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Determination of Diosgenin Present in the Extract of Stems of Fenugreek by HPTLC and FTIR

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ABSTRACT

A sensitive, fast, and reproducible high performance thin-layer chromatographic method has been developed for analysis of diosgenin from fenugreek stem extract using TLC aluminium plates precoated with silica gel G60F254. Among the different combinations of mobile phases used, best separation was achieved in methanol and water (9:1, v/v). Densitometric scanning of the plates directly at 275nm was used for analysis of diosgenin. For analysis of diosgenin, plates were scanned at 450nm after spraying and the retardation factor value of diosgenin was found to be 0.14 ± 0.1. in fenugreek stem extract sample. And also the interpretation of IR spectrum of both were compared and found to be similar. The present method is being reported for the first time and can be used for routine quality control and quantification of these marker compounds in various plant samples, extracts, and market formulations.

INTRODUCTION:

High-performance thin-layer chromatography (**HPTLC**) is an enhanced form of thin-layer chromatography (TLC). A number of enhancements can be made to the basic method of thin-layer chromatography to automate the different steps, to increase the resolution achieved, and to allow more accurate quantitative measurements.

Automation is useful to overcome the uncertainty in droplet size and position when the sample is applied to the TLC plate by hand.[1] One approach to automation has been the use of piezoelectric devices and inkjet printers for applying the sample.[2]

The spot capacity (analogous to peak capacity in HPLC) can be increased by developing the plate with two different solvents, using two-dimensional chromatography.[3]

Fig.1 Fenugreek stem

Fenugreek (*Trigonella foenum-graceum* Linn., Fam. Leguminosae) has a long history of being used as a medicinal herb and has been regarded as a treatment for just about every ailment known to man [4]. Right from early times, it has been extensively used in both Indian Ayurveda and Unani systems of medicines as well as traditional Chinese medicines for treatment of epilepsy, paralysis, gout, dropsy, chronic cough, diabetes, piles sinus, and lung congestion, inflammation, infection mitigation, hair treatment, breast enhancement, and aphrodisiac effects [5– 7]. The crop species has also long been used as a galactagogue to promote lactation in weaning mothers as well as for its ability to treat wounds and sore muscles [5, 8]. With the growing need for safer drugs, such type of traditional herbal medicines have been extensively preferred to prevent and cure human diseases because of easy accessibility by the local people and most importantly low toxicity [9]. Therefore, in view of this, human use of fenugreek is expected to increase day by day. Research literature survey suggests pharmacological properties of fenugreek seed are attributed to presence of

specific bioactive compounds like steroidal diosgenin, alkaloid trigonelline, flavonoid quercetin, galactomannan, and unusual amino acid 4-hydroxyisoleucine [10].

Diosgenin, a phytosteroid sapogenin, is the product of hydrolysis by acids, strong bases, or enzymes of saponins, extracted from the tubers of *Dioscorea* wild yam, such as the Kokoro. The sugar-free (aglycone) product of such hydrolysis, diosgenin is used for the commercial synthesis of cortisone, pregnenolone, progesterone, and other steroid products.

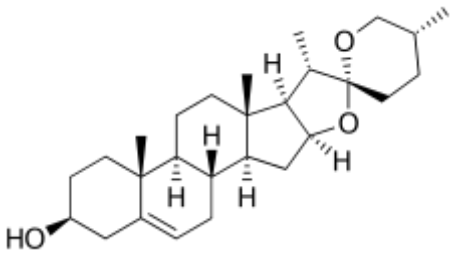


Fig.2. Structure of Diosgenin

Mainly diosgenin (25R)-spirost5-en-3 β-ol) that in addition to possessing anti-rheumatic and anti-viral properties, suppresses inflammation, inhibits proliferation, and induces apoptosis in a variety of tumour cells [11]. In the production of steroidal drugs and hormones such as testosterone, glucocorticoids, and progesterone, diosgenin is often used as a raw precursor [12].

The method is being reported for the first time and can widely be applied for routine analysis and quality assurance of related extracts, drugs, and polyherbal formulations.

MATERIALS AND METHODS

Method development:

Samples and Chemicals Standard diosgenin (98%) were procured from Chalapathi Institute of Pharmacetical Sciences.

Preparation of Standard Solution: Standard solutions of diosgenin (98%) were prepared with methanol: water (9:1) at a concentration of 1 mg mL−1 each in absolute methanol. Ultra sonication of mixture was required to ensure complete dissolution.

