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Comparative Qualitative and Quantitative Phytochemical Assay of Roots and Fruits of *Gokshur* Tribulus Terrestris Linn W.S.R to HPLC

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Abstract: *Ayurveda* science which deals with the various prevention and treatment modalities of every diseases also explained various drugs or *Dravyas* which are beneficial according to their parts, *Guna, Karma* etc for curing disease. *Dravyaguna* is one of the branch of *Ayurveda* which deals with the various drugs and its various properties according to parts, *Karma, Guna, Prabhava* etc. their detailed knowledge was explained under this concept. *Dravyaguna* is the branch of *Ayurveda* mainly deals with the medicinal plants. *Gokshur* is one of the plant which has main property of *Mootrala, Mootrakricchahara* and *Vatahara*. It is one of the important and known drug found in *Ayurveda* and general practise. In this comparative study its fruits and roots were studied according to their different properties and its assessment through HPLC.

Keywords: Gokshur, HPLC, Fruits, Roots

I. INTRODUCTION

Ayurveda describes four elements for successful management of the diseases. These four are *Vaidya* (Physician), *Dravya* (Drug), *Upasthata* (Nursing staff) and *Atura* (Patient)[1]. Among these four, *Dravya* is recognized as the *Karana* (tool)[2]. *Bheshaja* or drug is the tool for the physician and is described as the second most important factor for successful management of disease in *Ayurvedic* classical texts.

Gokshur is one of the herbs which are well as *Shothahara*. The root of *Gokshur* the important ingredients in *Dashmoola*. *Gokshur* was indicated in *Mootrakricha, Ashmari Vataja Vyadhi, Shotha* etc[3]. *Gokshur* (Tribulus terrestris Linn) is a herb of which the useful part is its root and fruit but the selection of plant part for formulation is still a controversy. In Kerala instead of roots, the fruits of *Gokshur* are being used in the preparations of Dashamoola. In *Ayurvedic* Pharmacopoeia of India, both root and fruit and the whole plant of *Gokshur* are indicated for medicinal preparations.

As there is growing demand for herbal pharmaceuticals, there is need to assure their quality. Various chemical and phytochemical test, analytical techniques, and hyphenated analytical techniques are used for determining the quality aspects of herbal materials. Both qualitative and quantitative measures are required for the quality assurance of them.

Methodology[4,5] –

A] Pharmacognostical Study *Gokshur* Seed and root -

Macroscopy -

The external features of the test sample (Seed) were documented using Canon XUS digital camera. The macroscopic features were compared to local flora for authentication. Colour, odour, taste, size, shape, and special features like touch, texture were studied.

Microscopy -

Sample was preserved in fixative solution of FAA (Formalin 5ml+ Acetic acid 5ml + 70% Ethyl alcohol 90ml). The materials were left in FAA for more than 48 hours.

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The preserved specimen was cut into thin transverse section using a sharp blade and sections were stained with Saffranine. The slides were also stained with iodine in Potassium iodide for detection of Starch. Transverse sections were photographed using Zeiss AXIO microscope attached to Zeiss AXIO Cam Camera under bright field light. Magnification of the figures were Indicated by the scale-bars.

B] Powder Microscopy –

A small quantity of powder seed drug placed on the slide and treated with chloral hydrate solution. Finally it was subjected to microscopical studies with trinocular microscope using 10x and 40x objective lenses and photographed.

Physiochemical Study -

Loss on Drying at 105°C -

10gm of the sample was placed in a tarred evaporating dish and dried at 150°C for S hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01g after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample.

Total ash -

2gm of sample was incinerated in a tarred platinum crucible at temperature not exceeding 450°C until carbon free ash was obtained. Percentage of ash was calculated with reference to weight of the sample.

Acid insoluble Ash -

25ml of dilute HCl was added to the Crucible containing the total ash and boiled The insoluble matter was collected on ash less filter paper (Whatmann 41) and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible dried on a hot plate and ignited to constant weight. The residue was allowed to cool in suitable desiccator for 30 min. and weighed without delay. The content of acid insoluble ash was calculated with reference to the air-dried drug.

