

PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF ROOT AND FRUIT OF GOKSHURA (*Tribulus terrestris* Linn.)

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ABSTRACT

Objective: *Gokshura* (*Tribulus terrestris* Linn) is a procumbent herb, having vast medicinal importance in Ayurveda. The use of the root of *Tribulus terrestris* Linn. is mentioned in many formulations but in practice, it is the aerial parts, especially the fruit, that is commonly used. Since the drug is using extensively in Indian traditional medicinal systems the present work is taken up with an objective to lay down detail pharmacognostical and preliminary phytochemical standards, that will contribute significantly to quality control of medicinally useful *Tribulus terrestris* Linn. **Methods:** The root and fruit of the plant were subjected to macroscopical and microscopical characterization, followed by preliminary physicochemical and phytochemical evaluations including chromatography and Atomic Absorption Spectroscopy (AAS) using standardized procedures. It also includes the qualitative tests for the detection of steroids, phenols, alkaloids, flavonoides, glycosides and tannins. **Results:** On microscopy of root, the cortex was embedded with rosette crystals of calcium oxalate and starch grains. Xylem was very wide and composed of scattered isolated xylem vessels embedded with a few prismatic crystals of calcium oxalate. The microscopy of fruit showed abundant simple unicellular trichomes in the epicarp. Elongated thick walled stone cells, fibrovascular strands and cluster crystals of calcium oxalate were found embedded in the mesocarp. Stony endocarp was also found with prismatic crystals of calcium oxalate. On preliminary phytochemical screening, the total ash value was found relatively high (10.08%) for fruits. The root showed the presence of different phytoconstituent groups such as alkaloids, steroids and tannins. The fruit showed the presence of alkaloids, steroids, tannin, saponin and flavonoids. AAS revealed that the plant contains insignificant quantities of heavy metals only. (delete- 'indicated the plant to contain insignificant quantities of heavy metals and considerable amounts of lead, cadmium, zinc and iron'.) **Conclusion:** The results of the study can serve as a valuable source of pharmacognostic information as suitable standards for its identification.

Keywords: *Gokshura*, *Tribulus terrestris* Linn, Pharmacognostic, phytochemical standards

INTRODUCTION

Gokshura (*Tribulus terrestris* Linn) is one among the ten drugs included under *Dasamoola* in the Ayurvedic classics. In clinical practice both roots and fruits of this plant are used. *Gokshura* (*Tribulus terrestris* Linn) is a procumbent herb and is a common plant of wasteland, chiefly in hot dry and sandy regions, throughout India. This herb is mainly known for its effect on filtration defects of renal system and urinary tract infections in Indian system of medicine. *Gokshura* has been known as a medicinal plant in India since ancient period itself. An ancient medical text *Caraka samhitha* identifies it as the best drug among the drugs curing obstructive uropathy, in the verse 'moothrakrichra anilahaaraanaam'¹. *Gokshura* root is included in the famous group of drugs *Dasamoola* and is said to be useful in the treatment of inflammation (*sopha*), fever (*jwara*), cough (*kasa*), dyspnoea (*swasa*)² etc. Fruit of *Gokshura* is an effective aphrodisiac (*vrushya*) and is useful in urolithiasis (*asmari*), dysuria (*mootrakrichra*)³ etc. Pharmacognostic study is the initial step to confirm the identity and to assess the quality and purity of crude drug. The adulteration of the crude drug can be prevented by means of studies including macro-microscopic study. Microscopy is an indispensable tool for authentication of crude drug and the study of powdered drugs. Setting up a standard of pharmacognostical and phytochemical characters of root and fruit of *Gokshura* (*Tribulus terrestris* Linn) will aid standardization, which can promise quality, purity and identity of samples.

MATERIALS AND METHODS

Sample collection- The sample drug *Gokshura* (*Tribulus terrestris* Linn.) was collected from wasteland near the coastal area of Thiruvananthapuram ('area' deleted) and the sample was authenticated by comparing it with different floras.