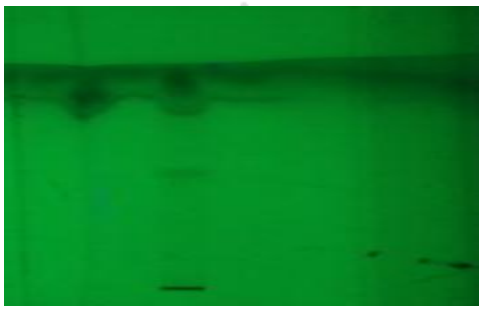
Sample Preparation: Accurately weighed fenugreek stems were refluxed in methanol for 5 hours and the extract was concentrated and dried and powder (1.0 g) of fenugreek stem

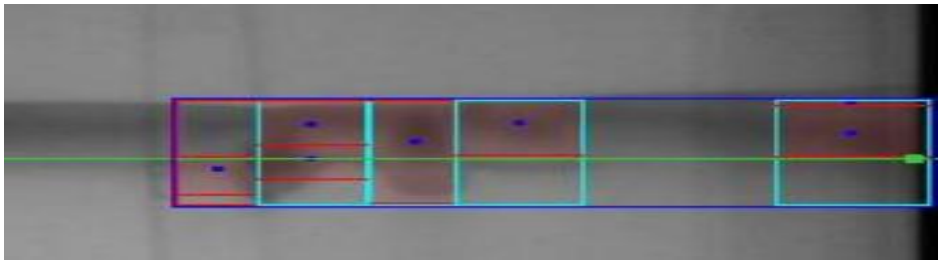
extract was taken separately in 100 mL round bottom flask. And then the different dilutions 0.1 -0.5ul of fenugreek stem extracts were prepared.

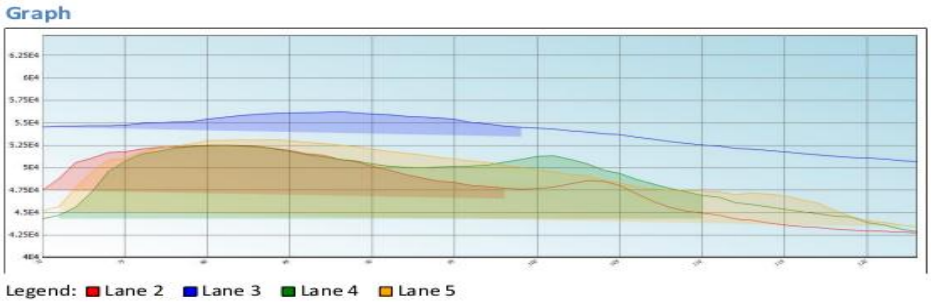
RESULTS AND DISCUSSION:

Estimation of Diosgenin for Preparation of Calibration Curves

Calibration was performed by application of variable concentrations of respective standard solutions (1 mg mL−1) of diosgenin on HPTLC plates. Both the standards were spotted at 0.1–0.5 μL in triplicate on 20 × 10 cm TLC plates for preparing six point linear calibration curves. The mobile phase was methanol and water (9:1, v/v). nonpolar ones. The calibration graph was plotted using the concentration versus average peak area at 450 nm and 275 nm for diosgenin. From each of the three samples, 4.0 μL of the extracts were applied in triplicate on the HPTLC plates so that the sample zone scan areas matched the scan areas of the standards in the middle of the calibration range, that is, 105 per band for diosgenin. The experimental parameters were identical for all the above analysis.







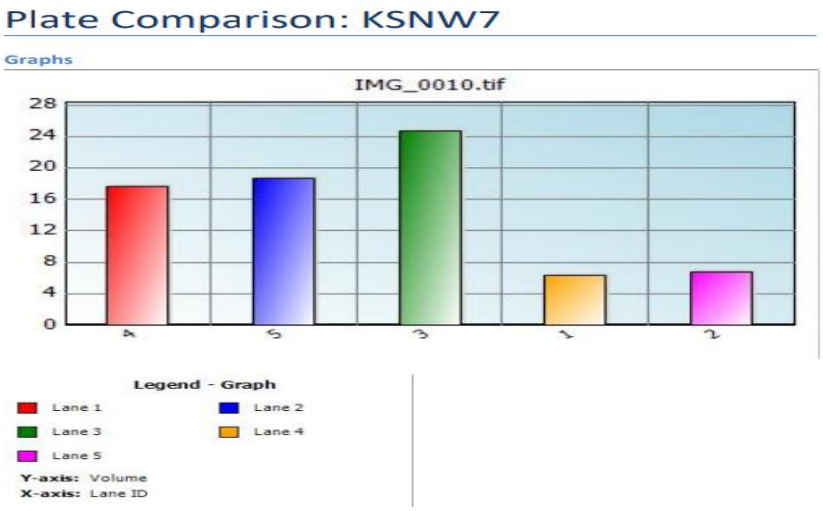


Fig. 3: Images of HPTLC separation of diosgenin and extracts of samples of fenugreek stem extract

Table. 1: Comparison data of R_f

Comparison Data

ID	Rf	Area	Volume
1_1	0.12	315	6.19
2_1	0.1	378	6.64
3_1	0.22	570	24.68
4_1	0.14	532	17.53
5_1	0.2	567	18.57

FTIR Comparison

IR spectrums of both the fenugreek extract and diosgenin were taken and interpreted.