Water insoluble Ash -

The ash was boiled for 5 min with 25ml of water and insoluble matter was collected on an ashless filter paper, washed with hot water and ignited for 15 min at temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash; the differences in weight represent the water-soluble ash with reference to the air dried sample.

Alcohol soluble extractive -

4 gm of the sample was weighed accurately in a glass stoppered flask. 100 ml of distilled alcohol (approximately 95%) was added, shaken occasionally for 6 hours and allowed to stand for 18 hours. The solution was filtered rapidly taking care not lose any solvent: 25ml of the filtrate was pipetted out in a pre-weighed 100ml beaker evaporated to dryness on a water bath. Then beaker was kept in an air oven at 150°C for 6 hours, cooled in desicator for 30 min and weighed. The percentage of alcohol extractable matter of the sample was calculated. The experiment was repeated twice and average value was taken.

Water soluble extractive -

4 gms of the sample was weighed accurately in a glass stoppered flask.100 ml of distilled water was added, occasionally shaken for 6 hours and allowed to stand for 18 hours.

The solution was filtered rapidly taking care not to lose any solvent. 25ml of filtrate was pipetted out in a pre-weighed 100ml of beaker, evaporated to dryness on a water bath.

Then the beaker was kept in an air oven at 150°C for 6 hours, cooled in desiccator for 30 min and weighed. The percentage of water extract soluble matter of the sample was calculated. The experiment was repeated twice and the average value was taken.

Determination of pH value -

The pH value is determined by glass electrode in pH meter. Standardize the pH meter by standard pH solution then electrode is dipped in the aqueous solution and pH is noted.

Qualitative And Quantitative Study of *Gokshur* -

Due to non- [feasibility] and non-compatibility in the methodology we intended to use, the method from UPLC was changed to HPLC due similar working, sensitivity and suitability to the study.

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HPLC

Chemicals -

HPLC grade solvents and other analytical grade solvents were purchased from Krishgen Biosystems, India.

METHOD FOR DIOSGENIN

1] Chromatographic conditions:

HPLC system: Shimadzu Prominence
Software: Lab solution
HPLC column: Hypersil ODS C18 (250 mm x 4.6 mm, 5 um)
Flow rate: 1ml/min
Injection Volume: 20 ul
Column thermostat temperature: 25oC
Autosampler temperature: Ambient
Elution: Isocratic
Detector: 210nm
2] Mobile preparation:
Acetonitrile: 80%
Methanol: 20%
Diluent: methanol
3] Standard sample preparation:
Weighed accurately 10mg of Diosgenin standards in to 100 ml of dissolved and make up to the mark with diluent.
Weighed 1gm of *Gokshur* powder was macerated with 70% ethanol at room temperature for 24 hr. The solution was centrifuged, supernated liquid was evaporated and the residue obtained was extracted with ethyl acetate and their volume was adjusted to 50 ml with the diluent.

METHOD FOR TIGOGENIN

1] Chromatographic conditions:
HPLC system: Shimadzu Prominence
Software: Lab solution
HPLC column: Hypersil ODS C18 (250 mm x 4.6 mm, 5 um)
Flow rate: 1ml/min
Injection Volume: 20 ul
Column thermostat temperature: 25oC
Autosampler temperature: Ambient
Elution: Isocratic
Detector: 209 nm
2] Mobile preparation:
Acetonitrile: 100%
Diluent: Water
3] Standard Sample preparation:
Weighed accurately 10mg of Tigogenin standards in to 100 ml vf dissolved and make up to the mark with diluent.
Weighed 1gm of the *Gokshur* powder were macerated with 70% ethanol at room temperature for 24 hr. The solution was centrifuged, supernated liquid was evaporated and the residue obtained was extracted with ethyl acetate and their volume was adjusted to 50 ml with the diluent.

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Observations and Results –

A] Pharmacognostical Study Of *Gokshur*[6] (Tribulus terrestris)

Macroscopy -

The observed macroscopical characters of root and fruit of Tribulus terrestris Linn. are given in Table.

