TITLE PAGE

IDENTIFICATION AND QUANTIFICATION OF DIOSGENIN IN ETHANOLIC EXTRACT OF *Trigonella foenum graecum (Fenugreek)* SEEDS

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ABSTRACT:

Diosgenin is an important steroidal sapogenin belonging to the triterpene group. Diosgenin is an precursor for synthesis of various semi synthetic steroids such as corticosteroids, sex hormones eg: estrogen, progesterone, testosterone and other steroidal drugs in pharmaceutical industry.. Diosgenin is used in the treatment of various diseases like in the management of stress, suppression of cholesterol absorption in body. Diosgenin can be isolated from various species of Dioscorea family , Costus species and from Trigonella species also. The present study is conducted to isolate and to quantity the diosgenin in fenugreek extract using chloroform solvent. The paper is concerned on the various studies like TLC, UV, IR, HPLC and HPTLC studies to

study the presence of diosgenin in the extract. The concentration of diosgenin obtained from fenugreek is 0.77% from fenugreek seeds.

Keywords: Diosgenin, Dioscorea, Fenugreek, TLC, HPTLC

1. INTRODUCTION

Medicinal plants and herbal medicines are very important and are in great demand for isolation of various chemical compounds which act as an intermediate and starting material for synthesis of various medicines. Steroids play an important role in drug and medicines. Steroidal compounds isolated from plants are used as a precursor for the synthesis of different compounds. Diosgenin **(Fig no.1)** is an important steroidal sapogenin belonging to the triterpene group. Diosgenin is an precursor for synthesis of various semi synthetic steroids such as corticosteroids, sex hormones eg: estrogen, progesterone, testosterone and other steroidal drugs in pharmaceutical industry.

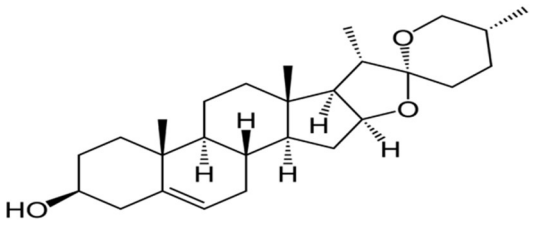


Fig No.1 Structure of diosgenin.

Diosgenin is reported in treatment of various diseases and disorders like it suppresses cholesterol absorption inside body, led to induction of apoptosis, induced differentiation of human erythroleukaemia cell line. It has estrogenic effects and also anticancer effects as it led to induced apoptosis. To date, diosgenin and other steroidal compounds were isolated in large quantity from *Dioscorea* species, it can also be isolated from other species like fenugreek, costus, tribulus etc.

Trigonella foenum graecum belonging to family- Fabaeace also known as methi, fenugreek contain a wide variety of constituents like alkaloids, flavonoids, saponins, amino acids, tannins and some steroidal glycosides, proteins etc. The seeds of fenugreek plant contain diosgenin in

more amount as compared to other aerial parts. The diosgenin present in the plant can be isolated for synthesis of various steroidal drugs. The present study is conducted to isolate and to quantity the diosgenin in fenugreek extract. The paper is concerned on the various studies like TLC, UV, IR, HPLC and HPTLC studies to study the presence of diosgenin in the extract.

2. MATERIALS AND METHOD:

2.1 Plant Material:

The seeds of *Trigonella foenum graceum* (fenugreek) were collected from local shop market of Raipur. The seeds were cleaned and dried over sunlight. The seeds were authenticated at Government Ayurvedic College, Post Graduate Department of Dravyaguna, GE Road, Raipur. The seeds are then grounded into powder form and passed through a 1 mm sieve. The fine powder was subjected to extraction.

2.2 Chemicals:

All the chemicals and solvents like ethanol, chloroform and methanol required for the extraction and identification process were of analytical grade and were procured from Merck.

2.3 Extraction of Diosgenin:

Diosgenin was extracted by the process of hydrolysis with ethanolic sulfuric acid. 65 gm of powdered plant material i.e seeds of fenugreek was mixed with 140 ml of 2.5M ethanolic sulfuric acid in a round bottom flask. 2.5M ethanolic sulfuric acid was prepared by dissolving 139ml of sulfuric acid to a sufficient quantity of ethanol to produce 1000 ml. (I.P 2007 page no:552) The filtrate was diluted to 100ml with water and then extracted with chloroform.(2×100ml). The chloroform extract was evaporated on water bath and the extract was then identified and quantified by TLC and other methods.

