins, minerals and other nutrients that sustain and strengthen the human body. They have been used by man since the beginning of our existence and have passed the test of time. History is an excellent source of herbal knowledge. Today's health-conscious public is now realizing that herbs, in conjunction with a proper diet and exercise program, can help them to achieve good health. Rasayana is one of the classes of Ayurveda that improve the general health of the body. Rasayan nourishes and rejuvenates the body and increases longevity, memory enhancement, immunomodulation and adoption^[1]. Herbs are a natural path to good health such as JIVANTI (or svarnajivantz) in Sanskrit literature, the name (jiv = life) indicates that the plant is considered to have the ability to bestow health and vigour. It is considered to be a rasayana and included among the 10 drugs constituting the jvaniya gana or 'vitalising group'^[2].

Leptadenia reticulata is a much branched twining shrub of family Asclepiadaceae. Flowers are greenish yellow, in many flowered cymes (in lateral or subaxillary cymes), the follicles are sub woody and turgid. Stem is cylindrical and bent occasionally at places. It is 5 to 10 cm long, 0.5 to 2.5 cm in diameter. The surface is rough, longitudinally ridged, wrinkled and furrowed, transversely cracked and with vertically elongated lenticels at places. Externally whitish brown, internally pale brown, fracture short and splintery, odor and taste are not characteristics. The bark is yellowish brown, corky, deeply cracked. Leaves are ovate to cordate, 4 to 7.5 cm long, 2 to 5 cm broad, entire, acute, subacute to mucronate, base symmetrical, petiole 1 to 3 cm long, glabrous above and pubescent below, green colour taste and odour not characteristics^[3].

The roots are externally rough, white or buff colored with longitudinal ridges and furrows and in transverse section the

wide cork, lignified stone cell layers and medullary rays can be seen. The root size varies from 3 to 10 cm in length and 1.5 to 5 cm in diameter. Flowering occurs in May and June, while fruiting begins in October and continues up to November^[4].

The present study was undertaken to evaluate the Pharmacognostical study and phytochemical investigation of roots of *Leptadenia reticulata*.

2. Materials and methods

2.1 Collection and authentication roots of *Leptadenia reticulata*

Fresh root material was collected from widely grown plant *Leptadenia reticulata* from Dhandhiya village of Rajkot district, Gujarat, India. Raw material was subjected to washing with distilled water and then allowed for drying for 5 days under shade and powdered to 60# separately and stored in well close container. The procured material of *Leptadenia reticulata* was authenticated by taxonomist

2.2 Pharmacognostical study roots of *Leptadenia reticulata*

2.2.1 Macroscopy of roots of *Leptadenia reticulata*

Roots of *Leptadenia reticulata* was taken and morphological evaluation was done by evaluating way like size, shape, taste, odour, colour, and features like touch, texture, etc.

2.2.2 Microscopical studies roots of *Leptadenia reticulata*

For microscopical studies, fine powder of Roots of *Leptadenia reticulata* were taken, cleared with chloral hydrate solution and studied. The lignified elements were visualized by staining the section with a drop of hydrochloric acid and phloroglucinol in the cut sections. Photomicrographs were shot for histological observation (Labomed).

2.2.3 Physicochemical parameters roots of *Leptedinia reticulata* ^[5]

The fresh extract of roots of *Leptedinia reticulata* was prepared by maceration method using different solvents. Physicochemical studies of roots of *Leptedinia reticulata* were carried out as per by using standard methods of pharmacopeia.

2.2.3.1 Determination of ash values

The ash remaining following ignition of medicinal plant materials was determined by three different methods which measures total ash, acid insoluble ash and water soluble ash.

2.2.3.1.1 Determination of total ash

Four crucibles were taken, cleaned and make it air dry and weigh. Place two grams of powder of roots of *Leptedinia reticulata* was taken in all four crucibles. The material was spread in even layer in the crucible and places it in furnace and ignites it by gradually increasing the heat to 500°C. Cool it by switching off plug and wait until temperature down up to 100 °C. The crucibles were taken and weigh it when hot. The total ash was calculated in mg per gram of air dried powder material.