Sr. no	Parameters	Characters observed- Fruit	Characters observed- Root
1.	Shape	Globose capsule with 5 indehiscent cocci Individual coccus is semi lunar in shape ripe fruit separating into five segments, starting from the middle	Cylinderical
2.	Size	1.5 cm in diameter and 0.7- 0.8 cm in thickness	0.8-1cm in diameter
3.	External Surface	Highly pubescent Woody and warty or tuberculate Five pairs of prominent short stiff spines Tips of spines almost meet in pairs	Small wiry rootlets present Surface is smooth but at places warty
5.	Central Portion	-	Woody

Figure no. 1 – Macroscopy of Roots of *Gokshur*

Figure no. 2 - Macroscopy of Fruit

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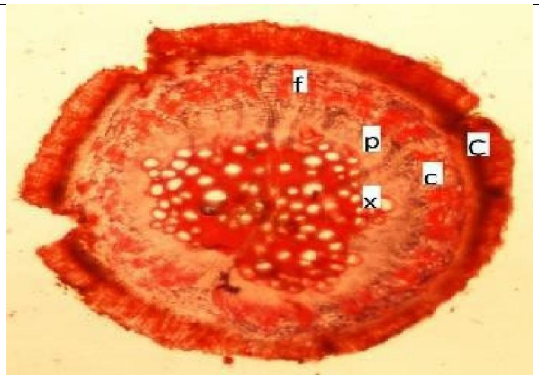
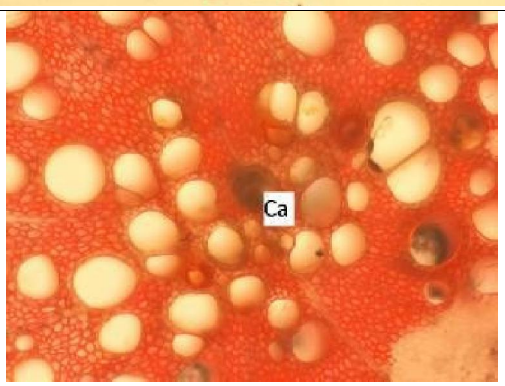
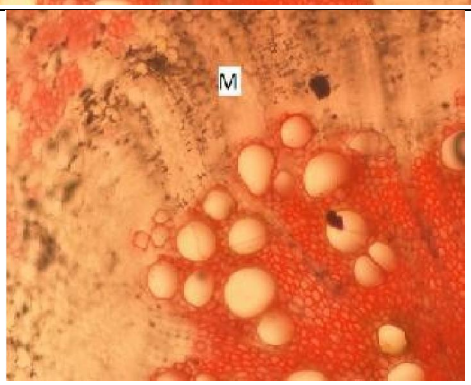
B] ORGANOLEPTIC CHARACTERS

Table showing Organoleptic Characters of Root and Fruit of *Gokshur* –

Dravya Lakshana	Root	Fruit
Touch	Rough and dry	Sharp with spikes
External features	Pale yellowish brown	Yellowish green
Odour	Slightly aromatic	nil
Taste	Sweetish and Astringent	Astringent to slightly bitter

Figure no. 3 – Microscopy of root

	f-fibre p-phloem X-Xylem C-Cork c-cortex
	ca- calcium oxalate crystals
	M-Medullary rays

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C] Microscopy –

Microscopy of root:

Transverse section of the root is circular in outline.

