

PHARMACOGNOSTICAL STUDY ON SHALPARNI (DESMODIUM GANGETICUM L. DC) MARKET SAMPLES

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ABSTRACT

Desmodium gangeticum (L.) DC, commonly known as *Shalparni* in Ayurveda is an important medicinal herb being used for therapeutic purposes. The root of *Desmodium gangeticum* (L.) DC has been accepted as official drug by Ayurvedic Pharmacopoeia of India. It has been described in *Agryaprakaran* as *Vrisya sarvdosharanam*. Till date maximum quantity of the crude drug is being procured from its natural habitat. Due to over exploitation of herb it has been entered the list of endangered plant species in various states of the country. Crude drug markets are important in supply chain of the drug in herbal drug industry. Present study has been an effort towards exploration of crude drug being sold in the name of *Shalparni* in major crude drug markets of northern India. The study includes market survey and pharmacognostical evaluation of crude drug samples procured from various crude drug markets along with comparative pharmacognostical

evaluation of the market samples with reference sample collected from the field and pharmacopoeial standards of the crude drug published in Ayurvedic Pharmacopoeia of India.

Aim: The aim of present study was to evaluate pharmacognostical standards of market samples of crude drug *Shalparni*. **Methods:** Comparative pharmacognostical evaluation of Macroscopic and microscopic, physicochemical, phytochemical and HPTLC characters of market samples of crude drug with reference sample and pharmacognostical standards published in API has been undertaken. **Results:** Results of the study demonstrate that cuttings

of aerial parts of *Desmodium gangeticum* along with the roots are being sold in the crude drug markets. **Conclusion:** The present study is helpful to supplement the information with regard to standardization and identification of botanical sources of crude drug being sold in the crude drug markets.

KEYWORDS: *Ayurveda*, *Shalparni*, *Desmodium gangeticum*, market samples, Pharmacognosy.

INTRODUCTION

Quality of therapeutic formulations is of paramount importance for the planning and execution of successful treatment. For quality therapeutic formulations, quality of crude drug utilized in the manufacture of formulations is the basic requirement. With the depletion of natural sources of crude drugs, supply chain of crude drugs has been put under strain and crude drug markets play an important role in the supply of crude drug to herbal drug industry. *Shalparni* is one of the most important classical drugs being utilized in the manufacture of a variety of classical and proprietary formulations. *Shalparni* has been classified in *Balya*,^[15] *Shenhopag*,^[16] *Sothhar*,^[17] *Angmardparashamana*,^[18] *Vayasthapan mahakashya*^[19] and in *Madhur skandh*^[20] in *Charaka samhita*. It has also been described in *Agryaprakaran* as *Vrisya sarvdosharanam*.^[21] *Acharya Sushruta* has classified it in *Vidarighandhadi gana*,^[12] *Laghupanchmool*^[13] and *Dashmoola*.^[14] Annual demand of *desmodium gangeticum* roots has been estimated between 500 to 1000 metric tons.^[22]

A large gap has been created between demand and supply of crude drug *Shalparni* due to its increasing demand. Considering the important role of crude drug markets in the supply chain of crude drugs in the herbal drug industry, present study was planned and executed with the aim of exploration of crude drug being sold in the name of *Shalparni* in crude drug markets to study market trends. The study included comparative pharmacognostical evaluation of market samples with reference sample collected from field and pharmacopial standards of crude drug published in *Ayurvedic Pharmacopiea of India*.

MATERIAL AND METHODS

Procurement of crude drug Samples and Authentication

To conduct the study, survey of crude drug market was carried out and market samples being sold in market in the name of *Shalparni* were procured from three major crude markets of Northern India including Delhi, Lucknow and Haridwar. The reference sample of *Shalparni*

was collected from Pilibhit Tiger Reserve after in-situ identification by experts from Post Graduate Department of Dravyaguna, Lalit Hari State Ayurveda College and Hospital, Pilibhit. A herbarium of the plant was also prepared and deposited in the Post Graduate Department of Dravyaguna, Lalit Hari State Ayurveda College and Hospital, Pilibhit. All the samples were labelled in following manner as Sample collected from field as Reference Sample, Sample procured from Delhi crude drug market as Sample - 1, Sample from Lucknow crude drug market as Sample - 2, sample from Haridwar crude drug market as Sample - 3.