Figure: 1 sample collection of *Tribulus terrestris* Linn



Macroscopic evaluation- The root and fruit of *Tribulus terrestris* Linn was subjected to organoleptic evaluation. A magnifying lens was used for a better evaluation of surface characters and the observed macroscopic characters were recorded.

Microscopic evaluation- The microscopic evaluation of sample drug including histological evaluation and powder microscopy was done according to the standard procedure detailed in API⁴.

Preliminary Physicochemical and Phytochemical evaluation - The preliminary physicochemical and phytochemical analysis of the root and fruit of *Tribulus terrestris* Linn. was done according to the standard procedures described in API⁴, which includes: physical evaluation like evaluation of moisture content, volatile oil content, total ash, acid insoluble ash, water soluble extractive, alcohol soluble

extractive, fibre content and sugar content. It also includes the qualitative tests⁵ for the detection of steroids, phenols, alkaloids, flavonoides, glycosides and tannins, Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC), and Atomic Absorption Spectroscopy (AAS).

RESULTS

a. Macroscopic evaluation

The observed macroscopical characters of root and fruit of *Tribulus terrestris Linn.* are given in Table 1.

Table 1: Macroscopic evaluation of root and fruit

CHARACTERS	ROOT	FRUIT
Shape	Cylindrical	<ul style="list-style-type: none"> ➤ Globose capsule with 5 indehiscent cocci ➤ Individual coccus is semi lunar in shape ➤ ripe fruit separating into five segments, starting from the middle
Size	0.3-0.8cm in diameter	10-15 mm in diameter and 7-8mm in thickness
External surface	<ul style="list-style-type: none"> ➤ Small wiry rootlets present ➤ Surface is smooth but at places warty 	<ul style="list-style-type: none"> ➤ Highly pubescent ➤ Woody and warty or tuberculate ➤ Five pairs of prominent short stiff spines ➤ Tips of spines almost meet in pairs
External surface colour	Pale yellowish brown	Yellowish green
Central portion	Woody	-
Fracture	Fibrous	-
Odour	Slightly aromatic	Nil
Taste	Sweetish and Astringent	Astringent to slightly bitter

Figure 2: Root of *Tribulus terrestris Linn*



Figure 3: Fruit of *Tribulus terrestris Linn*



Microscopic characters

a. Microscopic evaluation 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.

b. Microscopic evaluation 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.

Figure 4: Microscopy of root:

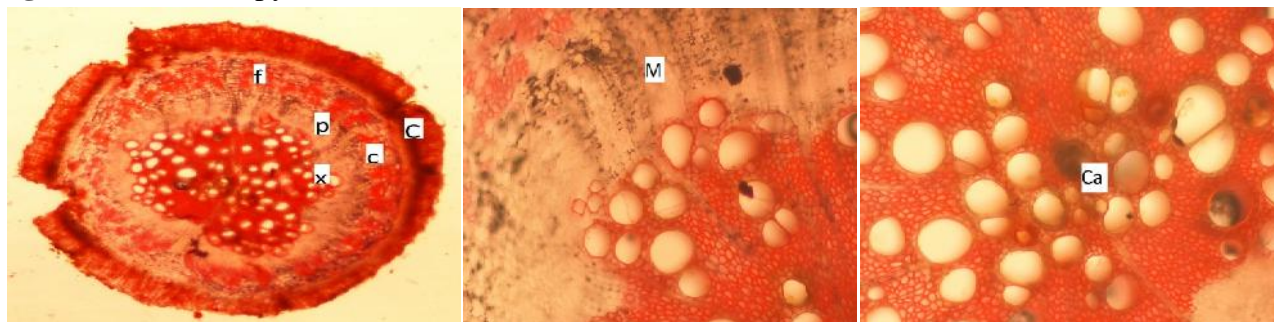
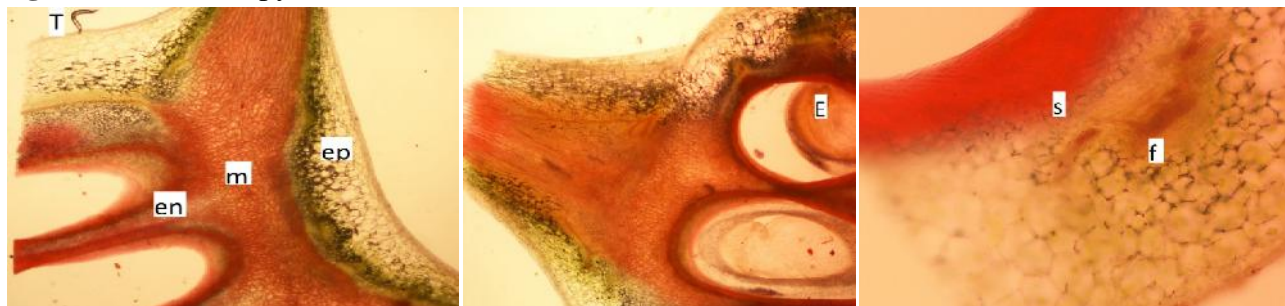