2.4 Yield Estimation:

Extract obtained was taken into a pre weighed china dish. The extract was then dried over water bath and subsequently weighed. The difference in weight was measured and the extract was kept in the dessicator for 7 days. The weight difference was used to calculate percentage yield. The percentage yield of the extract was calculated using the following formula:

$$\% \text{ yield} = \frac{\text{mass of extract}}{\text{mass of seed powder}} \times 100$$

2.5 Preliminary phytochemical screening:

Phytochemical screening of the extract was performed according to standard methods. Fenugreek extract obtained by above process was subjected to dryness and then the extract was used for the qualitative analysis of different phytoconstituents viz glycosides, alkaloids and steroidal compounds.

2.6 Characterization of diosgenin in extract:

2.6.1 TLC identification of extract:

The silica plate was placed in the developing chamber with mobile phase. Mobile phase was selected by hit and trial method. Various ratio of solvent system were prepared and the spots was visualized and Rf value was calculated. After visualization of spots solvent system or mobile phase was selected.

2.6.2 Qualitative estimation of diosgenin in extract by *UV* absorbance*:*

Diosgenin level in fenugreek extract was determined by measuring the absorbance at 430 nm, based on the color reaction with anisaldehyde, sulfuric acid and ethyl acetate. The absorbance was measured using chromophoric or color reactions. Two color developing reagent solutions were prepared (A) 0.5ml p-anisaldehyde and 99.5 ml ethyl acetate and (B) 50ml concentrated sulfuric acid and 50 ml ethyl acetate. 0.2 ml of extract was placed in a tube and dissolved in 2ml of ethyl acetate, 1ml of each of the reagents A and B were added to the tube and were mixed properly. The tubes was placed in a water bath maintained at 30°C for 10 min to develop color, then the tube was allowed to cool. The resultant yellow color chromogen was measured with spectrophotometer (Shimadzu, UV-Visible 1800) between wavelengths 300 nm -700 nm, using ethyl acetate solution as a blank for the process.

2.6 .3 Characterization and quantification of diosgenin in extract by HPTLC

Measured amount of extract were spotted on TLC plates for the development of solvent system for HPTLC method.

- **Preparation of sample:** 500 mg of given samples were solubilised in the respective solvent. Sonicator was used to make the samples complete soluble. After solubilisation samples were filtered using whatman filter paper to avoid residual impurities in samples.
- **Application and development of plates**: Commercially available precoated HPTLC plates (Merck) were used for the study.10µl of sample solutions were applied on the HPTLC plate using Linomat V applicator. The plates were then dried after application.
- **Solvent system:** Toluene: Ethyl acetate: formic acid (5:5:0.5)
- **Detection:** The developed plates were first observed under visible light and UV light (356 and 254 nm) for the detection of constituents.
- **Densitometry scanning:** The developed plates were scanned at a suitable wavelength for the qualitative analysis. Peak areas and peak heights were recorded from which the unknown concentration and RF value of the samples have been determined.

3. RESULT AND DISCUSSION:

Simple method was adopted for identification and characterization of diosgenin in fenugreek extract. As the quantity of diosgenin as an active constituent is less in fenugreek as compared to its rich source in dioscorea species , but due too its ease availability it can be used for isolation of steroidal compounds.

3.1 Percentage Yield: The percentage yield of the given extract was found to be **15.4% w/w.**

3.2 Preliminary phytochemical screening: Phytochemical tests of chloroform extract of fenugreek seeds revealed the presence of various phytoconstituents like

alkaloids, tannins and flavonoids which are responsible for its antibacterial and antimicrobial activity. It also shows the presence of steroidal saponin which can be isolated for synthesis of hormonal product. The plant extract was subjected to following tests.

S.No	Extract Constituents	Inference
1.	**Alkaloids**	**+ve**
2.	**carbohydrates**	**+ve**
3.	**Glycosides**	**+ve**
4.	**Steroids**	**+ve**
5.	**Flavonoids**	**+ve**
6.	**Tannins**	**-ve**
7.	**Amino acids**	**-ve**

+ve = present -ve = not present

TABLE NO: 1 Phytochemical screening of fenugreek extract

`3.3 TLC Profile:

TLC plates were run using solvent system **n-hexane: acetone: ethyl acetate** in the ratio of **1:8:1** respectively. The spots obtained were labeled and RF value was calculated.