2.2.3.1.2 Determination of acid insoluble ash

Two crucibles were taken, containing the total ash and 25 ml of hydrochloric acid was added in this. Beaker was covered with petridish and boiled gently for 5 min on water bath. Petridish was rinsed with 5 ml of hot water and that liquid was added in to the beaker. Two equal weight wattman filter paper were weighed. From that one filter paper was taken and filtered the solution of beaker. Wash the ash remains on the filter paper until filtrate become neutral with water. Same procedure was also done for the blank with another filter paper. Now, the filter paper containing ash was transferred in original crucible and blank filter paper was transferred in another crucible. Crucible was dried on the hot plate and placed it in to the furnace and ignites it by gradually increasing the heat to 500°C. Cool it by switching off plug and weigh until temperature down up to 100 °C. Crucible was removed and weighed it when hot. The content of acid insoluble ash was calculated in mg per gram of air dried powder material.

2.2.3.1.3 Determination of water soluble ash

Two crucibles were taken, containing the total ash and 25ml of water was added in this. Beaker was covered with Petri dish and boiled gently for 5 minutes on water bath. Petri dish was rinsed with 5 ml of hot water and that liquid was added in to the beaker. Two equal weight wattman filter paper were weighed. From that one filter paper was taken and solution of beaker was filtered from them. The ash remaining on the filter paper was washed with water until filtrate become neutral. Same procedure was also done for the blank with another filter paper. Now, the filter paper containing ash was transferred in original crucible and blank filter paper was transferred in another crucible. Crucible was dried on the hot plate and placed it in to the furnace and ignites it by gradually increasing the heat to 500°C. Cool it by switching off plug and weigh until temperature down up to 100°C. Crucible was removed and weighed it when hot. The content of water soluble ash was calculated in mg per gram of air dried powder material.

2.2.3.2 Determination of Extractive values

This method determines amount of active constituents extracted with solvents from a given amount of medicinal plant materials. Extractive values indicate the nature of the constituents present in a crude drug.

2.2.3.2.1 Alcohol soluble extractive

Accurately weighed 4 gm of air-dried powdered drug of roots of *Leptedinia reticulata* was macerated for 24 h with 100 ml of alcohol of the specified strength in a closed flask, shaken frequently during first 6 h and allowed to stand for 18 h. It was then filtered rapidly, taking precautions against loss of the solvent and 25 ml of the filtrate were evaporated to dryness in a tared flat-bottomed shallow dish and dried at 100° C to constant weight. The % w/w of alcohol soluble extractive value was calculated with reference to the air-dried drug.

2.2.3.2.2 Water soluble extractive

Accurately weighed 4 gm of air-dried powdered drug was macerated with 100 ml of water in a closed flask for 24 h, shaken frequently during first 6 h and allowed to stand for 18 h. It was then filtered rapidly and 25 ml of the filtrate were evaporated to dryness in a tared flat-bottomed shallow dish and dried at 100°C to constant weight. The % w/w of water soluble extractive value was calculated with reference to the air-dried drug.

2.2.3.3 Determination of moisture content roots of *Leptedinia reticulata*

2.2.3.3.1 Loss on drying

5 gm of root powder were dried into a pre- weighed flat and thin porcelain dish then dried it in the oven at 100°C or 105° C and Cool it in desiccators. The loss in weight was usually recorded as moisture.

2.3 Phytochemical screening of root of *Leptedinia reticulata*

Sample preparation

Root powder of *Leptedinia reticulata* were taken to perform phytochemical screening. All phytochemical tests were performed for root powder of *Leptedinia reticulata*. Root powder of *Leptedinia reticulata* were tests separately to check the presence of various phytochemicals visually like, alkaloids^[6], flavonoids^[7,8], saponins^[9,10], carbohydrates^[11], steroids, triterpenoids, carotenoids, amino acids, tannins^[12,13], phenolics^[14,15], coumarins^[16,17] and anthraquinones^[18] using standard procedures.