Figure No.4. Microscopy of root: C-Cork, c-cortex, f-fibre, p-phloem, X-Xylem, M-Medullary rays, ca- calcium oxalate crystals

Figure 5: Microscopy of fruit:



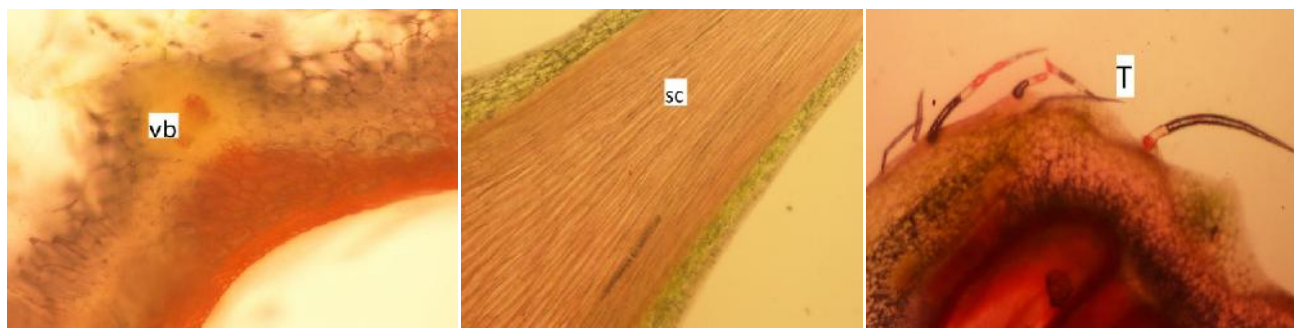


Figure No.5. Microscopy of fruit: T- Trichome, ep-epicarp, m-mesocarp, en-endocarp, E-Endosperm, s-stone cells, f-fibrovascular bundles, vb-vascular bundles, sc- sclereids(transverse section through spine)

Powder Microscopy

ROOT-The diagnostic characters of root powder of *Gokshura* (*Tribulus terrestris* Linn) were thin walled fibres, rosette crystals of calcium oxalate and starch grains which were scattered or embedded in the parenchymatous cells. The powder was very abundant with fragments of pitted vessels and trachieds.

FRUIT-The diagnostic characters of root powder of *Gokshura* (*Tribulus terrestris* Linn)

were abundant fragments of simple trichomes of various sizes and shapes, isolated or groups of thick walled sclereids from the endocarp, stone cells from the mesocarp, prismatic and cluster crystals from the mesocarp, longitudinally cut fragments of fibres, starch grains and oil globules.