After labeling of samples, pharmacognostical evaluation of samples was done in the Pharmacognosy Laboratory, National Botanical Research Institute (NBRI), Lucknow. For quality evaluation of crude drug samples, parameters laid by Ayurvedic Pharmacopeia of India (API) were strictly followed. Parameters taken into consideration are -

Pharmacognostic evaluation organoleptic evaluation

Organoleptic characteristics of all four crude drug samples of *Shalparni* were assessed by observing colour, odour, taste, size and shape according to quality control methods described in Ayurvedic Pharmacopeia of India for herbal crude drugs.

Microscopic evaluation preparation of sections

Free handed sections of all four crude drug samples were cut into thin sections manually with sharp cutting edge of blade. Then transferred on slide, cleared by warming with chloral hydrate, stained with phloroglucinol and Conc. HCl and mounted in glycerin. The lignified and cellulosic tissues were recognized by utilizing different staining techniques.^[9]

Powder microscopy

The powder microscopy was performed according to the method mentioned in Khandelwal.^[9]

Physicochemical analysis

Physicochemical parameters such as ash value, moisture content and extractive values were determined according to the procedures mentioned in WHO quality control methods for herbal materials.^[2]

Phytochemical analysis

Methanol extract of all the four samples was prepared and were subjected to quantitative chemical estimation.

HPTLC Chromatography

HPTLC Chromatograph of methanolic extract of all the four samples of *Shalparni* was developed using Linomat 5 Application (Camag), Volume applied 10.0 μ L, in Solvent System - Toulene: Ethyle acetate: Formic acid (7:3:1); Scan Wavelength - UV 600 nm; TLC Plate Development -pre-saturated Camag Twin Through Chamber.

RESULTS

Organoleptic characteristics of reference sample and three market sample of *Shalparni* was assessed by observing shape, size colour, surface, odour and tastes.

Table No. 1: Organoleptic characters of crude drug samples.

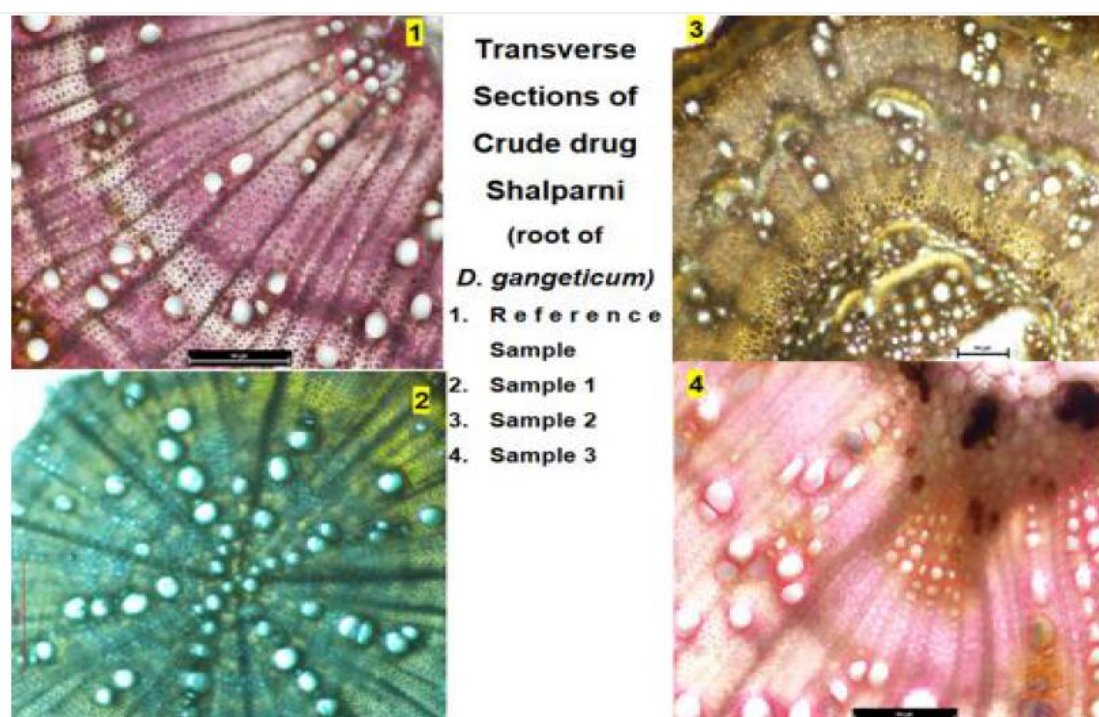
Organoleptic Characters of Reference and Market Sample of <i>Shalparni</i>				
Parameters	Reference sample	Sample 1 (Delhi)	Sample2 (Lucknow)	Sample 3 (Haridwar)
Shape	Uniformly Cylindrical	Irregular Cylindrical containing cuttings of ariel parts	Irregular Cylindrical	Irregular Cylindrical containing cuttings of ariel parts
Size	15 - 20 cm	2 - 3 cm	2.5 - 3 cm	2.5 -3 cm
Color	Light Brownish	Blackish Brownish	Yellowish Brown	Blackish Brownish
Surface	Rough	Rough	Rough	Rough
Odours	Odourless	Odourless	Odourless	Odourless
Taste	Feebly sweetish	Bitter	Bitter	Bitter



