

A PRELIMINARY PHYSICO-CHEMICAL ASSAY OF GOKSHURA-PUNARNAVA BASTI – A PILOT STUDYRamteke Rajkala^{1*}, Thakar AB², Shukla VJ³, Harisha CR⁴¹PhD Scholar, Department of Panchakarma, IPGT&RA, GAU, India²Reader, Department of Panchakarma, IPGT&RA, GAU, India³Head, Pharmaceutical Chemistry Laboratory, IPGT&RA, GAU, India⁴Head, Pharmacognosy Laboratory, IPGT&RA, GAU, India

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ABSTRACT

Tribulus terrestris Linn belonging to Zygophyllaceae family commonly known as Goksura is used in Ayurvedic system of medicine; commonly known as Puncture vine. The plant is considered as Diuretic used in Diabetes mellitus and heart diseases. *Boerhaavia diffusa* Linn belonging to Nyctaginaceae family known as Hogweed and in Ayurveda named as Punarnava. It is also well known for its diuretic and rejuvenating action. Gokshura and Punarnava have been practiced on urinary tract disorders since ancient time. This has been proven that Basti route is provided better effect in short term administration. As this *Basti* is combination of these only two drugs; this initial attempt was made to evaluate its physico-chemical profile. Pharmacognostically authenticated *Tribulus terrestris* and *Boerhaavia diffusa* was used for the preparation of Basti and it was analyzed through qualitative and quantitative analysis for physico-chemical parameters. Fingerprints of Thin Layer Chromatography (TLC) and High- Performance Thin Layer Chromatography study (HPTLC) also carried out.

KEYWORDS: *Tribulus terrestris*, *Boerhaavia diffusa*, Chromatography.***Corresponding author**PhD Scholar, Department of Panchakarma, IPGT&RA, GAU, India Email: vaidyarajkala@gmail.com**INTRODUCTION**

Acharya Charaka has explained that one should modify the potency of drugs from lower to higher side and vice versa, by combination, elimination, timing, processing and method of administration¹. Acharya Charaka, explained Goksura under Madhuraskandha dravya (Group of drugs possessing sweet taste), Mutravirechana gana (Group of drugs with Diuretic properties), Svyathuhara gana (Group of drugs with Anti inflammatory properties) and Anuvasanopaga gana (Group of drugs used in preparation of unctuous enema), where as Acharya Susruta included this drug under Vidarigandhadi gana and Kantak Panchamula (Group of five thorny drugs whose roots are used).

Punarnava explained by Acharya Charaka under Virechanadravyani (Group of drugs with purgative properties), Svedopaga (Group of drugs used in preparation of sudation), Anuvasanopaga (Group of drugs used in preparation of unctuous enema), Kasaharani (Group of drugs used in cough). Susruta included in Vidarigandhadi Gana (Group of drug

commencing from Vidarigandha) and Vatasamshana Varga (Group of drug pacify Vata).

Tribulus terrestris (Zygophyllaceae) is a procumbent annual or perennial herb with many spreading slender branches, the immature portions covered in a fine silky hair². It is commonly known as Caltrops, Puncture vine and it is distributed throughout tropical and warm temperate regions of the world³. Literature survey reveals the presence of Harman⁴, chlorogenin⁵, diosgenin and its acetate, furostanol glycoside, spirosterol saponin and a dihydroxy spirosteroidal sapogenin, saponins C & G, kaempferol, kaempferol-3-glucoside, kaempferol-3-rutinoside, kaempferol-3-β-D- (6''-p-coumaroyl) - glucoside, glucose, amino acids, alkaloids harmine, harmaline, harman⁶ and gitogenin in hydrolysed extracts of fruits, roots and leaves⁷. Plant is used as Diuretic⁸.

