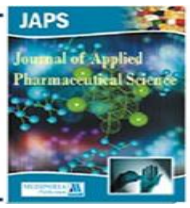
Journal of Applied Pharmaceutical Science
Available online at www.japsonline.com



ISSN: 2231-3354
Received on: 08-08-2012
Revised on: 18-08-2012
Accepted on: 24-08-2012
DOI: 10.7324/JAPS.2012.2820

Comparitive Pharmacognostical and Phytochemical Evaluation of Brihat and Laghu Gokshur

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ABSTRACT

Laghu Gokshur (*Tribulus terrestris)* and Brihat Gokshur (*Pedalium murex)* are well known drugs used in Ayurveda as diuretics. These are classified under mishrak varga as 'Dashmoola' in Ayurveda and in chemotaxonomy as Saponin glycosides. The objective of the work is to find out the diagnostic tool to identify the two varieties of Gokshur. The powder was studied for macroscopic, microscopic and physicochemical parameters. For HPTLC Stationary phase was Pre-coated silica gel GF 254 and mobile phase was Toluene: Ethyl acetate: Formic acid (7:2:1 v/v/v). The plate was scanned and quantified at 254 nm for Diosgenin.Results shows that microscopic characters like trichomes, stomata and crystals show some difference in the two varieties while physicochemical parameters show difference in extractive values. Phytochemical screening also shows similar findings. HPTLC analysis carried out using Diosgenin as reference standard revealed the presence of steroidal Saponin "Diosgenin". Quantitative estimation for Saponins found marked variation in the two varieties, where Laghu gokshur had 16% of Saponins while Brihat gokshur had 13% Saponins. The study can be used as a diagnostic tool for identification of these two varieties of Gokshur.

Keywords: Gokshur, Microscopy, Physicochemical parameters, HPTLC fingerprint.

INTRODUCTION

Brihat Gokshur *Pedalium murex* Linn. and Laghu Gokshur *Tribulus terrestris* Linn. are the plants under the mishrak varga, Dashmoola having diuretic property. Both plants are categorized under Glycosides steroidal Saponins. The chemical constituent of the plants includes Sapogenins, Tannins, Glycosides etc. Among these steroidal Saponins is the main active principle which is responsible for the diuretic activity of the plants.

P.murex Linn. is a branched succulent herb which grows up to 40 cm in height. Leaves simple, opposite, fleshy, serrate or crenate; flowers bright yellow, solitary and axillary. Fruits 4 angled with horizontal spines from the angles. *T.terrestris* Linn. is a annual or rarely perennial hirsute, prostrate herb with many slender spreading branches, one to two feet or more in length and about 0.05 inch in thickness, bearing unequal pairs of abruptly pinnate leaves and small solitary stalked yellowish flowers or spiny fruits at each node. The entire plant has a faint aroma and hence named as 'Ikshuu gandhika (Al-Ali *et al*.,. 2003).Diosgenin is a steroidal Sapogenin possessing estrogenic and antitumor properties found in both the species. The pharmacological property of a steroidal Saponins has been described including their hypocholesterolemic, antidiabetic and antioxidant activities (Trease and Evans, 1996).

In the present work, comparative Pharmacognostical, phytochemical profiles of whole plants of *Tribulus terrestris* and *Pedalium murex w.s.r* to the steroidal Saponin "Diosgenin''were studied.

Present study aims to carry out detailed Pharmacognostical and Phytochemical evaluation of Laghu Gokshur and Brihat Gokshur which includes macroscopic, microscopic and physicochemical analysis including HPTLC fingerprinting. In various Ayurvedic literature it has been mentioned that Laghu gokshur can be used as substitute for Brihat gokshur.Keeping this view under consideration the work was designed to find the characteristic feature where these two varieties shows similar characters.

MATERIAL AND METHODS

Collection of plants

The plants Brihat Gokshur and Laghu Gokshur were collected from surrounding areas of Jamnagar. The plants were washed, dried, powdered and taken for further study.

Macroscopic characters

Macroscopic details of the respective parts are noted by usual observation and with the help of flora, botanical texts. In the description, general condition of the drug color, odour, taste, texture etc. are noted. Drugs can be identified with the aid of the above, only in entire condition (Trease and Evans, 1996).

Microscopy characters

Transverse sections of root of both the plants had been taken and photomicrography had been done after proper mounting and staining. Powders of *Panchanga* of both the samples were studied microscopically for their characters (Trease and Evans, 1996).