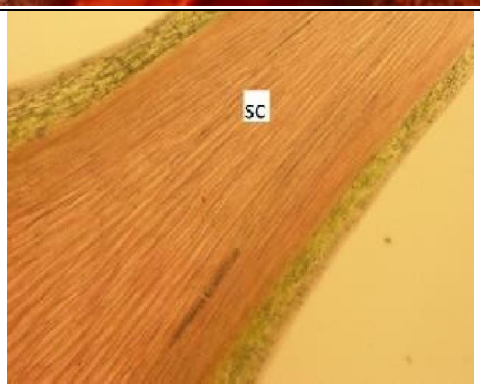
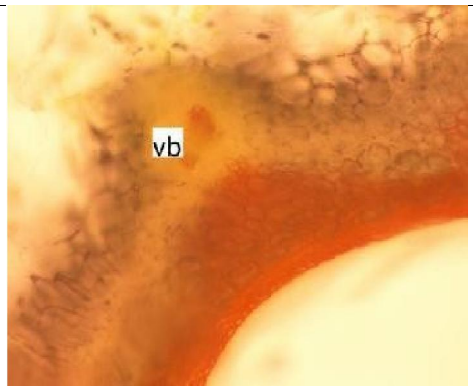
The Outermost cork tissue contained 5-7 rows of rectangular tangentially elongated cells and was irregular at some places.

Cortex comprised of 8-10 rows of parenchymatous cells and it contained rosette crystals of calcium oxalate and starch grains. pericyclic region found beyond the cortex, was traversed with group of fibres.

Phloem was wider composed of sieve tissue, parenchyma, small sized fibres and medullary rays.

Xylem was very wide and composed of scattered isolated xylem vessels associated with parenchyma and thin-walled fibres, embedded with a few prismatic crystals of calcium oxalate.

Figure no. 4 – Microscopy of fruit

	T- Trichome
	sc- sclereids(transverse section through spine)
	vb-vascular bundles

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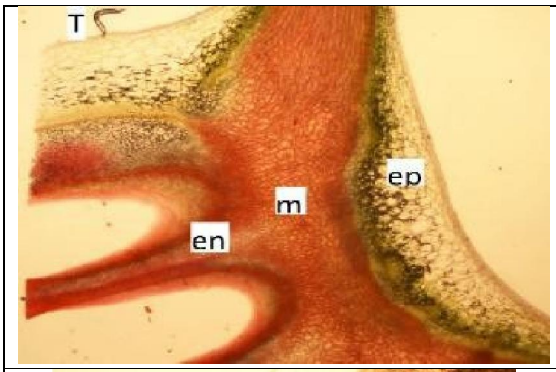
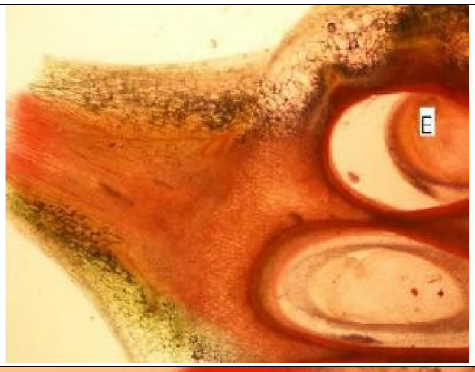
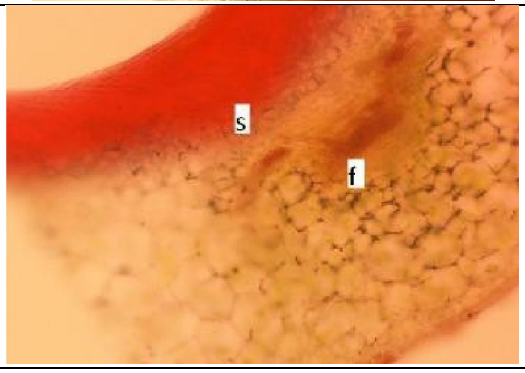
	T- Trichome ep-epicarp m-mesocarp en-endocarp
	E-Endosperm
	s-stone cells f- fibrovascular bundles

Microscopy of fruit:

Epicarp, made up of small tubular cells embedded at places with stomata, was noted to be covered with thick cuticle and contains abundant simple unicellular trichomes.

Rows of elongated thick walled stone cells, fibrovascular strands and cluster crystals of calcium oxalate were found embedded in the wide mesocarp.

Stony endocarp consisted of 10-15 rows of compactly arranged beaded cells and at places parenchymatous cells embedded with prismatic crystals of calcium oxalate.

Endosperm is wide; cells were filled with starch grains and fixed oil.