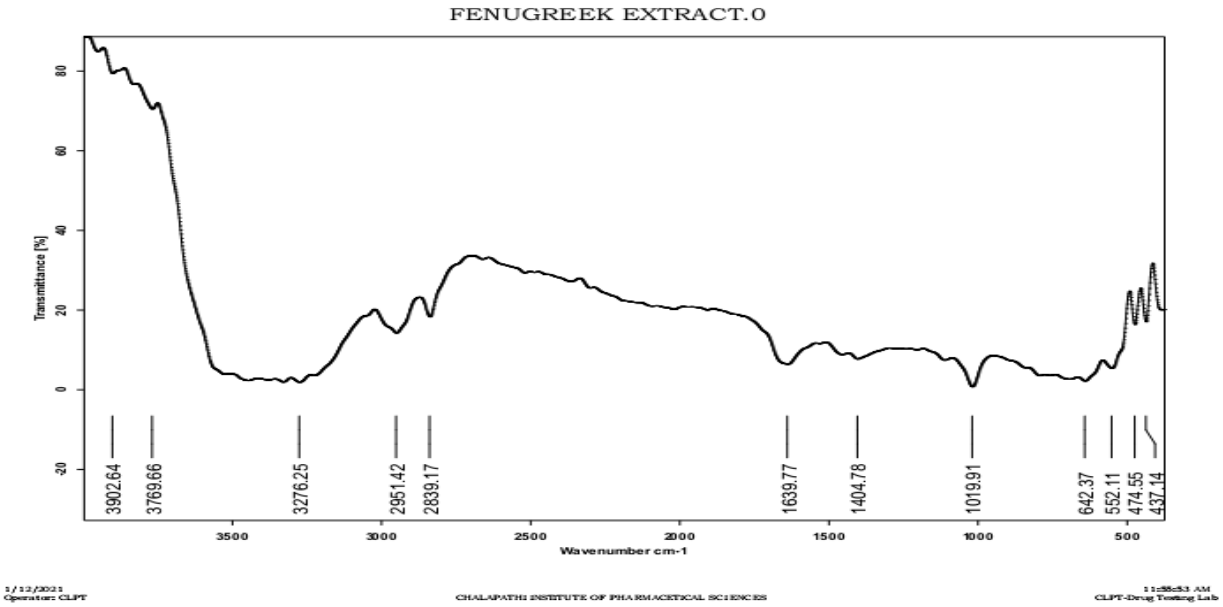


Fig. 4 IR Spectrum of Fenugreek stem extract

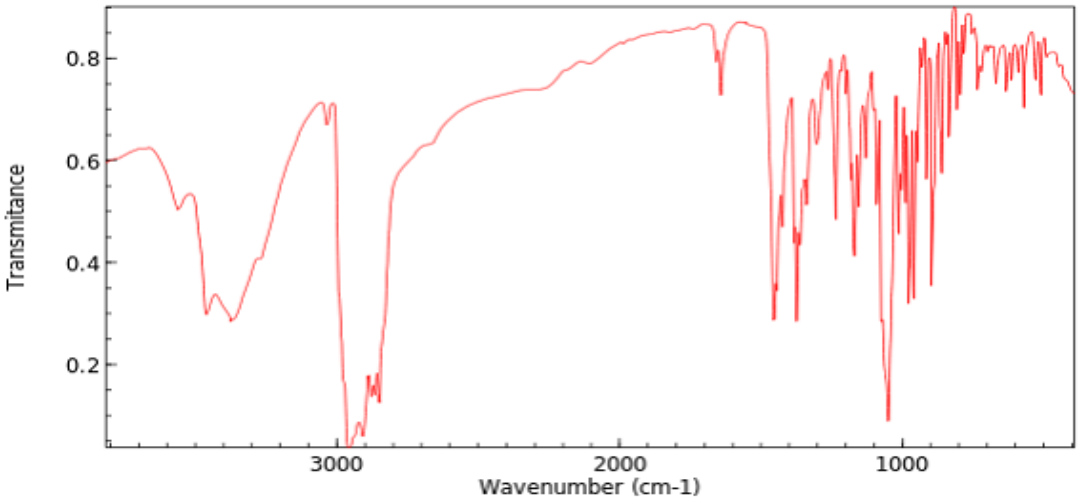


Fig. 5: IR Spectrum of Diosgenin

Table. 2: Interpretation of IR bands of fenugreek stem extract and diosgenin

Fenugreek stem extract		**Diosgenin**	
1000-1019	C=C Bending , Alkene	1000-1019	C=C Bending , Alkene
3276	OH Strething, Alcohol	3000	OH Strething, Alcohol
1639	C=N Stretching, Oxime	1639	C=N Stretching, Oxime
1404	S=O Stretching		

CONCLUSION:

A sensitive, fast, and reproducible high performance thin-layer chromatographic method has been developed for analysis of diosgenin from fenugreek stem extract, using HPTLC. Fenugreek stem extract samples were found to contain diosgenin in the retardation factor of 0.14 ± 0.1(w/w). And also the interpretation of IR spectrum of both were compared and found to be similar. The present method can be used for routine quality control and quantification of these marker compounds in various plant samples, extracts, and market formulations.

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