Figure 6: Powder microscopy of root

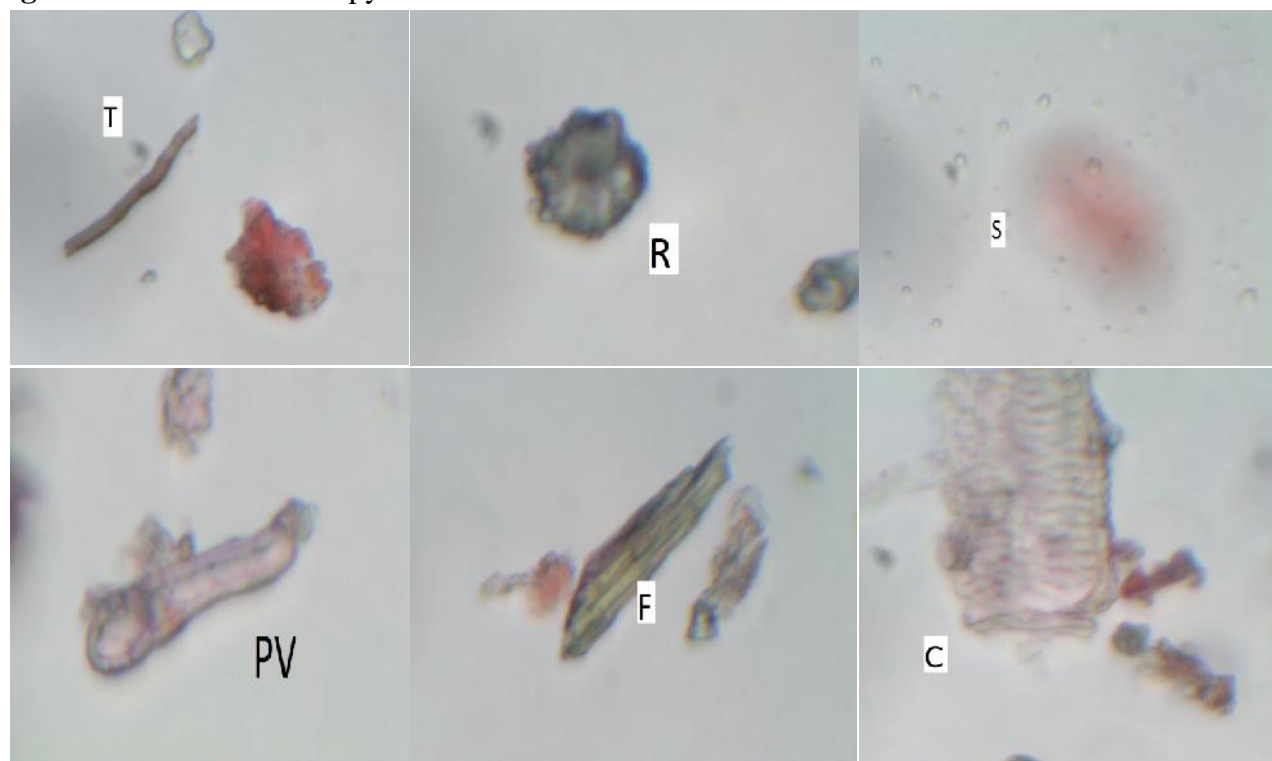


Figure No.6: Powder microscopy of root: T-Trachiedal fibre; R-Rosette crystals of calcium oxalate; S-Starch grains; PV-Simple Pitted Vessel attached to trachied; F-long Fibres; c-transversely and obliquely cut cork;

