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Qualitative and quantitative estimation of Diosgenin in coded ayurvedic formulation and its ingredient *Trigonella foenum-graecum* Linn. seeds used in diabetics

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Abstract

Background: *Trigonella foenum-graecum* (Methi) is a leguminous plant and botanically known as *Trigonella foenum-graecum* Linn, belong to the family Fabaceae. *Trigonella foenum-graecum* is used for a variety of health conditions, including digestive problems, bronchitis, tuberculosis, fevers, sore throats, wounds, arthritis, abscesses, swollen glands, skin irritations, diabetes, loss of appetite, ulcers, and menopausal symptoms, as well as in the treatment of cancer. *Trigonella foenum-graecum* seeds mainly contain Diosgenin [(3β,25R)-spirost-5-en3-ol], a plant-derived steroid sapogenin.

Results: The identification and quantification results by HPTLC and HPLC studies of *Trigonella foenum-graecum* seeds hydrolysed *Trigonella foenum-graecum* seeds, coded formulation, hydrolysed coded formulation extract with standard Diosgenin biomarker showed a significant highest peak in hydrolysed *Trigonella foenum-graecum* seeds and hydrolysed coded formulation. The standard Diosgenin is observed in the hydrolysed form of hydrolysed *Trigonella foenum-graecum* seeds and hydrolysed coded formulation. The literature on *Trigonella foenum-graecum* confirms its activity as antidiabetic, and the peak of standard biomarker Diosgenin is seen after derivatization with anisaldehyde sulphuric agent, which possesses medicinal phytoconstituents value. Further related to future scientific aspects, more studies on its potent antidiabetic activity and multipurpose action need to be carried out with medicinal composition and its effects on the human body.

Conclusion: This study aims to establish the qualitative and quantitative estimation of standard Diosgenin in reliable with coded ayurvedic formulation and *Trigonella foenum-graecum* seeds and its activity as antidiabetic by HPTLC and HPLC.

Keywords: *Trigonella foenum-graecum*, Coded formulation, Antidiabetic, Diosgenin, Ayurveda

Highlights

- HPTLC fingerprint profile along with Diosgenin.
- Quantification of Diosgenin by HPLC.

Background

India was enriched with a variety of herbs, shrubs, trees, and seeds used in the ancient Indian system of medicine and reported to possess beneficial medicinal effects in curing illness. *Trigonella foenum-graecum* is used in both forms in food and medicine in Asia. Seeds are eaten as sprouts and rich in secondary metabolites, potential sources of drugs, and essential oils of therapeutic importance. Due to their bitter taste, seeds are a good source of resin, protein, fibre, and mucilage. The critical advantages

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of medicinal plants' therapeutic uses in various ailments are their safety besides being economical, effective, and easy availability [1–3]. Our ayurvedic system praised this herb and documented its healing capacity in its ancient texts.