Stationary phase: silica gel G

Solvent system: **n-hexane: acetone: ethyl acetate (1:8:1)**

Visualization: Using Iodine Chamber

S.No	SOLVENT SYSTEM	RATIO	VISUALIZATION	RF VALUES
1.	**n-hexane: acetone**	**1:9**		
		2:8	**No spots visible**	**negative**
		3:7		
		4:6		
		7:3		
3.	**n-hexane: acetone: ethyl**	**(1:8:1)**	**2 visible spots**	**1. 0.2**

	acetate			**2. 0.6**
3.	**Chloroform:toluene:methanol**	**3:6:1 4:4:2**	**Not visible Light spots**	**Rf values cannot be identified**

Table no.2 Solvent system of TLC

`3.4 UV Absorbance Method: The resultant chromogen prepared was scanned between 300-700 nm to find the maximum absorbance region. One of the constituents present in the extract shows maximum absorbance at 410 nm, which is compared with that of the standard diosgenin.

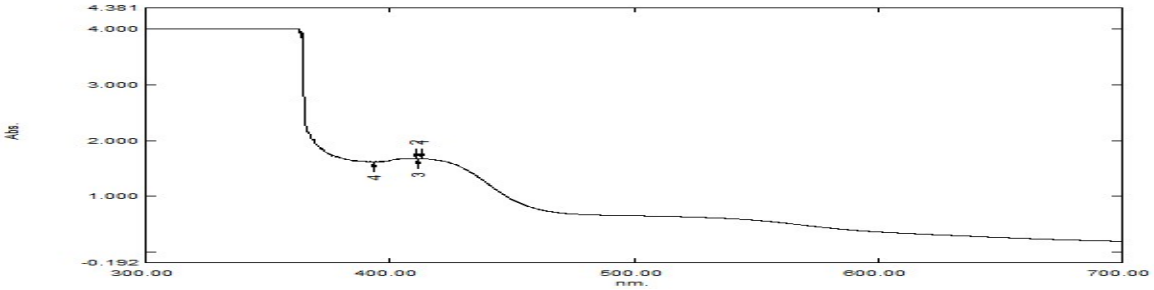


FIG NO 1: Absorption spectrum of fenugreek extract showing absorbance at 410 nm.

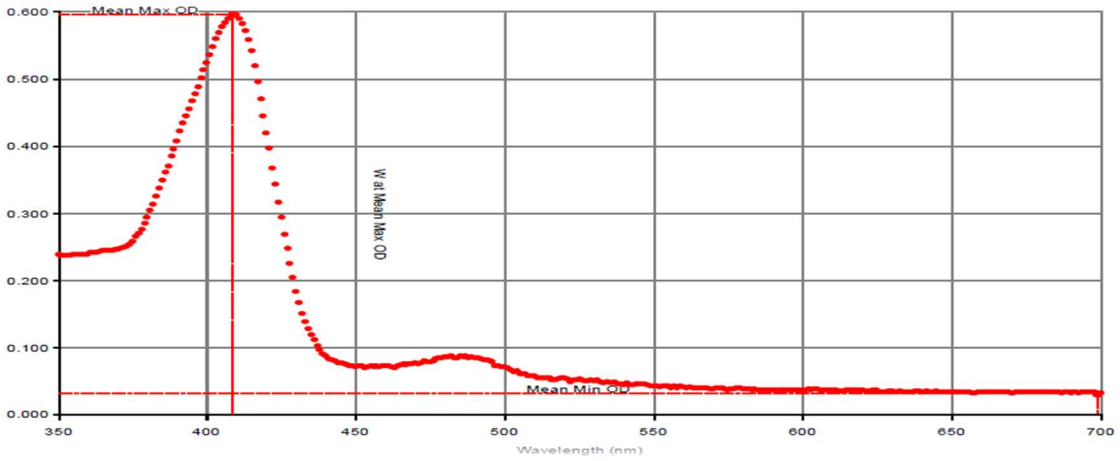
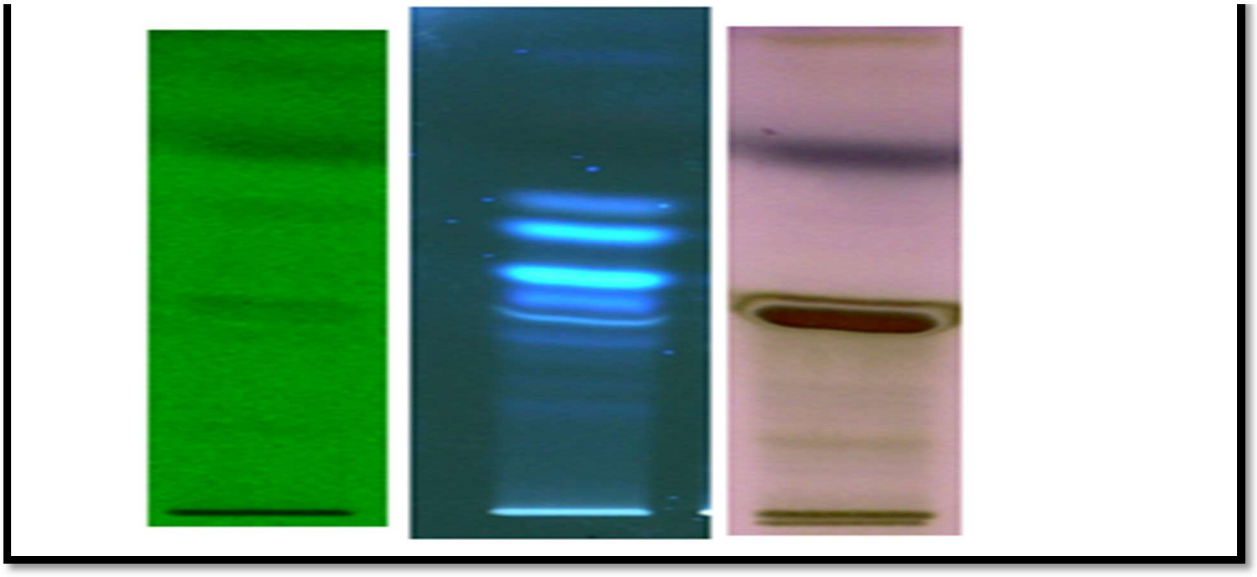