Figure 7: Powder microscopy of fruit

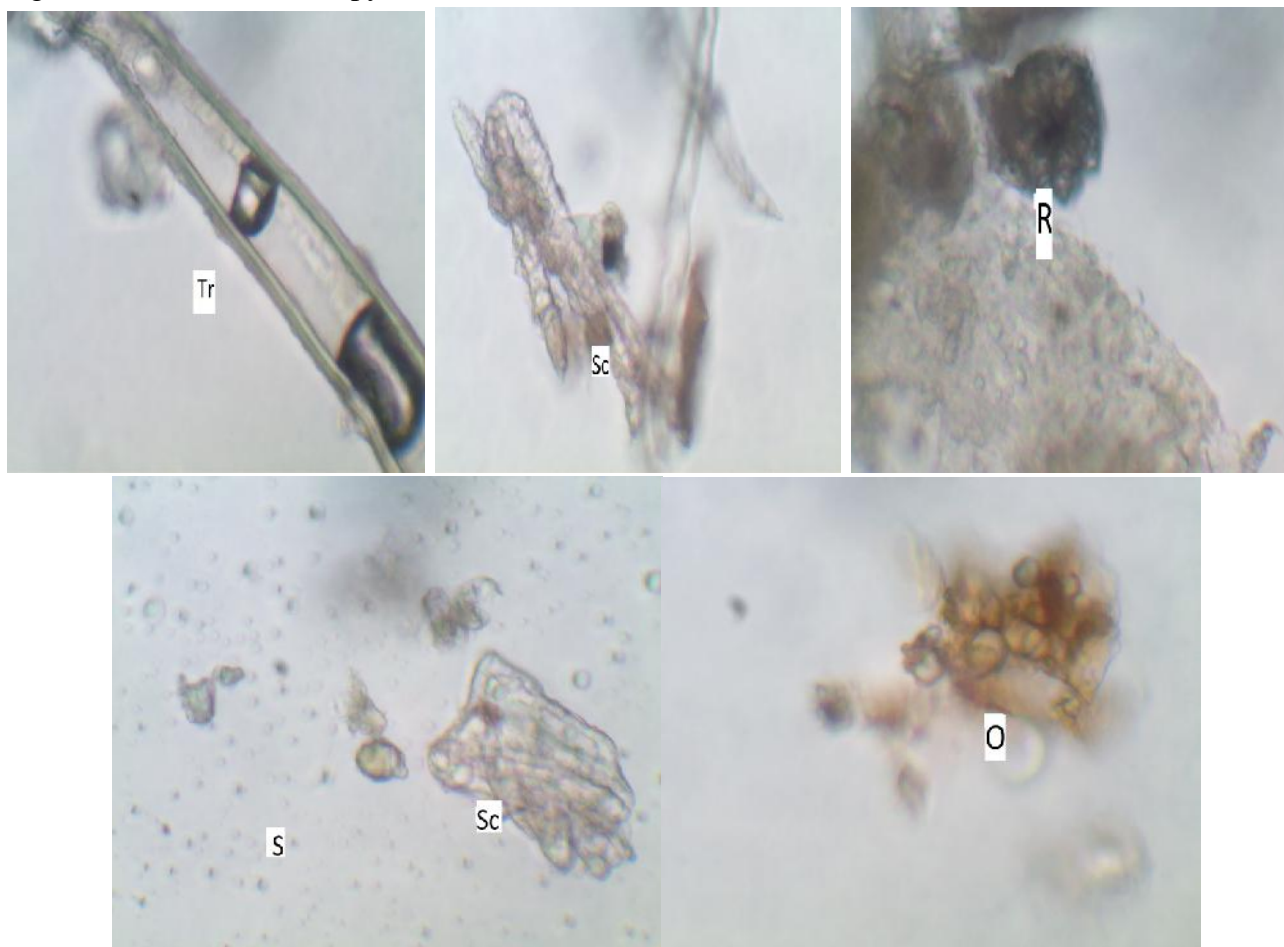


Figure No.7: Powder microscopy of fruit: Tr-fragments of Trichome; sc-isolated sclereids; R- Rosette crystals of calcium oxalate; S- Starch grains; O-Oil globules

Preliminary Physicochemical and Phytochemical evaluation

The results of Physico chemical evaluation of root and fruit of *Gokshura* (*Tribulus terrestris* Linn) are given in Table 2

Table 2: Physico chemical evaluation of root and fruit of *Gokshura* (*Tribulus terrestris* Linn):

Sl.no.	Experiment	Root	Fruit
1	Foreign matter (%)	Nil	Nil
2	Moisture content (%)	9	10
3	Volatile oil (%)	Nil	Nil
4	Total ash (%)	7.3	10.08

5	Acid insoluble ash (%)	0.69	0.62
6	Water soluble extractive (%)	11.3	10.9
7	Alcohol soluble extractive (%)	4.2	6.8
8	Fibre content (%)	46	38
9	Sugar content		
	Reducing sugar (%)	Nil	0.33
	Total sugar (%)	0.2	0.66

Qualitative analysis:

The results of Qualitative tests of root and fruit of gokshura (*Tribulus terrestris* Linn) are given in Table 3.

