

A PRELIMINARY PHYSICO CHEMICAL ASSAY OF *GOKSURA* GRANULES – A PILOT STUDY

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Abstract

Tribulus terrestris (Linn) of Zygophyllaceae family commonly known as *Goksura* is used in Ayurvedic system of medicine. It is distributed through out tropical and warm temperate regions of the world. Commonly it is known as Caltrops, Puncture vine. The Fruits of *Goksura* are considered as Diuretic, Aphrodisiac; used in Urolithiasis, Sexual dysfunctions and Infertility. As the powder is not palatable, its form has been converted in to granules form and attempts were made to evaluate its physico chemical profile. Pharmacognostically authenticated *Tribulus terrestris* was used for the preparation of granules and it was analyzed through qualitative and quantitative analysis of Physico – chemical parameters. Fingerprints of Thin Layer Chromatography (TLC) and High-Performance Thin Layer chromatography study (HPTLC) were also developed.

Keyword: *Tribulus terrestris*, Zygophyllaceae, *Goksura*, Phytochemical Analysis, Chromatography, Ayurveda.

Introduction:

In the description of *Shodasakala Chikitsa* (1) (Sixteen Pre-requisite Qualities), Ayurvedic classics have given special importance to the Physician's ability to understand the rationale behind the utility of natural components in modifying the disease conditions and in re-establishing the equilibrium of *Doshas* (Humour), *Dhatu*s (Tissues) and *Mala*'s (Waste Products) or in other words, maintaining the Health.

Acharya Charaka, explained *Goksura* under *Madhuraskandha dravya*(2) (Group of drugs possessing sweet taste), *Mutravirechana gana* (Group of drugs with Diuretic properties), *Svayathuhara gana* (Group of drugs with Anti inflammatory properties) and

Anuvasanopaga gana(3) (Group of drugs used in preparation of unctuous enema), where as *Acharya Susruta* included this drug under *Vidarigandhadi gana*, *Virataruvadi gana*(4) (Group of drug commencing from *Vidarigandha* and *Virataruvadi*), and *Kantak panchamula*(5) (Group of five thorny drugs whose roots are used).

Tribulus terrestris (Zygophyllaceae) is a procumbent annual or perennial herb with many spreading slender branches, the immature portions covered in a fine silky hair.(6) It is commonly known as Caltrops, Puncture vine and it is distributed through out tropical and warm temperate regions of the world. (7)

Literature survey reveals the presence of Chlorogenin, diosgenin and its acetate, gitogenin, astragalin, dioscin, gracillin, hecogenin, ruscogenin, trillin, furostanol glycoside, spirosterol saponin and a dihydroxy spirosteroidal saponin,

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trigogenin-3-diglucorhamnoside (terrestroside F), saponins C & G, kaempferol, kaempferol-3-glucoside, kaempferol-3-rutinoside, kaempferol-3- β -D- (6''-p-coumaroyl) - glucoside, glucose, rhamnose, rutin, harmine, neogitogenin, quercetin, reducing sugars, campesterol, β -sitosterol, stigmasterol, amino acids, alkaloids harmine, harmaline, harman and tetrahydroharmine; neogitogenin, amino acids (root), Tigogenin, Hecogenin and Gitogenin in hydrolysed extracts of fruits, roots and leaves.(8) Fruits of *Gokshura* are used as Diuretic(9), Aphrodisiac(10) and indicated in Urolithiasis(11), Sexual dysfunction as well as Infertility(12).

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(13). 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 physicochemical studies of a particular drug by making use of various parameters help in standardizing the drug and validate it. Chromatographic techniques were adopted for the separation of active moieties present in the formulation. Therefore, an attempt has been made to standardize *Gokshura* granules, an Ayurvedic medicine based on their TLC and HPTLC fingerprint profile.

Aims & objectives:

1. Pharmacognostical Study of powdered drug – Fruits of *Gokshura*
2. Physico-chemical analysis of *Gokshura* Granules

Method of preparation of the *Gokshura* Granules

The dried fruits of *Gokshura* were subjected to pulverizer to get fine powder. Equal quantity of sugar was taken and syrup was prepared by adding sufficient quantity of water in mild flame with

constant stirring till syrup reaches the *Tantumathvam* (thread like) stage. Then *Gokshura* powder was added to the sugar syrup and mixed thoroughly to prepare a homogeneous blend. The blended mass was sieved through a 40# sieve to obtain granule form and kept it for drying in room temperature.