Among the plants known for medicinal value in Indian medicine, the plants of genus *Trigonella foenum-graecum* Linn belong to the family Fabaceae are vital for their therapeutic potentials. It is extensively cultivated as a semi-arid crop in northern Africa, the Mediterranean, India, and Canada [4]. *Trigonella foenum-graecum* (Fig. 1) grows as an erect annual with long, slender stems reaching 30–60 cm in height. The plant bears grey-green, tripartite, toothed leaves. White or pale-yellow flowers appear in summer and develop into long, slender, sword-shaped seed pods with a curved, beak-like tip. It has been reported that *Trigonella foenum-graecum* could be employed in the medicinal, pharmaceutical, and nutraceutical fields [5]. It is used as an aphrodisiac, astringent, demulcent, carminative, stomachic, diuretic, emmenagogue, emollient, expectorant, galactagogue, restorative, and tonic [3]. *Trigonella foenum-graecum* is used to treat digestive issues, bronchitis, TB, fevers, sore throats, wounds, arthritis, abscesses, swollen glands, skin irritations, diabetes, loss of appetite, ulcers, and menopausal symptoms, as well as cancer. Antidiabetic, antifertility, anticancer, antibacterial, and antiparasitic effects have been described for *Trigonella foenum-graecum* [3, 6]. An infusion of the leaves is used as a gargle for recurring mouth ulcers in traditional medicine. It is used as an emollient in poultices for boils, cysts, and other skin irritations. It is used to control blood pressure and lower blood sugar levels. *Trigonella foenum-graecum* has been shown to reduce inflammation, alleviate congestion, and fight infection. It loosens and eliminates excess mucus and phlegm, as well as relieving sinus and lung congestion. The *Trigonella foenum-graecum* seed is used in Chinese medicine to treat abdominal discomfort, chilblains, cholecystitis, fever, hernia, impotence, hypogastric, nephrosis, and rheumatism [7]. *Trigonella foenum-graecum* is known to contain alkaloids, flavonoids, salicylate, and nicotinic acid. The chemical composition of *Trigonella foenum-graecum* (like seeds, husk, and cotyledons) showed that endosperm had the highest saponins (4.63 g/100 g) and protein (43.8 g/100 g) content [6, 8]. *T. foenum-graecum* contains 45–60% galactomannans, 20–30% proteins high in lysine tryptophan, 5–10% lipids, pyridine alkaloids, trigonelline (0.2–0.38%), choline (0.5%), carpaine gentianine, flavonoids luteolin, apigenin, quercetin, orientin, isovitexin vitexin, amino as 4-hydroxyisoleucine (0.09%), histidine, arginine lysine, calcium, saponins, glycosides steroidal sapogenins on hydrolysis (yamogenin, Diosgenin, neotigogenin, tigogenin), sitosterol, cholesterol, vitamin A, B1, C, and nicotinic acid [9–11]. *Trigonella foenum-graecum seeds* mainly contain Diosgenin [(3β,25R)-spirost-5-en3-ol], a plant-derived steroid sapogenin [3, 12]. Diosgenin [(3β,25R)-spirost-5-en3-ol] (Fig. 2), a phytosteroid sapogenin, possesses several biological activities, including anticancer, hypolipidemic, anti-inflammatory, and antidiabetic ones. It also plays a beneficial role in the cardiovascular system and reduces bone loss in osteoporosis [13]. Some reported literature quantifies Diosgenin in *Trigonella foenum-graecum* seeds by chromatographic methods specially HPLC [14–19]. In the current study, Diosgenin was selected for qualitative and quantitative estimation in the coded formulation and *Trigonella foenum-graecum* seeds by HPTLC and HPLC. The aim of the present study is to develop quality control protocols for the coded ayurvedic drug by analysing the reference standard of bioactive compounds present in the formulation and respective ingredient—evaluation of HPTLC fingerprint profiling along with dominant biomarkers and estimation of bioactive markers Diosgenin by HPLC.