Physicochemical parameters

Physicochemical study includes the parameters such as Loss on drying, Ash value, Acid insoluble ash, Extractive values in water and methanol, pH according to the various textual references (Trease and Evans, 1996).

Histochemical Tests

Some Histochemical tests were performed to detect various primary & secondary plant metabolites and cellular contents by using different reagents (Khandelwal , 2008).

Qualitative Study

In qualitative analysis various chemical tests were performed to find out the presence or absence of chemical constituents like Saponins, Alkaloids, Glycosides etc.

Quantitative Estimation

For quantitative evaluation of the Phytoconstituents the assay was carried out for total Saponins content by gravimetric method according to the references (Shaver *et al.,* 1964).

Fluorescence Analysis

The fluorescence analysis of Laghu Gokshur and Brihat Gokshur was done in different solvent extracts. For this chloroform, methanol, water, hydrochloric acid and sodium hydroxide were taken as solvents. The extracts of the plants were prepared in selected solvents. All the extracts were subjected to long U.V 366 nm and short U.V 254 nm to observe the difference in colour (Chase and Patter 1949).

Chromatographic fingerprint

Preparation of sample solution

10 gm of plant material was extracted with 5 ml of Methanol. The material was refluxed for half an hour. The extract was filtered and volume was made up to 10 ml to get solution concentration. The procedure was same for both samples (Lambert *et al.,* 1988).

Procedure

Before spotting, the plate was pre-washed with Methanol. Sample solutions were applied to the plate as sharp bands by means of Camag Linomat V sample applicator. The spots were then dried in a current of air. The mobile phase Toluene: Ethyl acetate: Formic acid (7:2:1 v/v/v) was poured into a twin trough glass chamber. Then whole assembly was left to equilibrate for 30 min & the plate was placed in the chamber .The plate was then developed until the solvent front had travelled at a distance of 80 mm above the base of plate. The plate was then removed from chamber & dried in a current of air. Detection was done with Camag TLC scanner 3 at a wavelength 254 nm and 366 nm and sprayed with Anisaldehyde Sulphuric acid reagent afterwards (Lambert *et al.,* 1988).

RESULTS AND DISCUSSION

Organoleptic values of powders of *P.murex* and *T.terrestris* showed both similarities and dissimilarities (Table 1). Dissimilar characteristics of the powders like colour and taste can be used as diagnostic values; *P. murex* powder is brown and bitter while that of *T.terrestris* is dark green and astringent. Powder of whole plant of *T.terrestris* and *P.murex* revealed distinguished characters like stomata, crystals, stone cells, fibers, vessels, trichomes (Table 2; Fig 1).

Table. 1: Macroscopic Study Results.

S.No	Parameter	Laghu Gokshur	Brihat gokshur
1.	Color	Greenish brown	Brown
2.	Odour	Characterstic	Characterstic
3.	Taste	Astrigent	Bitter

Table. 2: Microscopic Study Results.

S.No	Character	Laghu Gokshur	Brihat Gokshur
1	Trichome	Multiseriate	Uniseriate,sessile
2	Cork cells	Actinocytic,Stomata present	Stomata absent
3	Stone cells	Horse shoe shaped	Horse shoe shaped
4	Crystals	Rosette	Prismatic
5	Vessls	Pitted,reticulate	Pitted,spiral
6	Tracheids	Absent	Present

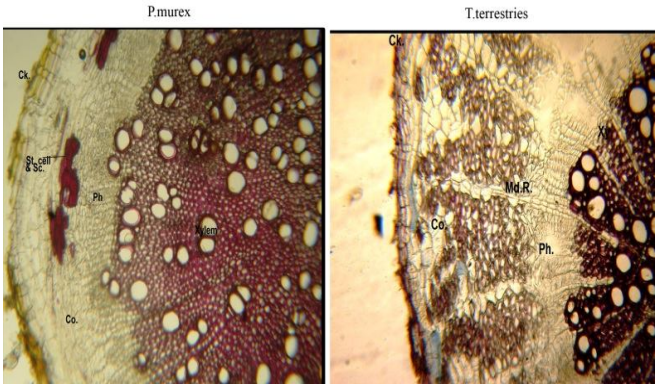

Fig. 1: T.S of Brihat and Laghu gokshur.