Microscopic evaluation

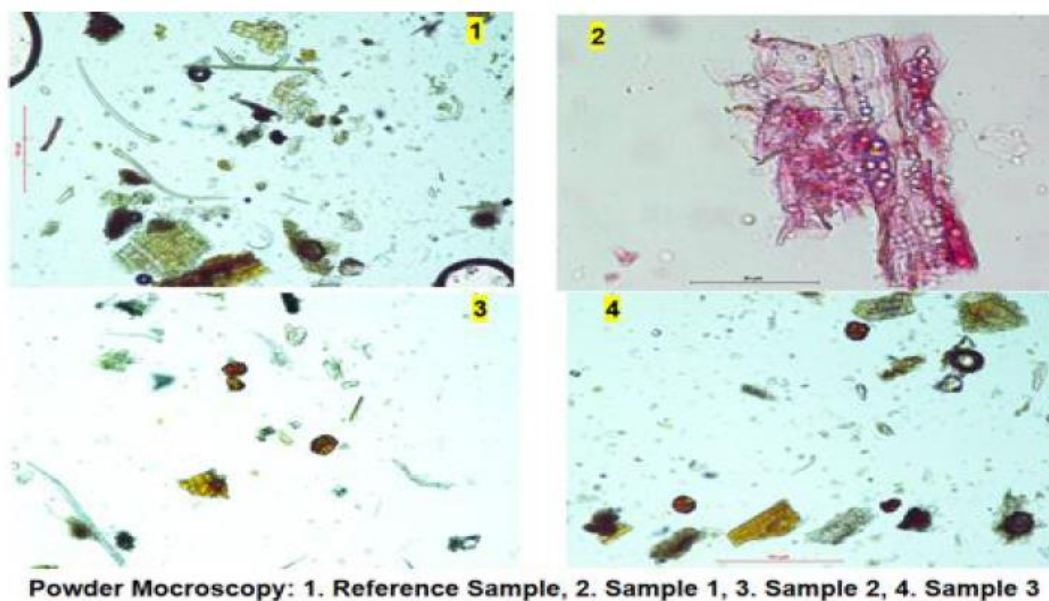
i. Preparation of section - Free handed sections of the root *Desmodium gangeticum* (One reference and three market sample) were cut into thin sections manually with sharp cutting edge of blade. Then transferred on slide, cleared by water and Safranin stain and mounted in glycerin.

Transverse section of roots - Compression of transverse section (T.S) of roots of *Desmodium gangeticum* procured from various sources has been represented in photographs given below. Microscopic observation reveals that microscopic structure of all the four sample are almost similar. Mature root shows cork, 3-7 layer of thin walled, tangentially elongated cells having a few prismatic crystals of calcium oxalate, cork cambium, single layered secondary cortex 4-10 layers of thin walled.



ii. Powder microscopy

Powder - Light brown; shows fragments of rectangular cork cells, vessels having reticulate thickening and bordered pits, xylem fibres, ray cells, prismatic crystals of calcium oxalate and simple round to oval starch grains, measuring 7-25 μ m dia. The presence of prismatic crystals of calcium oxalate, starch grain, lignified parenchyma, transversely elongated cork cells, septed fibers, pitted vessels, and simple fibers observed during the powder microscopy of *D. gangeticum* (L) DC.



