

ORIGINAL RESEARCH ARTICLE

A Study to Evaluate “Diosgenin” in Laghu gokshur (*T.terrestries*) and Brihat gokshur (*Pedalium murex*) by HPTLC Method

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ABSTRACT

Gokshur is one of the plant used in Ayurveda. The plant has diuretic activity. Brihat gokshur and Laghu gokshur are the plants used under the mishrak varga Dashmoola. Both the varieties contains sapogenins, tannins, glycoside etc.. chemical constituents. Among these steroidal saponin is the main active principle which is responsible for the diuretic activity of the plant. The present research work is aimed to find the difference in percentage of Diosgenin in Brihat and Laghu gokshur. A simple, accurate and sensitive HPTLC method was developed for estimation of diosgenin. Toulene : Ethyl acetate : Formic acid (7:2:1) was used as solvent system. The results shows difference in percentage of Diosgenin in two varieties of Gokshur where *T. terrestries* show 9.2 % and *P. murex* 8.4 % Of Diosgenin. The correlation coefficient was found to be 0.9989. The developed HPTLC method was simple, accurate, precise and cost-effective & can be utilized for the routine analysis of quantitative determination of Diosgenin.

Keywords: Diosgenin, HPTLC, Pedalium murex, Tribulus terrestries, , steroidal saponin.

INTRODUCTION

Laghu Gokshur (*Tribulus terrestries*) and Brihat Gokshur (*Pedalium murex*) are well known drugs used in Ayurveda as diuretic. These are classified under mishrak varga ‘Dashmoola’ in Ayurved and in chemotaxonomy under Saponin Glycosides ^[1]. Both plants contain sapogenins, tannins, glycoside etc. chemical constituents. Among these steroidal saponin is the main active principle which is responsible for the diuretic activity of the plant ^[2]. Diosgenin is a steroidal sapogenin possessing estrogenic and antitumour properties. The pharmacological property of a steroidal saponin has been described including their hypocholesterolemic, antidiabetic and antioxidant activities. Steroidal sapogenins are secondary metabolites whose biosynthetic precursors are sterols, particularly cholesterol ^[3]. High performance thin layer chromatography (HPTLC) is an invaluable quality assessment tool for the evaluation of botanical materials. It allows for analysis of a broad number of compounds both efficiently and cost effectively. Additionally, numerous samples can be run in a single analysis thereby dramatically reducing analytical time.

With HPTLC, the same analysis can be viewed using different wavelengths of light thereby providing a more complete profile of the plant than is typically observed with more specific types of analyses performing thin-layer chromatographic separation on HPTLC layers.

The main aim of the study is to find out the percentage of steroidal saponin Diosgenin in two varieties of Gokshur. A simple, accurate, precise and cost-effective HPTLC method was used for quantitative determination of Diosgenin in Gokshur sample. The results shows that there is no significant difference in Diosgenin content in Gokshur varieties.

MATERIALS AND METHODS

Diosgenin was purchased from Sigma chemicals Pvt. Ltd., Mumbai (India). Raw Gokshur plant was collected from Jamnagar. All the solvents and reagents used were of analytical grade.

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 conc. 1mg/ml.

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This solution was used for further dilutions. The dilutions were used for the analysis.^[4,5]

Preparation of standard solution

10 mg of Diosgenin was weighed and dissolved in 5 ml methanol by means of Ultrasonication for 15 min. The solution was diluted up to 10 ml with methanol (1 mg/ml). Pipette out 1 ml solution from stock solution and diluted up to 10 ml with methanol (100 µg/ml). From this stock solution further dilutions were made.