Table 3: Qualitative tests done in the root and fruit of gokshura (*Tribulus terrestris* Linn.)

Sl.no.	Experiment	Root	Fruit
1	Steroid- Libermann Burchard Test	Present	Present
2	Flavonoid- Shinoda Test	Absent	Present
3	Phenol- with FeCl ₃	Absent	Absent
4	Alkaloid- Dragendorff Test	Present	Present
5	Tannin- with FeCl ₃	Present	Present
6	Saponin- with NaHCO ₃	Absent	Present

Chromatography

a. Thin Layer Chromatography (TLC):

Different solvent systems were tested by trial and error method. Best separation was achieved by Toluene: Ethyl acetate-8:1 ration,

which was first observed through UV- fluorescence viewing cabinet (365nm) and the Rf values of the spots were noted (Table 4). Then the plates were developed in the iodine chamber and no changes were observed.

Table 4: Rf values of spots obtained in TLC with solvent system-Toluene: Ethyl acetate-8:1

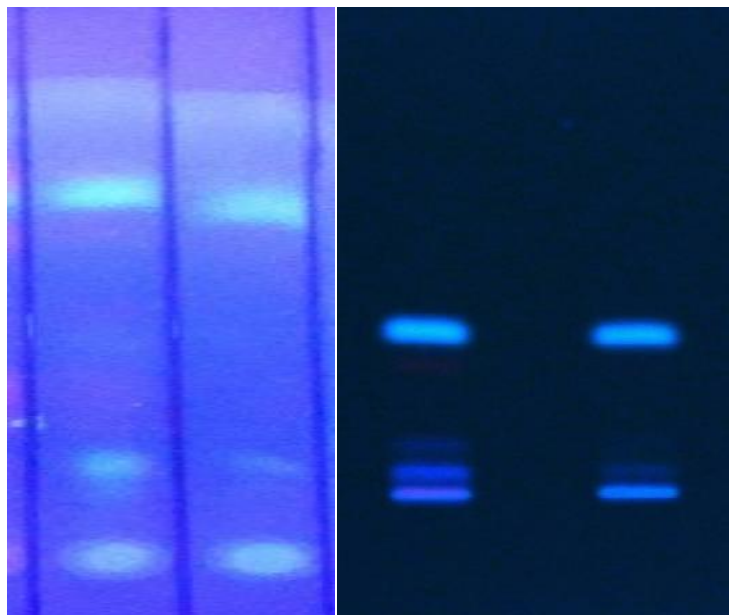
Solvent system with ratio	No. Of spots	Colour of spots	Rf value
Toluene : ethyl acetate (8:1)	Root- 2	Fluorescent blue	0.79
		Fluorescent blue	0.20
	Fruit- 2	Fluorescent blue	0.78
		Dull blue	0.21

b. High Performance Thin Layer Chromatography (HPTLC) :

HPTLC analysis of the alcohol extract of both root and fruit of *Tribulus terrestris* Linn were

done with the solvent system Toluene: Ethyl acetate-8:1.

Figure No 8: TLC and HPTLC plate with solvent system Toluene: Ethyl acetate (8:1)



Track a- TLC alcohol extract of root of *Tribulus terrestris* Linn
 Track b – TLC alcohol extract of fruit of *Tribulus terrestris* Linn
 Track A- HPTLC alcohol extract of root of *Tribulus terrestris* Linn
 Track B– HPTLC alcohol extract of fruit of *Tribulus terrestris* Linn

Atomic Absorption Spectroscopy (AAS):

The results of Heavy metal content screened in the samples of root and fruit of *Tribulus terrestris* Linn. are given in the Table 5.