Physicochemical analysis

All the four samples were subjected to Physicochemical Analysis. Observations Physicochemical values have been listed in following table.

Table No. 2: Physicochemical parameters of crude drugs.

Physicochemical parameters of crude drugs						
S. N	Parameters	Values in %				
		Reference Sample	Sample 1	Sample 2	Sample 3	API standard ³
1.	Foreign matter	0.0000	1.3000	1.0000	1.200	Not more then 2%
2.	Moisture content	7.2600	5.4600	5.8200	5.6900	Not Available
3.	Total Ash	4.9136	6.0544	5.3705	4.0419	Not more then 6%
4.	Acid Insoluble Ash	2.1783	3.0533	2.3250	1.5166	Not more then 2%
5.	Water soluble Ash	3.8500	4.2550	3.3600	4.3430	Not Available
6.	Alcohol soluble extractive	2.2700	0.9800	2.1600	1.6100	Not less than 1%
7.	Water soluble extractive	3.8500	4.2550	3.3600	4.3430	Not less then 6%
8.	N-Hexane soluble extractive	1.3402	0.5104	0.4832	0.4890	Not Available

Phytochemical analysis

All the four sample have been subjected to quantitative Phytochemical analysis. Observations of Phytochemical analysis of sample of *Shalparni* collected from various sources revealed that -

Table No. 3: Phytochemical concentrations in crude drug samples.

Phytochemical concentrations in crude drug samples					
S.N	Phytochemicals	Values in %			
		Reference sample	Sample 1	Sample 2	Sample 3
1.	Total sugar	0.02100	0.02800	0.03700	0.02200
2.	Total starch	0.12100	0.05400	0.09900	0.07600
3.	Total protein	0.01370	0.02180	0.02190	0.06980
4.	Total tannin	0.03150	0.02510	0.00510	0.04010
5.	Total alkaloid	7.00660	12.18160	6.67830	4.19000
6.	Total flavonoid	1.46540	0.88450	0.20230	0.57800
7.	Total Phenolics	0.00012	0.05077	0.08748	0.00012

HPTLC Chromatograph of Methanolic Extract of Crude Drug Samples

Table No. 4: Rf Values of Phytoconstituents in HPTLC Chromatogram.

Rf Values of Phytoconstituents in HPTLC Chromatogram						
S.N.	Reference Sample	Sample 1	Sample 2	Sample 3	Standard Markers	
					B Sitosterol	Lupeol
1.	0.008	-	0.004	-	-	-
2.	-	0.010	-	0.010	-	-
3.	0.042	-	-	-	-	-
4.	-	-	0.258	0.269	-	-
5.	0.279	-	-	-	-	-
6.	-	-	0.340	0.339	-	-
7.	0.351	0.347	-	-	-	-
8.	-	-	0.453	-	-	-
9.	-	0.496	-	0.492	-	-
10.	0.511	-	0.507	-	-	-
11.	0.546	-	0.540	-	-	-
12.	-	-	-	0.568	-	-
13.	-	-	0.646	-	-	-
14.	-	0.738	-	0.724	0.726	-
15.	-	-	-	0.749	-	-
16.	0.767	-	-	-	-	-
17.	-	-	0.779	-	-	-
18.	0.838	0.810	0.849	0.819	-	0.835
19.	-	-	-	-	-	-
20.	-	-	-	0.915	-	-
21.	-	0.967	-	0.968	-	-

DISCUSSION

Shalparni is one of the most frequently utilized drug for therapeutic purposes in Ayurveda. Therapeutic utility of drug has been evidenced from references of the drugs in classical texts of Ayurveda ranging from Samhita texts, Chikitsa Grantha, Kosha Grantha and Nighantu texts. It has been considered to be the drug capable of alleviating all type of pathology by *Acharya Charak* in *Agryapakaran* “*Vidarigandha Vrisya sarvdos haranam*”.