Experimental conditions

Chromatographic Conditions:

The following Chromatographic Conditions were used to quantify the Diosgenin:

1. Stationary phase: silica gel GF 254(E.Merck) precoated TLC plates
2. Mobile phase : Toluene:Ethyl acetate:Formic acid (7:3:1 v/v/v)
3. Sample volume : 5µl
4. Sample for HPTLC: Methanol extract of Laghu Gokshur
:Methanol extract of Brihat Gokshur
:Standard Diosgenin solution
5. Spray reagent : Vaniline sulfuric acid

Instrumental Conditions:

- Application mode : Camag Linomat V
- Development Chamber : Camag Twin trough Chamber.
- Plates : Precoated Silica Gel GF254 Plates.
- Chamber Saturation : 30 min.
- Development Time : 30 min.
- Development distance : 7 cm.
- Scanner : Camag Scanner III
- Detection : Deuterium lamp.
- Data System : Win cats software

Procedure:

Before spotting, the plates were pre-washed with methanol. Standard & sample solutions were applied to the plates as sharp bands by means of Camag Linomat V sample applicator. The spots were dried in a current of air. The mobile phase (20 ml) was poured into a twin trough glass chamber, 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 & quantification was performed with Camag TLC scanner 3 at a wavelength of 254 nm.

Quantification:

Sample solutions (5µl) and standard solution (2.5µl,5µl,7.5µ) were spotted on HPTLC plate (E.Merck). The percentage of Diosgenin present in Brihat and Laghu Gokshur extract was calculated by comparison of the areas measured for standard solution.

Linearity:

The linearity of diosgenin was determine by applying standard solution of different concentrations ranging from 2.5-7.5 µg/ml spot on 20x20 cm HPTLC plates, precoated with silica gel GF 254 (E.Merck) in the form of sharp 6 mm bands, the distance between 2 adjacent bands was 9.5 mm .The plate was developed in a solvent system of Toulene: Ethyl acetate: Formic acid (7:3:1v/ v/v), up to a distance of 80 mm, at room temperature. The plate was dried in air.

The detector response for Diosgenin was measured for each band at wavelength of 254 nm, using Camag TLC scanner & win CAT software. The peak areas of Diosgenin was obtained by plotting a graph of peak vs applied concentration of Diosgenin (µg).

RESULTS

The method described utilizes silica gel GF 254 HPTLC plates as stationary phase and Toulene : Ethyl acetate: Formic Acid (7:2:1 v/v/v) as mobile phase which gives good separation of Diosgenin (Rf.=0.55) standard. The results show that there is difference in percentage of Diosgenin in Brihat and Laghu Gokshur. The percentage of Diosgenin was found to be **8.4 %** in Brihat gokshur (*P.murex*) and **9.2 %** in Laghu gokshur (*T.terrestries*). Results are shown in (Table 2). The calibration curve was linear in the range of 2.5-7.5 µg/ spot & the correlation coefficient was determined (Fig 2). The correlation coefficient was found to be 0.9989. After derivatisation with vanillin sulfuric acid the sample shows similar colour reaction as that of standard. Bluish green colour spots were observed in sample & standard at Rf value 0.55

Table 1: HPTLC results (Rf value)

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	11	0.04,0.06,0.14,0.22 0.26, 0.36,0.42,0.44 , 0.55 ,0.63,0.66	8	0.05,0.08,0.13,0.20, 0.25,0.28, 0.55 ,0.48	2	0.32, 0.55