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D] PHYSIO-CHEMICAL STUDY -

Table showing Physio chemical analysis of *Gokshur* (Tribulus terrestris)

Parameter	Results	
	Root	Fruit
Loss on drying	9.48	9.02
Total Ash	7.3	10.08
Acid insoluble ash	0.70	0.63
Water soluble ash	0.5	0.7
Alcohol soluble extractive value	4.3	6.7
Water soluble extractive value	11.3	10.8
pH	9.36	9.43

E] PHYTOCHEMICAL SCREENING OF *Gokshur* (Tribulus terrestris)

Table showing Preliminary Phytochemical constituents screening of *Gokshur* (Tribulus terrestris)

Test	Inference	
	Root	Fruit
Alkaloid	+	+
Steroid	+	+
Carbohydrate	-	-
Tannin	+	+
Flavanoid	-	+
Saponins	+	+
Phenols	+	-
Amino acids	+	+
Reducing sugar	+	+

Discussion

The use of the root of Tribulus terrestris Linn. is mentioned in many formulations but, in clinical practice, the aerial parts, especially the fruit, are commonly used. Since the drug is used extensively in Indian traditional medical system, the present work was taken up with an objective to lay down detailed pharmacognostical and phytochemical standards, which would contribute significantly to quality control of medicinally useful *Gokshur* (Tribulus terrestris).

HPLC of the alcohol extract of root and fruit of *Gokshur* with HPLC systems were carried out. Two important constituents of saponins- diosgenin and tigogenin were studied using the technique in both roots and fruits and it was seen that both the roots and fruits contained diosgenin and tigogenin.

The HPLC study of *Gokshur* root showed multiple peaks representing various phytoconstituents when compared to the standard tigogenin. The RT being 9.896 in 100% area of tigogenin observed in *Gokshur* fruit and 100% area is observed in *Gokshur* root with RT 9.924 and 9.904 respectively. Similarly, the HPLC study for presence of diosgenin in *Gokshur* root and fruit was carried out comparing with RT 9.477 of standard diosgenin.

The results observed with 9.641 of diosgenin for RT of *Gokshur* fruit and 9.702 for *Gokshur* root covering 100% area. Fruits contained diosgenin (2.63 mg) and tigogenin (1.74 mg) wheras roots contain diosgenin (2.15mg) and tigogenin (1.45mg). Fruits contain comparatively higher amount of these saponins as compared to the roots. This shows that both roots and fruits contain saponins.

II. CONCLUSION

Macro-morphology and microscopy along with the preliminary phytochemical evaluation of root and fruit confirm the quality and purity of plant and its identification. On microscopy of root, the cortex was embedded with rosette crystals of calcium oxalate and starch grains.

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The HPLC study was done instead of UPLC due to non-feasibility and non-compatibility of method that was intended for use for identification of diosgenin and tigogenin (saponins). The HPLC study of *Gokshur* root showed multiple peaks representing various phytoconstituents when compared to the standard tigogenin. The RT being 9.896 in 100% area of tigogenin observed in *Gokshur* fruit and 100% area is observed in *Gokshur* root with RT 9.924 and 9.904 respectively.

Similarly, the HPLC study for presence of diosgenin in *Gokshur* root and fruit was carried out comparing with RT 9.477 of standard diosgenin. The results observed with 9.641 of diosgenin for RT of *Gokshur* fruit and 9.702 for *Gokshur* root covering 100% area. The study revealed that the fruits contained diosgenin (2.63 mg) and tigogenin (1.74 mg) whereas roots contained diosgenin (2.15mg) and tigogenin (1.45mg). Fruits contain comparatively higher amount of these saponins as compared to the roots.

We can conclude that the fruits and roots have similar saponin quantities and therefore the substitution which is done for roots by fruits of *Gokshur* Tribulus terrestris Linn for its pharmacological use in different formulations can be done in situations where there is shortage of *Gokshur* roots. Here the observations and results obtained are useful for further pharmacological and therapeutical evaluation along with the standardization of plant material. Hence the fruits can be used for substitution of roots without the worry of any unintended side effects.

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