Boerhaavia diffusa Linn (Nyctaginaceae) is a small perennial creeping herb, commonly known as "Red hogweed" and distributed widely all over in India, and in many other countries. The root and the whole plant are used as an Ayurvedic medicine in India and Unani

medicine in Arab countries for the treatment of diabetes⁹, stress, inflammation¹⁰ and congestive heart failure. The plant is known to possess anti-inflammatory¹¹, diuretic¹² and immunomodulatory¹³ activities. It has also been reported to be useful in the treatment of elephantiasis and nephrotic syndrome¹⁴.

The *Boerhaavia diffusa* plant contains a large number of such compounds as flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins, and glycoproteins. Also punarnavine, boeravinone and punarnavoside. The herb and roots are rich in proteins and fats. The herb contains 15 amino acids, including 6 essential amino acids, while the root contains 14 amino acids, including 7 essential amino acids.

In the description of Chatuspada (Four Limbs of Treatment), Drugs have the second most importance in treating the diseases and also the drug should possess the good qualities in it. So, proper identification and standardization of the drug is essential. Each and every drug has its own physical and chemical characteristics that help for separating it from other closely related drugs. Hence physico - chemical studies of a particular drug by making use of various parameters help in standardizing the drug and validate it. Thus the present study was aimed at evaluating the pharmacognostical features and physico - chemical analysis of the Goksura – Punarnava Basti.

AIMS & OBJECTIVES

1. Pharmacognostical study of powdered drug – Goksura and Punarnava
2. Physico-chemical analysis of *Goksura- Punarnava Basti*

MATERIALS AND METHODS

Plant Material

The dried fruits of *Tribulus terrestris* and roots of *Boerhaavia diffusa* were collected from the Pharmacy, Gujarat Ayurved University, Jamnagar. Both drugs were pulverized; raw powder and fine powder was collected. The powder was subjected to powder microscopy.

Pharmacognostical Study

Morphological, Organoleptic and Microscopic study of the powdered drug was done as per the guidelines of Ayurvedic pharmacopoeia of India¹⁵ at Pharmacognosy Laboratory, I.P.G.T & R.A, Jamnagar.

Preparation of Gokshura-Punarnava Basti

The Gokshura - Punarnava oil prepared in the Pharmacy of I.P.G.T & R.A. and the Basti was prepared using these raw powder for decoction of Basti and fine powder for paste of Basti in Panchakarma Department of I.P.G.T. & R.A., Gujarat Ayurved University, Jamnagar.

Asthapana Basti was prepared by taking Honey (75 ml) and Rock salt (5 gm) first in mortar and continues stirred with pestle; then Gokshura - Punarnava oil (150 ml) is added and mixed well. Then paste of Gokshura - Punarnava fine powders (15 gm) was added and at last decoction of Gokshura - Punarnava (350 ml) added in mixture and churned well.

Physico - chemical study

This Gokshura-Punarnava Basti was analyzed by using, qualitative and quantitative parameters at Pharmaceutical Chemistry Laboratory of I. P. G.T & R. A., Gujarat Ayurved University, Jamnagar.

RESULTS AND DISCUSSION

Pharmacognostical Study

A. Organoleptic characters

1. **Gokshura:** The powder was greenish yellow in colour, has pleasant odour, sweet and bitter in taste.
2. **Punarnava:** The powder was brownish white in colour, has none odour and bitter in taste.

B. Powder Microscopy

The dried powder of Goksura (*Tribulus terrestris*) and Punarnava (*Boerhaavia diffusa*) were mounted in the distilled water to detect microscopic characters.

1. Gokshura

The unicellular trichomes, starch grains, stratified fibers, oil globules, prismatic crystals of calcium oxalate. When stained with Phloroglucinol and conc. HCl, lignified cells with polygonal cells of mesocarp cells, lignified parenchyma, stone cells were observed. (**Plate No.1**)

2. Punarnava

Acicular crystal, cork cells in transverse and surface view, starch grains, prismatic crystal, pitted vessel, fibres. All the microscopic characteristics identified were equivalent to the standard profile. (**Plate No.2**)

Physico - chemical Study

Organoleptic Characters: The characters of the sample are tabulated in **table 1**.