Root is the universally accepted useful part as evidence by its inclusion in *Laghupanchmool* and *Dashmool* by Acharya Sushruta.

It possesses wide range of pharmacological activities like Antioxidant activity,^[11] Anti-fertility activity,^[1] Anti-ulcer activity,^[4] Anti-inflammatory activity,^[7] Antimicrobial activities^[6] and effects on cardio vascular system^[10] and Central nervous system.^[8] It has also been demonstrated to possess anti-viral properties^[5] and effective in the treatment of Dengue.

Due to ever increasing demand and non judicious harvesting of the drug from wild, *Desmodium gangeticum* has entered into the list of endangered medicinal plants in various state of India.

Organoleptic Study of the four crude drug samples namely one reference sample and three market samples reveals that the taste of Reference sample was feebly sweetish as compared to the taste of crude drug samples procured from the market which has a bitter taste. This may be due to the Storage condition of the crude drugs procured from the market and Mixing of other plant parts like stem, leaves, fruits and inflorescence along with the root in market samples. Though, most of the texts consider *Shalparni mool* sweet and bitter in taste. The taste of the crude drugs may change due to post harvesting processing like washing, cutting, drying and storage. All other organoleptic characters of market samples like shape, size, surface and odour are almost similar. The difference observed in color of crude drug samples may also be result of post harvest processing and storage conditions.

Differences observed in the organoleptic characters of crude drug powders reflect slight difference in the color, odour and taste of the powder drugs. Color of powder of Reference sample and sample no.1 collected from Delhi are light brown were as the sample no. 2 and sample no. 3 procured from Lucknow crude drug market and Haridwar crude drug market respectively are yellowish green. Powder of Reference sample had sweet odour and was madhur tikt in taste where as powder of all the market samples was odourless and bitter in taste. This difference in color, odour and taste of reference sample as compared to market samples may be attributed to Post harvest processing like washing, cutting and drying, Storage conditions, Duration / time period of storage of market samples since harvesting, Mixing of roots with other plant parts like stem, leaves, inflorescence and fruits.

The differences observed in microscopic are transverse section (T.S) of reference sample and market sample procured from crude drug markets Delhi, Lucknow and Haridwar may again be attributed to the post harvest handling, drying and storage conditions of crude drug samples. Long term storage of crude drugs may alter the presence of various bio-molecules in the cell wall, cell membrane, cytoplasm of the cells.

The result of Physicochemical analysis of the crude drug samples namely Reference sample and market sample comparatively analyzed with crude drug standards described in Ayurvedic Pharmacopoeia of India (A.P.I) has been under taken. As mentioned earlier, a Reference sample of the crude drug was collected from field.

In Foreign matter analysis, Reference sample was found to be free from presence of any foreign matter and market samples were found to contain some amount of foreign matter which appeared to be cuttings of stem, leaves, inflorescence and fruits of *D. gangeticum*. Value of foreign matter was within the limits of crude drug standard described in API i.e. below 2%. Reference sample was subjected to proper post harvesting processing, therefore was devoid of any foreign matter. Analysis of Moisture content in crude drug sample reflected highest moisture content in Reference sample. Permissible limit of moisture content in crude drug has not been mentioned in API. Reference sample was freshly collected, washed, cut in to pieces and dried. Therefore has highest moisture content.

Analysis of Total Ash value reveals that sample no.1 collected from Delhi contained highest value of total ash content which was 6.0544%, slightly higher then the permissible limit of total ash value. The findings suggest that the sample procured from market of Delhi, due to presence of highest foreign matter might had highest total ash value. Total ash value of other samples namely Reference sample, sample no. 2 collected from Lucknow and sample no. 3 purchased from Haridwar crude drug market was within the limits of API Standards.

Analysis of acid insoluble ash reveals that all the crude drug samples namely Reference sample, sample no.1 collected from Delhi and sample no. 2 collected from Lucknow had acid insoluble ash value above permissible limit of 2%. Only sample no. 3 procured from Haridwar crude drug market had acid insoluble ash value 1.5166% which was within API Standards. Acid insoluble ash value reflects the presence of impurities, minerals, siliceous materials and the inorganic residue remaining after treatment of ash with acid. In the present study, acid insoluble ash value of reference sample was 2.1783%, slightly higher then API

Standards where as acid insoluble ash value of market samples collected from Delhi and Lucknow crude drug markets were much higher then the permissible limit. The higher acid insoluble value may be attributed to higher mineral contents in the soil from where the crude drug have been collected and inorganic impurities present in the market samples.