3. Results

3.1 Collection and authentication of root of *Leptedinia reticulata*

Roots of *Leptedinia reticulata* was collected when it fully grown and flowering. Total 1500 gm of herb was collected to get 1000gm of dry powder. Authentication was done by taxonomist and Herbarium sheet (PH/15/010) was deposited at the Pharmacognosy and Phytochemistry department of K. B. Institute of Pharmaceutical Education and research.

3.2 Pharmacognostical study of root of *Leptedinia reticulata*

Leptedinia reticulata is climber or straggler throughout the greater part of India. Macroscopical observation of the plant

was done according to size, shape, surface with a naked eye which provided a great deal of information about the drug material under consideration. For microscopical studies, fine powder of Roots of *Leptedinia reticulata* were taken, cleared with chloral hydrate solution and studied.

3.2.1 Morphology of root of *Leptedinia reticulata*

The roots of *Leptedinia reticulata* are externally rough, white or buff colored with longitudinal ridges and furrows and in transverse section the wide cork, lignified stone cell layers and medullary rays can be seen. The root size varies from 3 to 10 cm in length and 1.5 to 5 cm in diameter as shown in Figure 1.



Fig 1: Roots of *Leptedinia reticulata*

3.2.2 Microscopical studies roots of *Leptedinia reticulata*

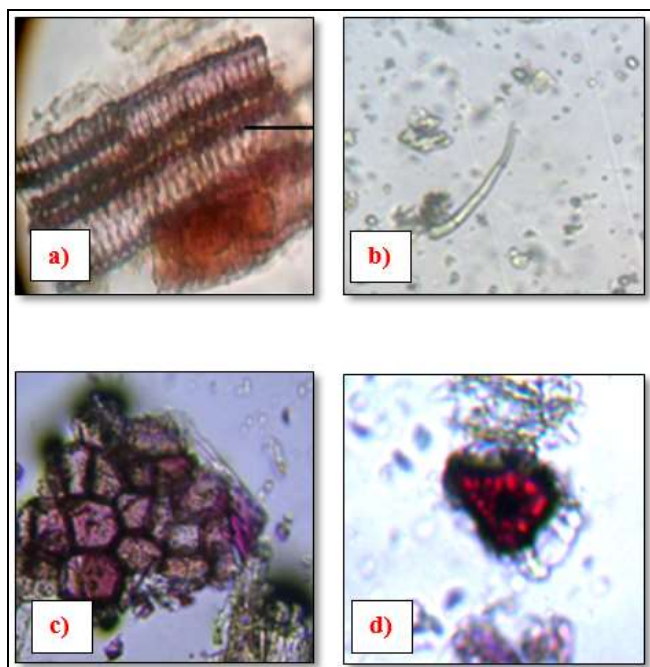


Fig 2: Microscopy of root powder of *Leptedinia reticulata* a) Spiral xylem vessels b) Unicellular trichome c) Cork cell d) stone cells

3.2.3 Physicochemical parameters roots of *Leptedinia reticulata*

The quantitative determination of some pharmacognostical parameters is useful for setting standards for crude drugs.

The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of the drug is not too high, thus it could discourage bacteria, fungi or yeast growth. Equally important in the evaluation of crude drugs, is the ash value and acid-insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica.

3.2.3.1. Ash value of powder of root of *Leptedinia reticulata*

The results of ash values of crude powder of leaf, stem and root of *Cocculus hirsutus* were shown in table 1.

Table 1: Percentage ash value of powder of roots of *Leptedinia reticulata*

Name of plant	ASH Values (% w/w)		
	Total Ash	Acid Insoluble Ash	Water Soluble Ash
Powder of root of <i>Leptedinia reticulata</i>	06.50	00.87	02.70
Standard range	NMT 16.50	NMT 03.00	--

3.2.3.2. Extractive value of powder of root of *Leptedinia reticulata*

The results of extractive values of root of *Leptedinia reticulata* were showed in table 2. The water extractive value of powder of root of *Leptedinia reticulata* was higher than alcoholic extractive value.