Materials & methods:

Plant Material: The dried fruits of *Tribulus terrestris* were collected from the Pharmacy, Gujarat Ayurved University, Jamnagar. The fruits were pulverized and sieved through # 80 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 India(6) at Department of Pharmacognosy, I.P.G.T & R.A, Jamnagar.

The powder was converted in to granules at the Pharmacy, Gujarat Ayurved University, Jamnagar.

Physico - chemical study: *Gokshura* granules were analyzed by using, qualitative and quantitative parameters at Pharmaceutical Chemistry Laboratory of I. P. G.T & R. A., Gujarat Ayurved University, Jamnagar.

RESULTS & DISCUSSION:

Pharmacognostical Study:

Organoleptic characters: The powder was greenish yellow in color, has pleasant odor and sweet and bitter in taste.

Powder Microscopy: The dried powder was mounted in the distilled water to detect 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). All the microscopic characteristics identified were equivalent

Physico - chemical Study:

Organoleptic Characters: The characters of the sample are tabulated in table no.2

Physico-chemical parameters: The granules were evaluated for physico chemical parameters like Total Ash Value, loss on drying, pH value, Sugar estimation (Total sugar, Reducing Sugar, Non - Reducing Sugar), Acid soluble and water-soluble extractive values. The results were placed at table no.3

The Common parameters mentioned for *Goksura* in Ayurvedic Pharmacopoeia of India are total ash, pH Value, water and alcohol soluble extractives (6). On its basis the parameters like total ash content, water and methanol soluble extractives etc., were selected. Presence of more moisture content in a sample can create preservation problem. Hence loss on drying was also selected as one of parameters. Since, the sample was in the form of granules has the possibility of containing significant quantity of sugar, hence Sugar estimation was considered as another parameter. Total sugar was found to be 50.73 % w/w suggesting presence of considerable amount of sugar in the sample. The water-soluble extractive and methanol soluble extractive values were found to be 51.6 % and 27 % respectively, indicating considerable amount of polar compounds in the sample.

Qualitative Test of *Goksura* Granules:

The methanol extract of the sample was analyzed qualitatively for different functional groups. Details are placed at table no.4.

Thin layer chromatography:

Methanol Extract:

Granules weighing 5 gm are taken with 100 ml of alcohol kept for twenty-four hours. Filtrate was prepared and evaporated till it gets dried in a flat-

to the standard profile. (6)

bottomed shallow dish and concentrated on water bath to volume of requirement.

TLC is mentioned as a primary tool for identification as part of monographs on all medicinal plants. (14) Alkaloid fraction 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 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.5). Then after spraying with the Anisaldehyde Sulphuric acid reagents and drying in a hot air oven and the number of spots viewed under daylight (Table no.6). (Plate No.2)

TLC of alcoholic extract of drug on silica gel "G" plate using Toluene (8 ml): Ethyl acetate (2 ml): Glacial acetic acid (0.5 ml) shows five spots Under 366 nm U.V. at R_f 15, 43, 50, 72 and 97. Where as in 254 nm three zones visible at R_f 15, 72, 97. On running mobile phase over stationary phase, well distributed, distinct, clear spots were observed without clumping.

Thin Layer Chromatography of alcoholic extract of *Goksura* Granules after spraying Anisaldehyde Sulphuric acid followed by heating and then visualized in day light shows 7 prominent spots at R_f 22, 40, 54, 65, 77, 86, 97

High Performance Thin layer chromatography:

Methanol extract of *Goksura* Granules 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 ml), Ethyl acetate (2 ml), Glacial acetic acid (0.5 ml) (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). The slit dimensions were 6 mm x 0.45 mm and the scanning speed was 20 mm s⁻¹ (Table no.7). Then the plate was sprayed with Anisaldehyde Sulphuric acid followed by heating and then visualized in day light shows 4 prominent spots (Table no. 8).

Visual observation under UV showed multiple spots, but on analyzing under densitometer all the spots were not detected. This may be due to the limitations of the integrative system Savitsky – Golay 7 with following specifications like Slope – 5; Minimum height – 10AU; Minimum area – 50 AU and Maximum height was 990 AU. However, chromatogram shows 7 prominent spots at hRf 3, 16, 42, 61, 68, 84, 98 in short wave uv 254 nm and 2 prominent spots at hRf 3, 98 in long wave uv 356 nm.