Physico-chemical parameters: The Basti was evaluated for physico - chemical parameters like Specific gravity, Refractive index, Iodine value, Acid value, pH value, Sugar estimation (Total sugar), Saponification value and Viscosity. The results were placed at **table 2**.

There are no parameters for Basti is explained in Ayurvedic Pharmacopoeia of India so this is an attempt to break new ground for Basti; as this Basti contains only two drugs i.e. Gokshura and Punarnava, Madhu (Honey) and Saindhava (Rock salt). Every Basti have specific consistency and some physical parameters so above parameters were studied. The difficulties with oil content which was not suitable for many instruments some parameters cannot report.

Qualitative Test of Goksura – Punarnava Basti: The methanol extract of the sample was analyzed qualitatively for different functional groups. Details are placed at **table 3**.

Thin layer chromatography

Chloroform Extract

Acid hydrolysis process was used for this extraction¹⁶. 20 ml of methanolic extract in round bottom flask was taken. 20 ml of 2M HCl was added, and refluxed it for 2 hours. Then chloroform added in it and chloroform layer separated with the help of separating funnel. Then it was evaporated in clean and dried evaporating dish; the residue was diluted with chloroform and used for further study i.e. TLC and HPTLC.

TLC of Chloroform Extract of Goksura – Punarnava Basti

Chloroform extract was used for the spotting of the TLC plate (Silica gel - G Precoated plates). Then the spotted TLC was run with the solvent systems (Toluene – 8.5 ml, Ethyl acetate - 2 ml, Glacial acetic acid - 0.5 ml) separately. And the resulting TLC pattern was viewed under long wave ultra violet light at 366 nm or Short wave ultra violet light at 254 nm (Table no. 4). Then after spraying with the Anisaldehyde Sulphuric acid reagent and drying in a hot air oven and the number of spots viewed under daylight (Table no. 5). (Plate No.3)

The findings of thin layer chromatography of Goksura-Punarnava Basti at 366nm and 254nm UV light are as follows-

Adsorbent: Silica gel – G

Solvent system: Toluene : Ethyl acetate : Glacial acetic acid (8.5: 2: 0.5)

TLC of chloroform extract of *Basti* shows four spots under 366 nm U.V. at hR_f 0.16, 0.5, 0.64 and 0.84.; where as in 254 nm three zones visible at hR_f 0.16, 0.73 and 0.98. On running mobile phase over stationery phase, well distributed, distinct and clear spots were observed without clumping.

TLC of chloroform extract of Gokshura-Punarnava Basti after spraying Methanolic Sulphuric acid (5%) followed by heating and then visualized in day light shows 5 prominent spots at hR_f 0.16, 0.2, 0.52, 0.66 and 0.9.

High Performance Thin layer chromatography:

Chloroform extract of Gokshura - Punarnava Basti were spotted on precoated silica gel - GF 60254 aluminium plate as 5mm bands, 5mm apart and 1cm from the edge of the plates, by means of a Camag Linomate V sample

applicator fitted with a 100 μ L Hamilton syringe. Toluene (8.5ml), Ethyl acetate (2ml), Glacial acetic acid (0.5ml) (v/v) (20ml) was used as a mobile phase. The development distance was 6.4 cm (development time 30 min.). After development, densitometric scanning was performed with a Camag T.L.C. scanner III in reflectance absorbance mode at 254 nm and 366 nm under control of win CATS software (V 1.2.1 Camag) (Fig No.1, 2). The slit dimensions were 6 mm x 0.45 mm and the scanning speed was 20 mm s⁻¹ (Table no.6). Then the plate was sprayed with Methanolic Sulphuric acid (5%) followed by heating and then visualized in day light shows eight prominent spots (Table no. 7).