Table 2: Percentage extractive value of powder of roots of *Leptedinia reticulata*

Name of plant	Extractive values (%)	
	Water Extractive value	Alcohol Extractive Value
Powder of root of <i>Leptedinia reticulata</i>	09.40	08.80
Standard range	NLT 22.40	NLT 05.20

3.2.3.3. Moisture content of powder of root of *Leptedinia reticulata*

The results of loss on drying of powder of root of *Leptedinia reticulata* were showed in table 3.

Table 3: Percentage moisture content of powder of roots of *Leptedinia reticulata*

Name of plant	% Moisture content
Powder of root of <i>Leptedinia reticulata</i>	06.50
Standard range	NMT 08.00

3.3 Phytochemical screening of powder of roots of *Leptedinia reticulata*

The root powders of *Leptedinia reticulata* were screened for alkaloids, tannins, glycosides, triterpenes, and steroids etc. using different chemical test to establish its identity. The chemical tests shows the presence of Alkaloids, glycosides, Tannins, Phenols, Flavonoids, etc. as shown in Table 4.

Table 4: Phytochemical screening of powder of roots of *Leptedinia reticulata*

Class of phytochemicals	Name of test performed	powder of roots of <i>Leptedinia reticulata</i>
Alkaloids	Dragondorff's test	+
Steroids	Liebermann Burchard test	-
Triterpenoids	Salkowski test	-
Cardiac glycosides	Borntrager's Test	+
Tannins	Lead acetate test	+
Phenolics	Folin-Ciocalteu reagent test	+
Saponins	Foam test	+
Flavonoids	Shinoda's test	+

4. Discussion and Conclusion

In many cases botanically different plant species are sold in the place of Ayurvedic or Unani drugs. Sometimes different species of the same genus, which are more easily and cheaply available, are used in place of that which has been prescribed in the literature. Also, there are certain well known drugs around which confusion still prevails with regards to their correct identity. Another reason for the confusion is that different plants species are sold under one Ayurvedic name or one plant species under different names [19].

Today with the present surge of interest in the phyto-therapeutics, the availability of genuine plant material is becoming scarce [20]. This is partly due indiscriminate exploitation of medicinal plant wealth and partly due to depletion of other resources out of greed to obtain maximum drug yield. Since crude plant drugs form the basis for the manufacture of numerous medicinal preparations, accurate determination of drug identity forms an essential part of its study [21].

Morphological and Microscopical study helps to identify the standard material and which makes them differ from adulterating species. Physicochemical studies of leaf, stem and root of *Cocculus hirsutus* were determined by ash values, extractive values and loss on drying according to the WHO guidelines. The total ash method was designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which was derived from the plant tissue itself, and "non-physiological ash", which was the residue of extraneous matter adhering to plant surface. Acid insoluble ash is the residue obtained after the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is the difference in weight between the total ash and residue after treatment of total ash with water [22,23]. The percentage of total ash of powder of root of *Leptedinia reticulata* was 06.50%. The percentage of extractive value of water (09.40%) was greater than extractive value of ethanol (08.80%) extractive value. The percentage of Loss on drying of root of *Leptedinia reticulata* was 06.50%. all the values of physico-chemical parameters were compared with standard and it comply in the range of it.

The phytochemical screening of root powder of *Leptedinia reticulata* showed the presence of alkaloids, saponins, flavonoids, glycosides and tannins. These all phytoconstituents may be responsible for many different activities. Although the root powder of *Leptedinia reticulata* helps to cure and management of various diseases and disorder.

5. Acknowledgement

The authors are thankful to department of Science and Technology for providing DST INSPIRE FELLOWSHIP.

6. References

1. Chauhan NS, Saraf DK, Dixit VK. Effect of vajikaran rasayana herbs on pituitary-gonadal axis. *European Journal of Integrative Medicine*. 2010; 2(2):89-91.