Water soluble ash value was minimum in sample no. 2 and maximum in sample no. 3. The API Standard for water soluble ash value has not been defined in API Standard. Water soluble ash value is the indicated of water soluble inorganic salts present in ash. Higher water soluble ash value indicates higher amount of inorganic contents present in ash and soluble in water.

Alcohol soluble extractive value was found to be highest in Reference sample and lowest in sample no. 1 collected from Delhi crude drug market. According to API Standards alcohol soluble extractive value should not be less then 1%. Thus the crude drug sample procured from Delhi crude drug market was below standard. Crude drug samples namely Reference sample and sample procured from Lucknow crude drug market and sample procured from Haridwar crude drug market were within quality Standards.

Water soluble extractive value of crude drug sample collected from Lucknow crude drug markets was lowest. Water soluble extractive value of all the crude drug samples analyzed were found to be below API Standard which is minimum 6%. Low water soluble extractive value reflect that Either immature plants have been collected, Or the process of extraction of water soluble extractive has been prematurely terminated due to which water soluble extractive value might have gone down. Value of N-Hexane soluble extractive value was found to be highest for Reference sample. N-Hexane soluble extractive value signifies presence of non polar Bio- molecule in the crude drug sample. Though N-Hexane soluble extractive value has not been described in API.

Phytochemical Analysis of crude drugs samples reveals that sugar contents in Reference sample was lowest. The total sugar content in sample no. 2 procured from Lucknow crude drug market (0.037%) was highest. The concentration of sugar content in any plant tissue is determined by Geo climatic condition and the age (Maturity) of plant tissue. The total starch content was found to highest in Reference sample where as total protein content was highest in sample no. 3 collected from Haridwar crude drug market. Total alkaloid content were highest in crude drug sample procured from Delhi followed by reference sample where as

total flavonoid was found in Reference sample. The concentration of phenolic compound was highest in sample no. 2 procured from Lucknow crude drug market.

The concentration of primary and secondary metabolites present in any crude drug may be effected by Geo-climatic condition in which plant grow, Developmental and environmental factor like sun light, temperature, water salinity, genetic factors and plant parts utilized.

HPTLC Fingerprinting of all the four crude drug samples was carried out using B- Sitosterol and Lupeol as standard markers compound. Methanolic extract of crude drugs was used for HPTLC Chromatogram. The Chromatogram reveals that Sample no. 1 reveals of a bio-molecule having Rf value 0.738, sample no. 3 reveals a bio-molecule having Rf value 0.724, Reference sample reveals presence of bio-molecule having Rf value 0.767 and sample no. 2 reveals presence of bio-molecule having Rf value 0.779. All these Rf value may be approximate with Rf value of B-Sitosterol (0.726). All the four crude drug sample analyzed show presence of B-Sitosterol, Reference sample shows presence of bio-molecule with Rf value 0.838, Sample no. 1 shows presence of bio-molecule having Rf value 0.810, Sample no. 2 show presence of bio- molecule having Rf value 0.849 and sample no. 3 shows presence of bio-molecule having Rf value 0.819. All these Rf values of bio-molecules present in samples analyzed correspond to the Rf value of Standard marker Lupeol (0.835). Therefore all the four samples analyzed using HPTLC Chromatogram may be considered to demonstrate presence of Lupeol.

CONCLUSION

From the study it may be concluded that Crude drug sample procured from the market were botanically the roots of *Desmodium gangeticum* having slightly lower quality. Quality of crude drug *Shalparni* procured from market has been found to be below standards published in Ayurvedic Pharmacopoeia of India due to supply of crude drug adulterated with other plant parts in place of official crude drug “Root of *Desmodium gangeticum*”. This trend of supply of whole plant in place of root adversely affects quality and therapeutic efficacy of finished herbal drugs and needs to be checked with the development of monitoring system of supply chain of crude drug and crude drug markets.

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