HPTLC of Methanolic extract of *Gokshura* Granules after spraying Anisaldehyde Sulphuric acid followed by heating and then visualized in day light shows 4 prominent spots at hRf 3, 46, 71, 78.

CONCLUSION:

The plant *Tribulus terrestris* is used from the ancient time for its medicinal values and most of the Ayurvedic formulations prescribed for various diseases have *Gokshura* as one of the ingredients. Most of the formulations are clinically effective but cumbersome for the patient compliance. In the present study, the granule form was evaluated for pharmaceutical feasibility and for patient palatability.

The plant *Tribulus terrestris* was identified and authenticated

pharmacognostically and was used as a unique ingredient. The formulation namely, *Gokshura* Granules were subjected to phytochemical, physico-chemical, TLC and HPTLC studies. It is inferred that the formulation meets the minimum qualitative standards as reported in the API at a preliminary level.

On the basis of our observations and experimental results, we created method of preparation of *Gokshura* granules for the first time and are economical in terms of time and machinery. This study may be used as reference standard in the further quality control researches. Further studies may be carried out based on identification and separation of active ingredients with the help of Biomarkers like Protodioscin.

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Table No.2 Organoleptic Parameters of Goksura Granules

S.No	Parameters	Goksura Granules
1	Texture	Rough
2	Color	Yellowish brown
3	Taste	Sweet, Bitter
4	Odor	Pleasant

Table No. 3 Physico-chemical parameters

S. No.	Parameters	Sample - Goksura Granules
1.	Loss on drying	4.88 % w/w
2.	Water soluble Extract	51.6 % w/w
3.	Alcohol soluble Extract	27 % w/w
4.	Total Ash	8.4 % w/w
5.	pH Value (5% of Aqueous solution)	5.76
6.	Sugar Estimation Total Sugar Reducing Sugar Non - Reducing Sugar	50.73%w/w 11.53%w/w 39.2% w/w

Table No. 4 Functional Groups

S.No	Test	Reagents	Results
1	Carbohydrate	Molish's test	Positive
2	Steroid	Libermann – Burchard test	Positive
3	Saponin Glycosides	Foam Test	Positive
4	Flavonoids	Lead acetate test	Positive
5	Alkaloids	Dragendroff's test	Positive
		Wagner's test	Positive

Table No. 5 TLC of Methanol Extract of Goksura Granules

Extract	Solvent System	Wavelength	No. of spots	hRf value	Observation under UV light
Methanol Extract	Toluene (8 ml): Ethyl acetate (2 ml): Glacial acetic acid (0.5 ml)	366 nm	5	15, 43, 50, 72, 97	Parrot Green, Sky Blue, Red, Green, Blue spots
		254 nm	3	15, 72, 97	Blue, Blue, Blue spots

Table No. 6 TLC - After spraying with Anisaldehyde Sulphuric acid

Extract	Solvent System	Spray	No. of spots	hRf value	Observation under Daylight
Methanol Extract	Toluene (8 ml): Ethyl acetate (2 ml): Glacial acetic acid (0.5 ml)	Anisaldehyde Sulphuric acid	7	22, 40, 54, 65, 77, 86, 97	Dark Blue spots

Table No. 7 HPTLC of Methanol Extract of Goksura Granules

Extract	Solvent System	Wavelength	No. of spots	hRf value
Methanol Extract	Toluene (8 ml): Ethyl acetate (2 ml): Glacial acetic acid (0.5 ml)	366 nm	2	3, 98
		254 nm	7	3, 16, 42, 61, 68, 84, 98

Table No. 8 HPTLC - After spraying with Anisaldehyde Sulphuric acid

Extract	Solvent System	Spray	No. of spots	hRf value
Methanol Extract	Toluene (8 ml): Ethyl acetate (2 ml): Glacial acetic acid (0.5 ml)	Anisaldehyde Sulphuric acid	4	3, 46, 71, 78