Fig. 1 Plant material of *Trigonella foenum-graecum* Seeds

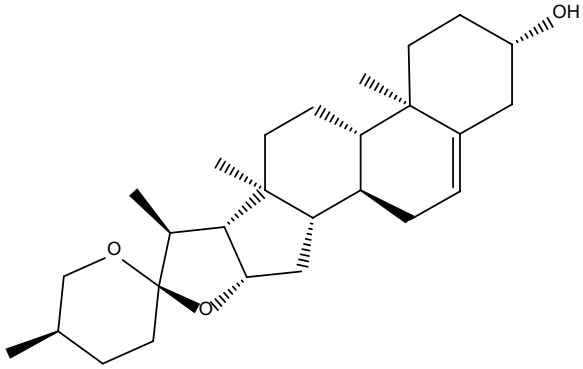


Fig. 2 Chemical structure of Diosgenin

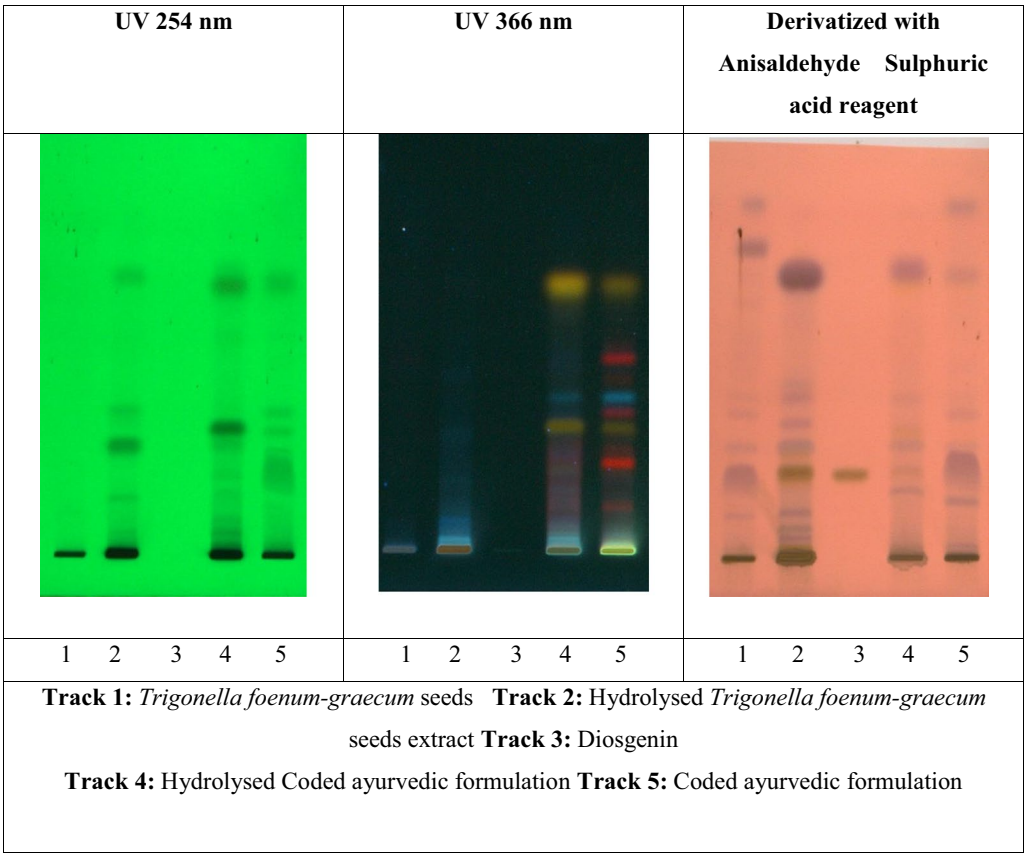


Fig. 3 HPTLC fingerprint profiling of *Trigonella foenum-graecum*, coded ayurvedic formulation and Diosgenin reference standard

Methods

Procurement of samples

CCRAS, New Delhi, supplied coded formulation and *Trigonella foenum-graecum* seeds.

Reagents and standards

All chemicals and solvents were used analytical grade or HPLC grade and obtained from E-Merck and other renowned companies. The Diosgenin reference standard was procured from Natural remedies, Bengaluru, India.

Extraction procedure

Preparation of Trigonella foenum-graecum seed extract

The dried powdered seed 10.4896 g of *Trigonella foenum-graecum* was extracted with 200 ml of methanol using Soxhlet for 24 h. The extract was evaporated to dryness under reduced pressure. The obtained extract was collected, dried, weighed, and stored separately for further studies. The obtained extracted residue weight of extraction was 2.1845 g.

Preparation of formulation extract

The powdered 10.3038 g coded ayurvedic formulation was extracted with 200 ml of methanol using Soxhlet for 24 h. The extract was evaporated to dryness under reduced pressure. The obtained extract was collected, dried, weighed, and stored separately for further studies. The obtained residue weight of extraction was 1.2939 g.

Preparation of standard Diosgenin solution

Stock solutions of Diosgenin (0.52 mg /ml) were prepared by dissolving 5.2 mg accurately weighed standards in a small amount of HPLC grade methanol and made up the volume to 10 ml in a standard volumetric flask. The stock solution was further diluted as per the requirement for the preparation of working solutions.

Preparation of test solutions

The residues obtained from coded ayurvedic formulation and *Trigonella foenum-graecum* seeds were accurately weighed 50 mg each and dissolved in methanol using 10-ml

Table 1 R_f values of *Trigonella foenum-graecum* seeds, coded ayurvedic formulation, and Diosgenin reference standard