The findings of High performance thin layer chromatography of Goksura – Punarnava Basti at 366nm and 254nm UV light are as follows-

Adsorbent: Aluminium - backed Silica gel GF 60₂₅₄ HPTLC plates

Sample Application: By Auto-sampler CAMAG Linomat V

Solvent system: Toluene : Ethyl acetate : Glacial acetic acid (8.5:2:0.5)

HPTLC of chloroform extract of Basti shows five spots under 366 nm U.V. at hR_f 0.21, 0.27, 0.34, 0.49 and 0.58.; where as in 254 nm six spots visible at hR_f 0.19, 0.34, 0.49, 0.57, 0.76 and 0.93.

HPTLC of chloroform extract of Gokshura-Punarnava Basti after spraying Methanolic Sulphuric acid (5%) followed by heating and then visualized in day light shows eight spots at hR_f 0.27, 0.42, 0.55, 0.62, 0.71, 0.78, 0.85 and 0.95.

CONCLUSION

The plant *Tribulus terrestris* and *Boerhaavia diffusa* are used from the ancient time for its medicinal values and most of the Ayurvedic formulations prescribed for various diseases have Goksura and Punarnava as one of the ingredient. In present study, Basti form was chosen as its immediate action. This study was aimed to prove genuinity of the drug used and to assess the physico - chemical characteristics in Basti form. The results were found to be significant and encouraging towards the goal for standardizing Goksura – Punarnava Basti.

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Table 1 - Organoleptic Parameters of Goksura – Punarnava Basti

Sr. No.	Parameters	Results
1.	Colour	Brownish black
2.	Taste	Sweet, Bitter
3.	Odour	Non Irritant
4.	Consistency	Liquid

Table 2: Physico-chemical parameters

Sr. No.	Parameters	Gokhura- Punarnava	
		Taila	Asthapana Basti
1.	Specific gravity	0.915	1.075
2.	Refractive index	1.475	--
3.	Iodine value	97.028	--
4.	Acid value	4.50	--
5.	pH Value	5.76	5.0
6.	Sugar Estimation (Total sugar)	--	24.1 %w/w
7.	Saponification value	136.98	--
8.	Viscosity	--	30.83

Table 3: Functional Groups

Sr. No	Test Group	Name of Test	Results
1	Carbohydrate	Molisch's test	Positive
2	Steroid	Liebermann – Burchard test	Positive
3	Saponin Glycosides	Foam test	Positive
4	Flavonoids	Lead acetate test	Positive
5	Alkaloids	Dragendroff's test	Negative
		Wagner's test	Negative
6.	Proteins	Biuret test	Positive
7.	Tannin	General test	Negative

Table 4: TLC findings of Gokshura – Punarnava Basti

Sr. No.	Wavelength	Number of spots	R _f value
1.	366 nm	4	0.16
			0.5
			0.64
			0.84
2.	254 nm	3	0.159
			0.727
			0.977

Table 5: TLC - After spraying Methanolic Sulphuric acid (5%)

Sr. No.	Number of spots	R _f value	Observation
1.	5	0.16	Blue spot
		0.2	Blue spot
		0.52	Green spot
		0.66	Purple spot
		0.9	Orange spot

Table 6: HPTLC of Chloroform Extract of Gokshura – Punarnava Basti

Sr. No.	Wavelength	No. of spots	R _f value
1	366 nm	5	0.21
			0.27
			0.34
			0.49
			0.58
2	254 nm	6	0.19
			0.34
			0.49
			0.57
			0.76
			0.93

Table 7: HPTLC after spraying with Methanolic Sulphuric acid (5%)

Sample	Solvent System	No. of spots	R _f value
CHCl ₃ Extract of <i>Gokshura</i> – <i>Punarnava</i> <i>Basti</i>	T : E.A : G.A.A (8.5:2:0.5)	8	0.27
			0.42
			0.55
			0.62
			0.71
			0.78
			0.85
			0.95