Table 2: Percentage Diosgenin

S. No	<i>T.terrestris</i>	<i>P.murex</i>
1	9.2%	8.4%

Fig 3: 3D Densitogramme 254 nm

Fig 1: Representing the HPTLC spots

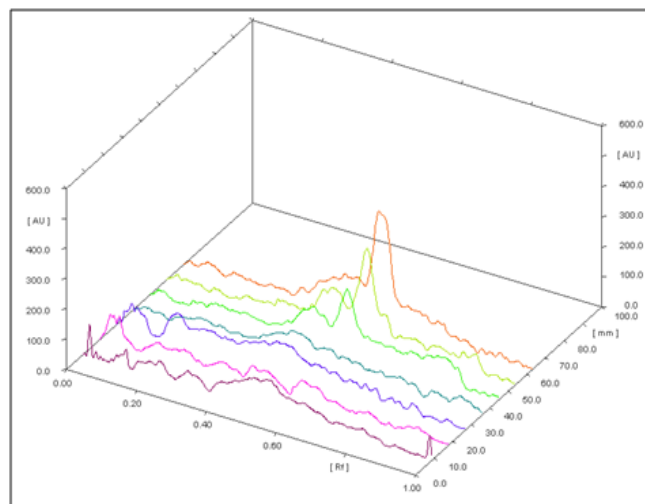
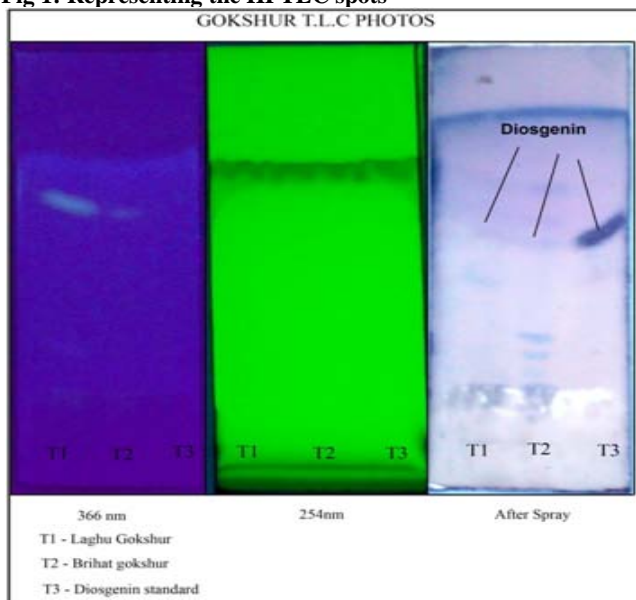
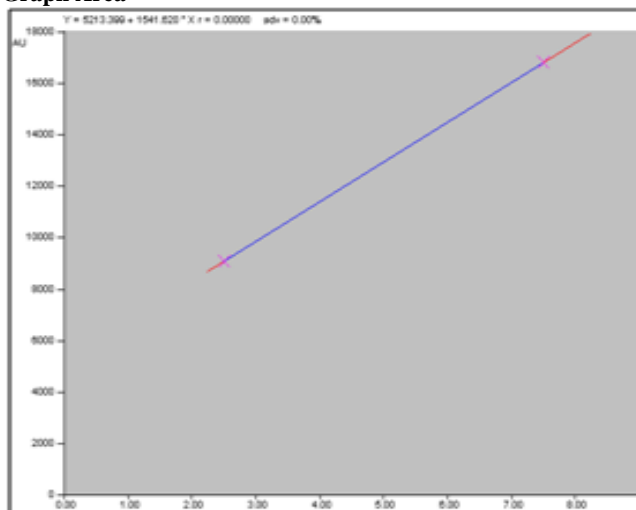
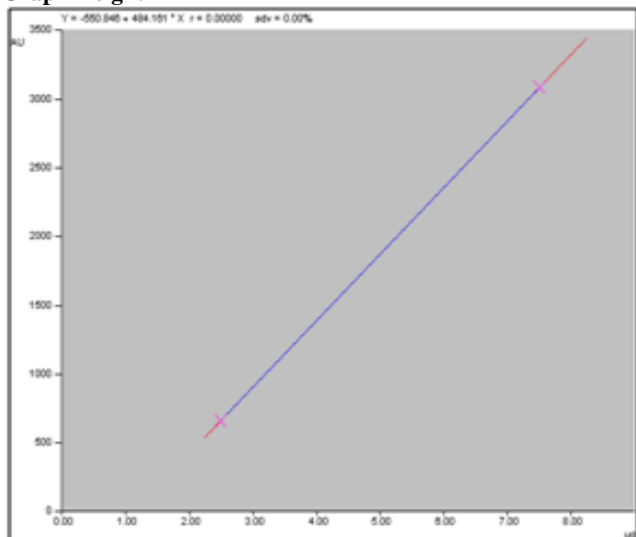


Fig 2: Linearity Graph Graph Area



Graph Height



DISCUSSION

HPTLC fingerprint shows no significant difference in percentage of Diosgenin in Laghu gokshur (*T.terrestris*) and Brihat gokshur (*P.murex*). In Ayurveda Brihat gokshur is used as an substitute for Laghu gokshur. The study shows that both varieties contains Diosgenin and there is no significant difference. So study shows that Brihat Gokshur can be used as substitute for Laghu Gokshur.

CONCLUSION

The developed HPTLC method was simple, accurate, precise and cost-effective and can be utilized for the routine analysis of quantitative determination of Diosgenin in Gokshur sample.

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