2. Kasera PK, Shukla JK. Bio-Medicinal properties and cultivation of *L.reticulata*- an endangered plant of the desert, India, *Scientific Correspondance, Current Science*. 2003; 84:877-889.
3. Kirtikar KR, Basu BD. *Indian Medicinal Plants*, Bishen Singh Mahendra Pal Singh, Dehradun. 1994; 2(2nd edition):1629-1630.
4. Prajpathi ND Kumar. *Agro's Dictionary of Medicinal Plants*, Agrobios, India, 2003, 189.
5. Anonymous. *Physicochemical parameters, WHO Guidelines 1st ed.* A.I.T.B.S. Publishers and distributors, Delhi, 2002, 40-43.
6. Geissman A, Peach K, Tracy MV. *Modern Methods of Plant Analysis*, ed. Heidelberg, Berlin, Springer Verlag. 1955; 3:471.
7. List PH, Horhammer L, *Chemical tests. Hager Hand buch der pharmazeutis chem praxis*. Berlin, Springer Verlag Band. 1967; 1:256.
8. Geissman A, Peach K, Tracey MV. *Morden methods of plant analysis*, Springer Verlag, Berlin, Gottingen, Heidelberg. 1955; 3:473.
9. Fishcher R. *Praktikum der Pharmakognosic*, 3rd ed, Berlin, Springer Verlag, 1952, 362.
10. Evans WC, Evans D. *Trease and Evan's Pharmacognosy*, 15th ed. W.B. Saunders Company Ltd., London, 2002, 193.
11. Griffin WJ, Owen WR, Perkin JE. A Phytochemical survey of eastern Australian plants for saponins. *Planta Medica*. 1968; 16(1):75-81.
12. Simes JH, Tracey JG, Webb LJ, Dunstan WJ. An Australian phytochemical survey - saponins in eastern australian flowering plants, *Australia common wealth scientific Industrial Research organization Bulletin*, 1959, 281.
13. Wilson JA, Merrill HB. *Analysis of leather and material used in making it*, 1st ed. The McGraw Hill Book Co. Inc., New York, 1931, 290-293.
14. Freudenberg K, Weinger K, Geissman A. *The chemistry of flavonoid compounds*, Pergamon Press. Oxford, 1962, 211.
15. Robinson T. *The organic constituents of higher plants, their chemistry and interrelationships*, Minneapolis 15 Minn., Burgers publishing company. 1947; 4(1):1964.
16. Clerk JD, Descamps A, Vander ME. *Colorimetric method for determining tannin*, *Bulletin Association Anciens etud*. Brass, University Louvain, 43(4), 68-76.
17. Harborne JB. *Chemical test In: Flavonoids, Phytochemical Methods*, 2nd ed. Champan and Hall Ltd. London, 1973, 42.
18. Feigl F. *Identification of individual organic compound*. In: *Spot Tests in Organic Analysis*, 4th ed. Elsevier Publishing Company, London, 1956, 237.
19. Malati G, APG Pillai. *Microscopic profile of powdered drugs used in Indian systems of medicine; Vol 2: Leaf drugs* Institute of Ayurvedic Medicinal Plant Sciences, Jamnagar, 2005.
20. Mammen D, Daniel M, Sane RT. *Pharmacognostic and Phytochemical studies on Leptadenia reticulata (retz.) wight & arn. and Ichnocarpus frutescens r. br. for identification of distinguishing biomarkers*. *Pharmacognosy Journal*. 2011; 2(18):7-12.
21. Malati G, APG Pillai. *Microscopic profile of powdered drugs used in Indian systems of medicine. Vol 1: Bark drugs*. Institute of Ayurvedic Medicinal Plant Sciences, Jamnagar, 2005.
22. Syed YH, Khan M, Bhuvaneshwari J, Ansari JA. *Phytochemical investigation and standardization of extracts of flowers of Woodfordia fruticosa; a preliminary study*. *J Pharm Biosci*. 2013; 1:134-140.
23. Pradhan N, Gavali J, Waghmare N. *WHO (World Health Organization) guidelines for standardization of herbal drugs*. *International Ayurvedic Medical Journal*. 2015; 3:2238-2243.