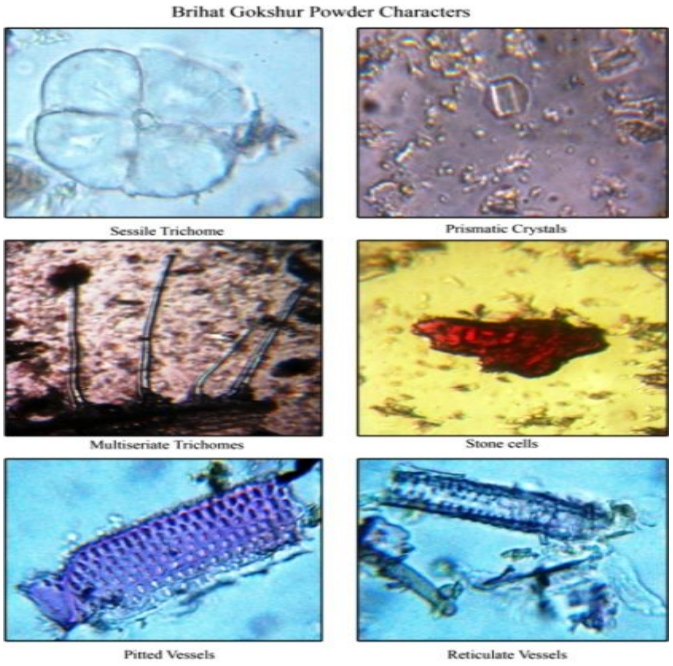

Fig. 2a: Powder characters Brihat gokshur.

T.S of *P. murex* shows presence of cork, cortex, sclerides, stone cells, lignified xylem, and phloem. *T.terrestris* root showed similar characters with medullary rays (Fig. 2) Histochemical test results showed similar findings in both varieties of Gokshur and no difference was observed (Table 3). Loss on drying of powder of *T.terrestris* was more similar to that of *P. murex* (Table 4). No significant difference could be observed in Ash values of powders. More values for total Ash showed the presence of inorganic material such as silica, silicate etc. Extractive values also showed distinct variation. The water soluble extractive value of *T.terrestris* was more than *P. murex* powder. Alcohol soluble extractive values were same for both samples. Qualitative phytochemical analysis of whole plant extracts of *T.terrestris* and *P.murex* showed similar constituents like Alkaloids, Glycosides, Saponins , Tannins and Flavanoids. Estimation of phytochemical constituents confirmed more amount of Total Saponins in *T.terrestris i.e.* 16% while *P. murex* had 13% Total Saponins (Table 4). Fluorescence study of powders of both species also revealed distinguished characters (Table 7).

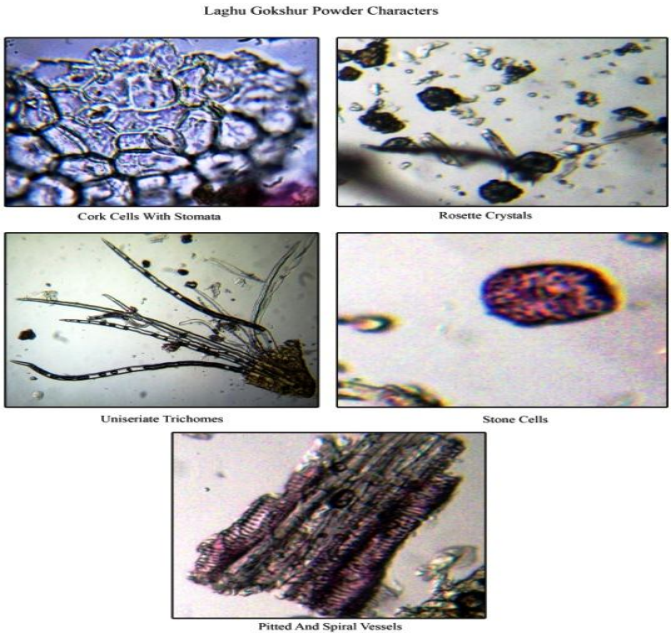

Fig. 2b: Powder characters Laghu gokshu.