FIG NO 2: Absorption spectrum of standard diosgenin

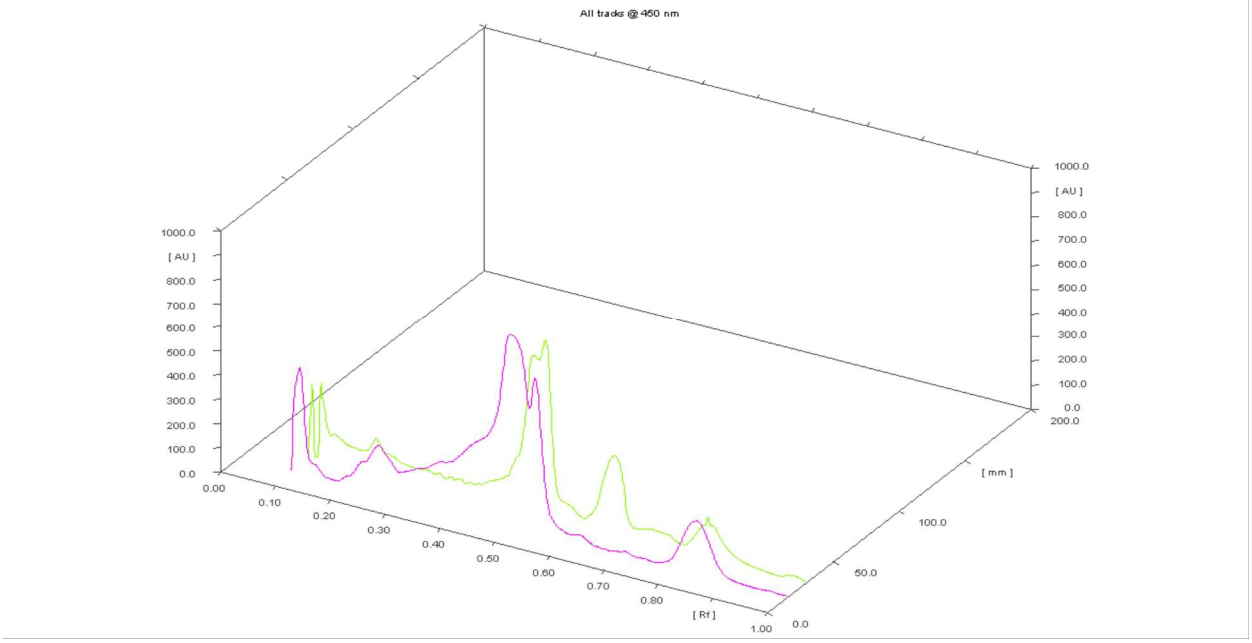
3.4 HPTLC Chromatogram of the extract: HPTLC result of the extract shows presence of various constituents along with diosgenin. In the extract the quantity of diosgenin present was 0.77% having Rf value between