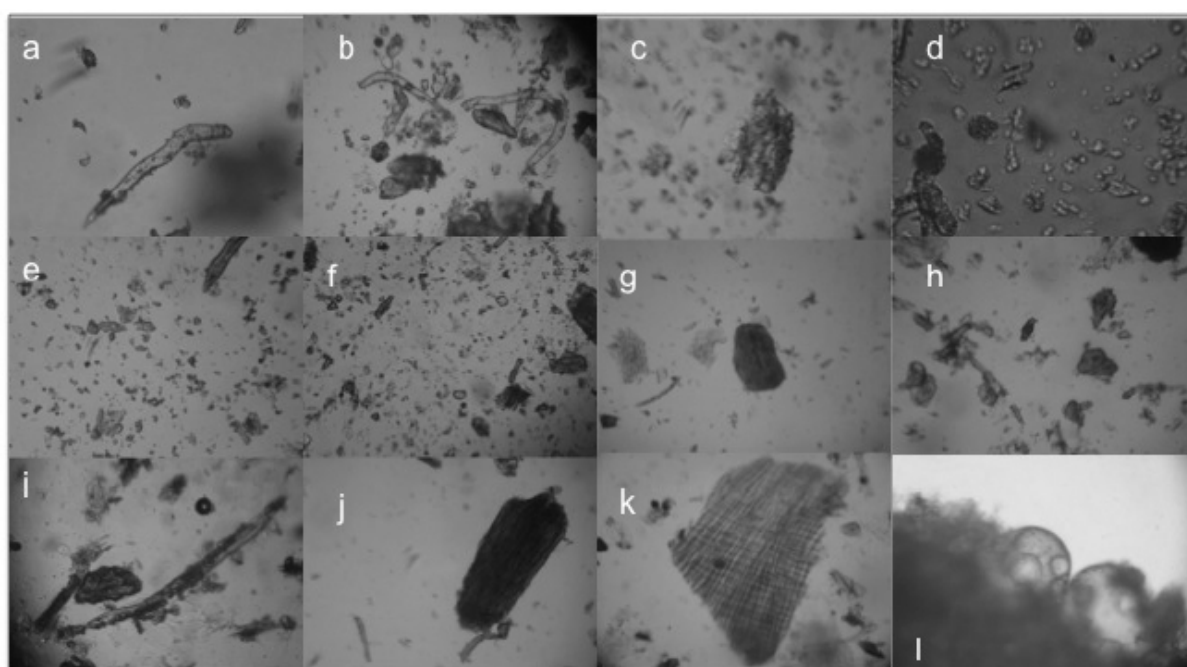


Plate no: 1 - a. Trichome, b. Trichome with Starch grain, c. Parenchymal cells, d. Rosette crystals, e. Starch grains, f. Prismatic crystals, g. Mesocarp cells, h. Stone cells, i. Fiber with stone cells, j. Lignified fibers, k. Lignified cells with polygonal cells of mesocarp, l. Oil globules.