Though in most of the reagents the colour characteristics were similar, treatment with ammonium hydroxide, hydrochloric acid, methanol and chloroform showed diagnostic colour for powders of *T.terrestris* and *P.murex* when subjected to 254 nm and 366 nm which could be used as diagnostic characters. HPTLC profiles of methanol extracts of *T.terrestris and P.murex* and showed distinct characteristic with only one common spot with similar Rf value (Table 6, Fig. 3). When the plate was observed at 366nm *T.terrestris* shows 2 spots while 1 spot was found in *P.murex*. No spot was detected when plate was observed in short U.V. that is 254 nm. When derivatisation (Anisaldehyde spray reaction) was carried out for identification of Diosgenin in the sample, blue colored spot with Rf value 0.62 was observed in both varieties of Gokshur which were identical to standard Diosgenin spot. The colour reaction shows the presence of Diosgenin in Laghu Gokshur and Brihat Gokshur. Hence, TLC profile could be used as a diagnostic tool to identify the two varieties.

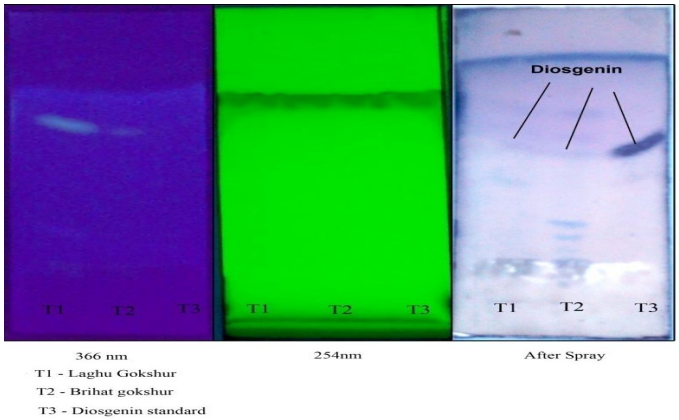

Fig. 3: HPTLC of Brihat and Laghu gokshur.

Table. 3: Histochemical Test Results.

Sr. No.	Test for	Test	Observation	Result	
				T. terrestries	*P. murex*
1.	Starch	Section/ Powder + Iodine solution	Starch turned blue in colour	++	++
2.	Calcium oxalate	Section/ Powder + HCl	Crystal dissolves	++	++
3.	Lignified elements	Section/ Powder + Phloroglucinol + HCl	Lignified tissues turned pink in colour.	++	++
4.	Mucilage	Sudan III	Red	++	++

Table. 4: Physicochemical Parameter Results.

S.No.	Parameter	Brihat Gokshur	Laghu Gokshur
1	L.O.D	6.2 %w/w	7.1 % w/w
2	Ash value	8.1 % w/w	8.4 % w/w
3	Acid insoluble ash	0.03 % w/w	1.7 % w/w
4	Extractive value(water)	17.90% w/w	24.71% w/w
5	Extractive value(Alc.)	9.51% w/w	9.35% w/w
6	% Saponin	13 %	16%

Table. 5: Qualitative Test Results.

S.No	Test	Laghu Gokshur	Brihat Gokshur
1	Tannin	Present	Present
2	Saponin	Present	Present
3	Alkaloid	Present	Present
4	Glycoside	Present	Present
5	Carbohydrate	Present	Present
6	Flavanoid	Present	Present
7	Mucilage	Present	Present

Table. 6: HPTLC Results of Brihat and Laghu gokshur .

Condition	Laghu Gokshur		Brihat Gokshur		Diosgenin standard	
	No of spots	Rf value	No of spots	Rf value	No of spots	Rf value
366 nm	2	0.34,0.82	1	0.82		
254 nm	-	-	-	-	-	-
After spray	8	0.17,0.2,0.28, 0.34,0.50,**0.62**, 0.73,0.79	8	0.17,0.2,0.29,0.35, 0.50,**0.62**,0.73,0.79	1	**0.62**

Table. 7: Fluorescence Activity Results.

S.No	Solvent	Laghu Gokshur		Brihat Gokshur	
		254 nm	366 nm	254 nm	366 nm
1	Methanol	Nil	Nil	Nil	Purple
2	Water	Light green	Light green	Light green	Golden green Florescence
3	Chloroform	Nil	Greenish white	Nil	Pink
4	Acid	Light green	Florescence green	Light green	Florescence green

CONCLUSION

All the above parameters can be used as diagnostic tool to identify and differentiate the two varieties of Gokshur i.e. *T.terrestris* and *P.murex*. The study shows that both the plants have differentiating as well as similar characteristics.

ACKNOWLEDGEMENT

Author expresses sincere thanks to the Institute of Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University for their encouragement, co-operation, support, suggestions and affection throughout my Studies.

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