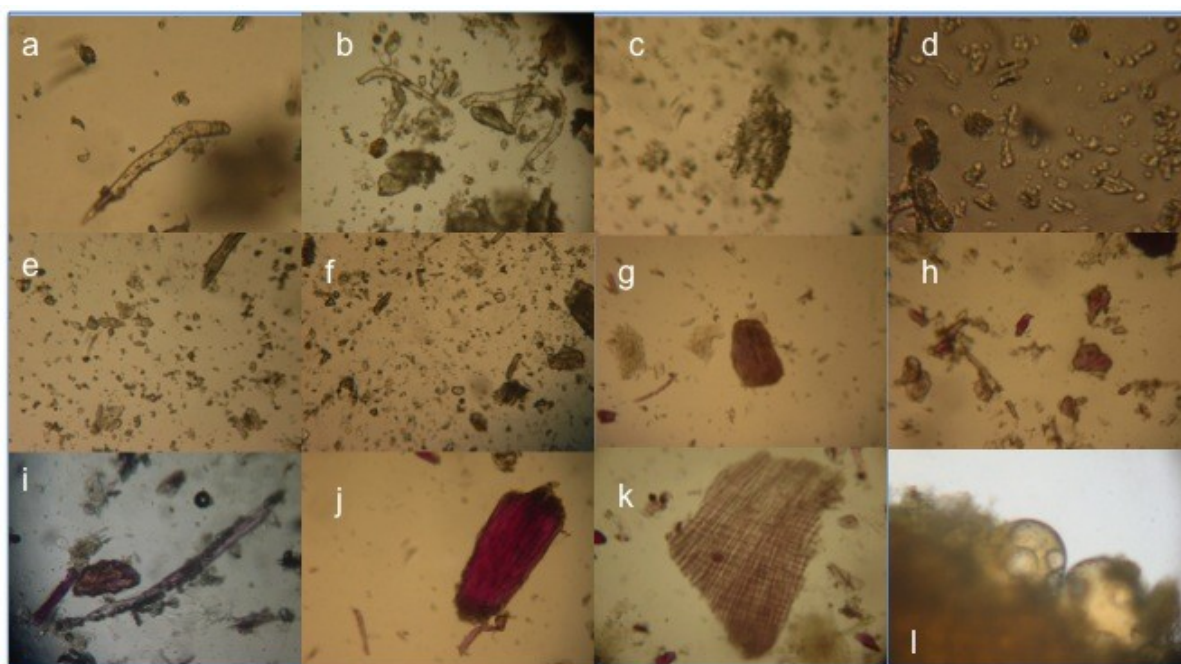
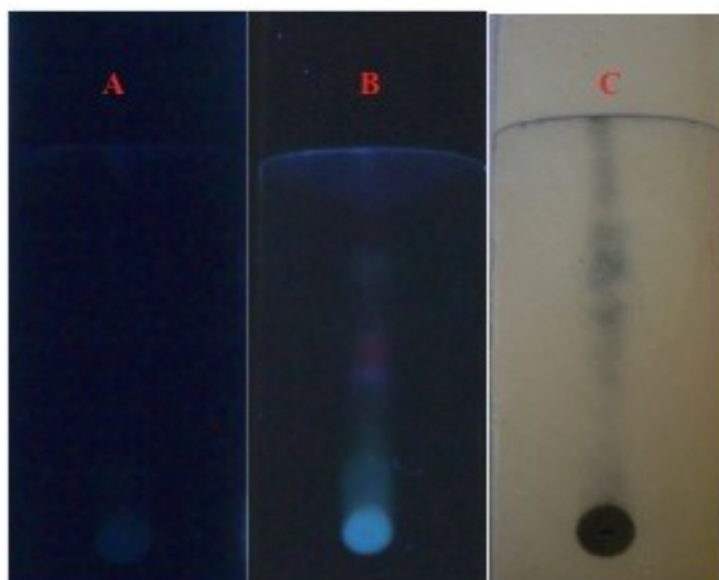


Plate no: 1 - a. Trichome, b.Trichome with Starch grain, c. Parenchymal cells, d. Rosette crystals, e. Starch grains, f. Prismatic crystals, g. Mesocarp cells, h. Stone cells, i. Fiber with stone cells, j. Lignified fibers, k. Lignified cells with polygonal cells of mesocarp, l. Oil globules.

Plate No:2 TLC of *Gokshura* Granules



A. 254nm UV light

B. 366nm UV light

C. After spraying with Anisaldehyde Sulphuric acid

Figure No.1 Densitogram curve of *Gokshura* Granules Extract in 254nm & 366nm