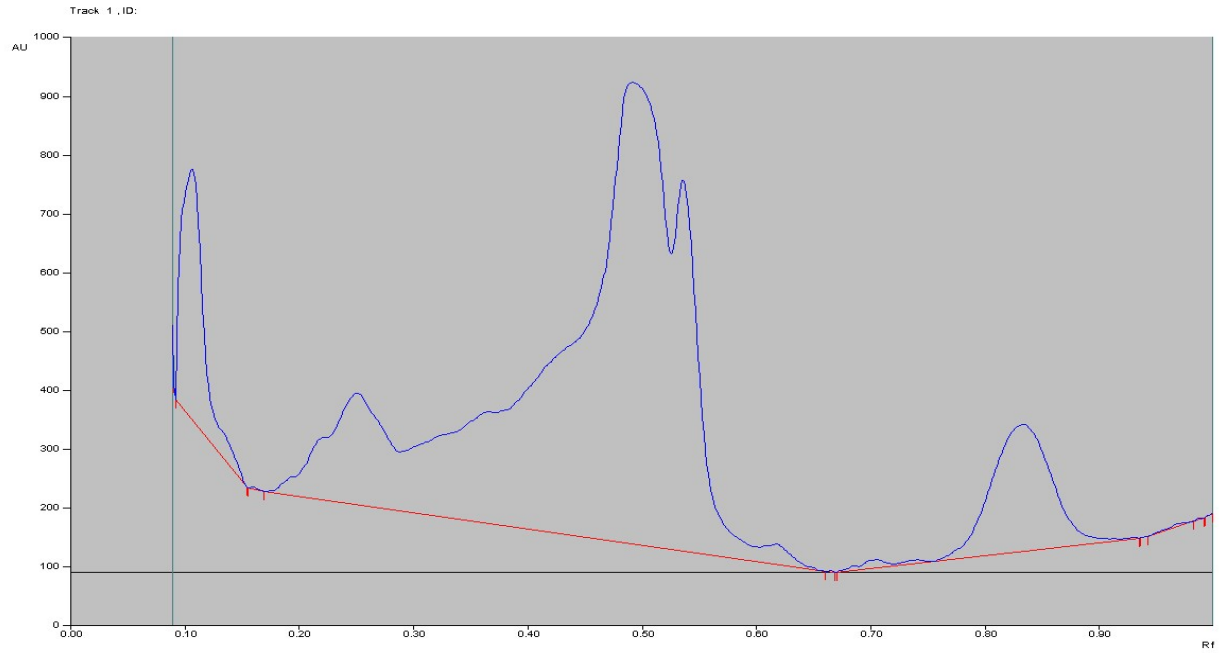
A **B** **C**

FIG NO-3 (**a**) HPTLC plate at 254nm (b) HPTLC plate at 365nm

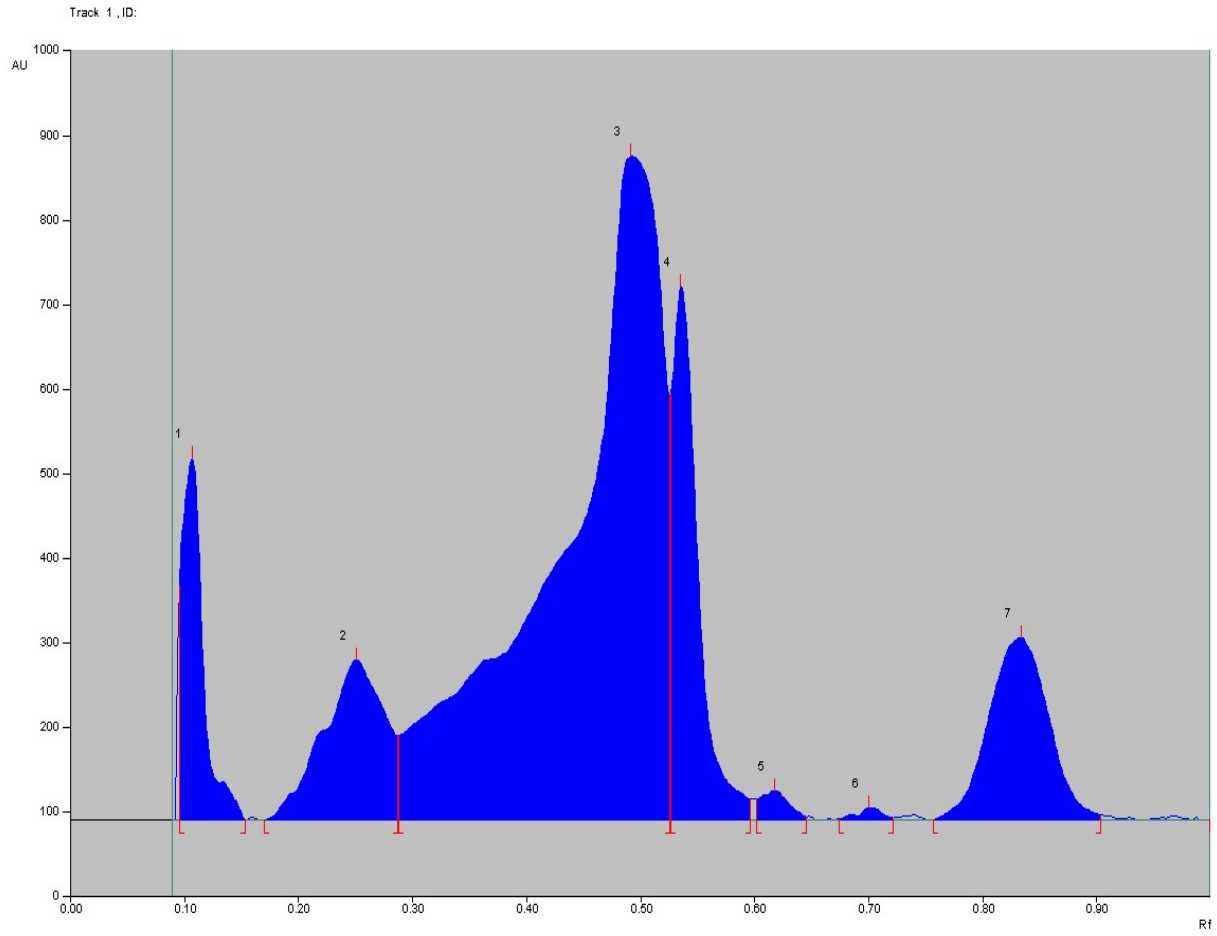
(c) HPTLC plate after derivatization with Anisaldehyde H2SO4



GRAPH NO .1 3Dspectrum of fenugreek extract



GRAPH NO. 2 **The HPTLC Spectrogram of the extracted compound.**



GRAPH NO .3 Densitometrry scan of fenugreek extract

s.no	StartPosition	StartHeight	MaxPosition	MaxHeight	Max%	EndPosition	EndHeight	Area	Area%
1.	0.10 Rf	276.2 AU	0.11 Rf	427.8 AU	18.64%	0.15 Rf	0.2 AU	7480.7 AU	7.11%
2.	0.17 Rf	0.1 AU	0.25 Rf	189.3 AU	8.25%	0.29 Rf	99.2 AU	9342.6 AU	8.88%
3.	0.29 Rf	99.5 AU	0.49 Rf	784.3 AU	34.17%	0.53 Rf	501.9 AU	63614.1 AU	60.45%
4.	0.53 Rf	504.1 AU	0.54 Rf	629.8 AU	27.44%	0.60 Rf	25.2 AU	13206.2 AU	12.55%
5.	0.60 Rf	24.9 AU	0.62 Rf	35.0 AU	1.52%	0.65 Rf	3.7 AU	807.6 AU	0.77%
6.	0.68 Rf	1.6 AU	0.70 Rf	13.7 AU	0.60%	0.72 Rf	2.3 AU	286.1 AU	0.27%
7.	0.76 Rf	0.2 AU	0.83 Rf	215.5 AU	9.39%	0.91 Rf	6.1 AU	10504.1 AU	9.98%

TABLE NO:2 Table showing rf value and area covered of diosgenin and other compound

4. CONCLUSION:

This study deals with the extraction, photochemical screening, TLC, UV, HPTLC of Fenugreek seed extract.TLC of the extract was performed to obtain a good solvent system for the isolation of diosgenin. With the help of UV absorbance of the diosgenin in the extract was found. The result of HPTLC shows the quantity of diosgenin in the extract.

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