Table 5: Atomic absorption spectroscopy (in parts per million-ppm)

Heavy metal concentration in ppm	Root	Fruit	Maximum permissible limits ⁶
LEAD	0.05240	0.2963	10
CADMIUM	0.01	0.012	0.3
IRON	8.6856	4.5503	27.4
ZINC	2.2606	0.8670	20(for edible herbs)

DISCUSSION

Tribulus terrestris Linn (Zygophyllaceae) is a commonly used medicinal plant in *Ayurveda*. 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 pharmacognos-

tical and phytochemical standards, which would contribute significantly to quality control of medicinally useful *Tribulus terrestris* Linn.

The root of *Tribulus terrestris* Linn is cylindrical in shape with woody central portion. The root can be easily identified with its pale yellowish brown colour and warty surface. The fruit is yellowish green coloured, globose capsule with 5 indehiscent semi lunar cocci.

The surface is woody and warty or tuberculate and with five pairs of prominent short stiff spines.

The transverse section of root shows important demarcating characters like wider phloem composed of sieve tissue, parenchyma, small sized fibres and medullary rays and very wide xylem embedded occasionally with prismatic crystals of calcium oxalate. Abundant simple unicellular trichomes, vascular bundles and stone cells in the mesocarp, fibrovascular strands, crystals of calcium oxalate and wide endosperm are the characteristic features of fruit microscopy. On powder microscopy, the root powder are characterised with the presence of fibres, fragments of pitted vessels and trachieds. Rosette crystals of calcium oxalate and starch grains were scattered or embedded in the parenchymatous cells. The diagnostic characters of fruit powder are, abundant fragments of simple trichomes of various sizes and shapes, isolated or groups of thick walled sclereids, prismatic and cluster crystals from the mesocarp, longitudinally cut fragments of fibres starch grains and oil globules. These microscopic characteristics of *Tribulus terrestris* Linn were found to be consistent with earlier reports⁷.

The physicochemical parameters were shown in Table 2. The ash values of a drug give an idea of the earthy matter or inorganic composition and other impurities present along with the drug and the value was more in fruit (10.08%) than root (7.3%). Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The high water solubility of the contents than alcohol solubility of both root and fruit suggests aqueous extract for future studies.

The preliminary phytochemical screening of root showed the presence of different phyto-constituent groups such as alkaloids, steroids and tannins. The fruit showed the presence of alkaloids, steroids, tannin, saponin and flavonoids. TLC and HPTLC of the alcohol extract of root and fruit of Gokshura with different solvent systems were carried out. The best separation was achieved using Toluene-Ethyl acetate as a solvent system in a ratio of 8:1. Atomic absorption spectroscopy was done for four heavy metals namely lead, cadmium, zinc and iron, all were found to be under the permissible limits.

CONCLUSION

Macromorphology 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. Xylem was very wide and composed of scattered isolated xylem vessels embedded with a few prismatic crystals of calcium oxalate. The microscopy of fruit showed abundant simple unicellular trichomes in the epicarp. Elongated thick walled stone cells, fibrovascular strands and cluster crystals of calcium oxalate were found embedded in the mesocarp. Stony endocarp was also found with prismatic crystals of calcium oxalate. On phytochemical analysis the fruit was found with relatively high total ash value (10.08%). The preliminary phytochemical screening of root showed the presence of different phytoconstituent groups such as alkaloids, steroids and tannins. The fruit showed the presence of alkaloids, steroids, tannin, saponin and flavonoids. AAS evidences that the plant contains heavy metals in

insignificant quantities only .(delete-‘indicated the plant to contain insignificant quantities of heavy metals and considerable amounts of lead, cadmium, zinc and iron’)Here the observations and results obtained useful for further pharmacological and therapeutical evaluation along with the standardization of plant material.

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