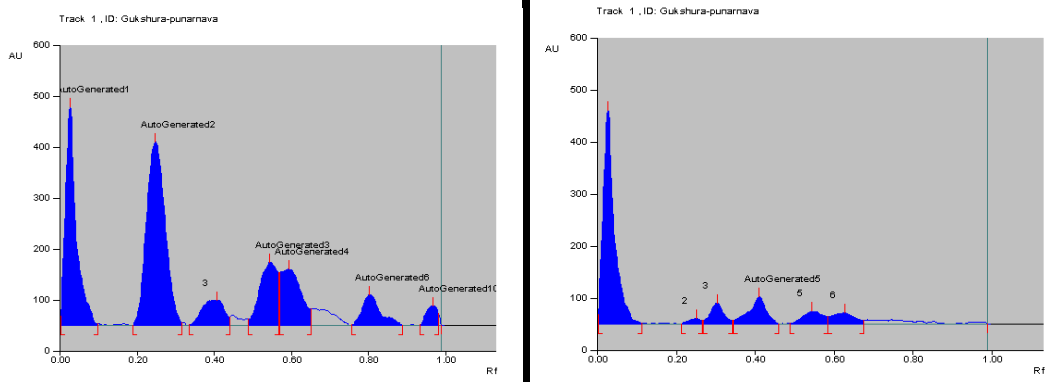
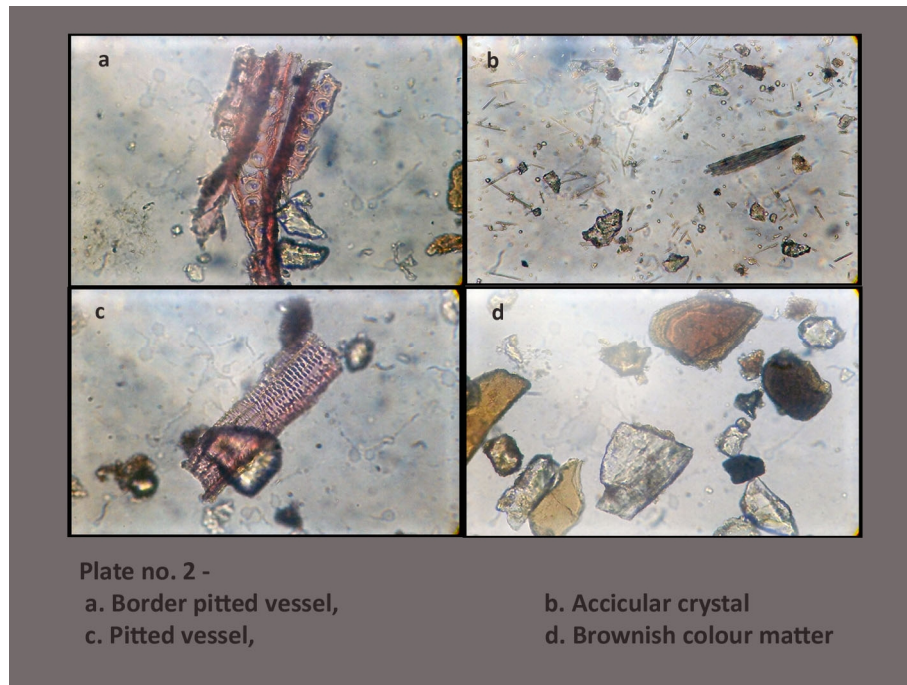


Figure 1: Densitogram curve of Gokshura-Punarnava Basti Extract in 254nm & 366nm

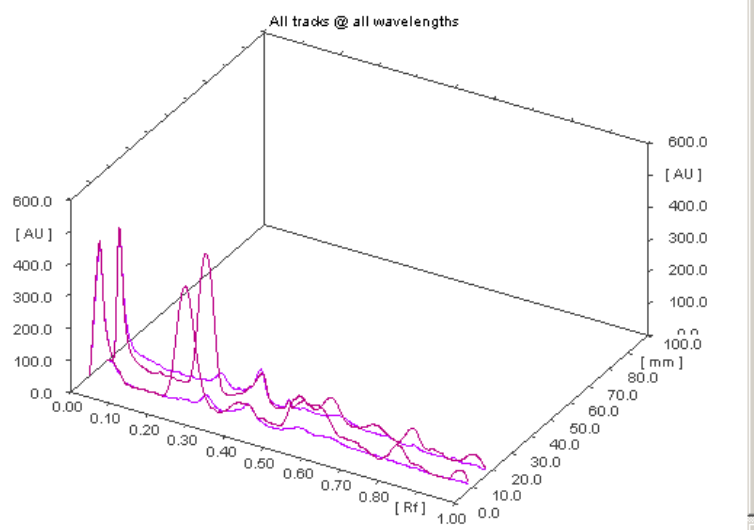


Figure 2

Source of support: Nil, Conflict of interest: None Declared