Wavelength	Track 1: *Trigonella foenum-graecum*		Track 2: Hydrolysed *Trigonella foenum-graecum*		Track 3: Diosgenin		Track 4: Hydrolysed coded formulation extract		Track 5: Coded formulation extract	
UV 254 nm	R_f	**Colour**	R_f	Colour	R_f	Colour	R_f	Colour	R_f	Colour
	–	–	–	–	–	–	0.04	Green	0.05	Green
	–	–	0.08	Green	–	–	0.07	Green	–	–
	–	–	–	–	–	–	0.12	Green	0.13	Green
	–	–	0.18	Green	–	–	0.19	Green	–	–
	–	–	–	–	–	–	0.24	Green	0.24	Green
	–	–	0.32	Green	–	–	0.33	Green	0.30	Green
	–	–	–	–	–	–	0.37	Green	0.36	Green
	–	–	0.41	Green	–	–	–	–	0.43	Green
	–	–	–	–	–	–	0.64	Green	–	–
	–	–	0.80	Green	–	–	0.78	Green	–	–
	–	–	–	–	–	–	0.84	Green	–	–
UV 366 nm	–	–	0.04	Pale blue	–	–	0.04	Pale blue	0.04	Pale blue
	–	–	0.07	Dark blue	–	–	–	–	–	–
			0.09	Blue			0.11	Purple	0.11	Purple
	–	–	0.22	Blue	–	–	0.24	Purple	0.25	Red
	–	–	–	–	–	–	0.27	Purple	0.27	Purple
	–	–	0.34	Blue	–	–	0.37	Yellow	0.37	Yellow
	–	–	–	–	–	–	–	–	0.41	Red
	–	–	–	–	–	–	0.45	Blue	0.45	Blue
	–	–	0.52	Blue	–	–	–	–	0.51	Purple
	–	–	–	–	–	–	–	–	0.56	Red
	–	–	–	–	–	–	0.76	Yellow	0.76	Yellow
Derivatized with anisaldehyde sulphuric acid reagent	0.04	Grey	0.07	Grey	–	–	–	–	0.04	Grey
	0.12	Grey	0.11	Grey	–	–	–	–	0.09	Grey
	–	–	0.15	Grey	–	–	–	–	0.17	Grey
	–	–	0.20	Grey	–	–	0.20	Yellow	–	–
	0.22	**Grey**	**0.22**	**Green**	**0.22**	**Green**	**0.22**	**Green**	**0.22**	**Grey**
	0.34	Grey	0.34	Grey	–	–	0.34	Grey	0.32	Grey
	–	–	–	–	–	–	0.37	Grey	–	–
	0.41	Grey	0.41	Grey	–	–	0.41	Grey	0.41	Grey

volumetric flask, filtered through 0.22 µ membrane filters, and used for HPTLC fingerprint profiling and identification of reference standard Diosgenin biomarker compound.

Preparation of hydrolysed formulation and *Trigonella foenum-graecum* test solution

433.4 mg of *Trigonella foenum-graecum* seeds were weighed and extracted in 5 ml of methanol, and 4 ml of 3 N HCl was added. The above extract was refluxed for 1 h on a water bath. The solution was cooled and adjusted to pH 7 using aqueous ammonia. Make up the solution by using methanol in a 10-ml standard flask. Similarly, Ayush D was hydrolysed by weighing 500 mg extract in 5 ml of methanol, and 4 ml of 3 N HCl was added refluxed for 1 h on a water bath. The above solution was cooled and adjusted to pH 7 using aqueous ammonia. Made up to the mark in a 10-ml standard flask using methanol, filtered through 0.22 µ membrane filters, and used for HPTLC fingerprint profiling and identification of reference standard Diosgenin.

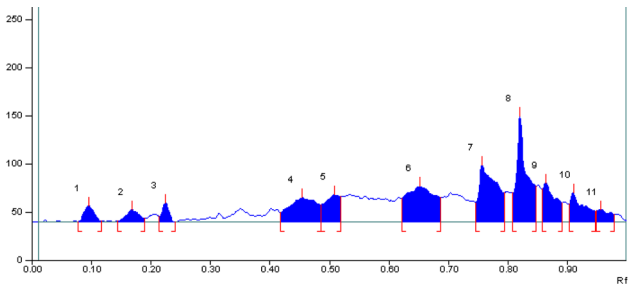
Instrumentation

HPTLC analysis

HPTLC analysis was performed on a CAMAG HPTLC system (Muttenz, Switzerland) equipped with an automatic TLC sampler IV, twin trough development chamber, TLC Scanner 3 linked with WINCATS software version 1.4.4 [20–26].

Track 1:

Trigonella foenum-graecum seeds extract



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.08 Rf	0.4 AU	0.10 Rf	16.8 AU	4.26 %	0.12 Rf	0.5 AU	260.8 AU	2.82 %
2	0.14 Rf	0.5 AU	0.17 Rf	13.0 AU	3.32 %	0.19 Rf	4.0 AU	254.4 AU	2.76 %
3	0.21 Rf	5.5 AU	0.22 Rf	19.8 AU	5.04 %	0.24 Rf	0.0 AU	209.9 AU	2.27 %
4	0.42 Rf	9.9 AU	0.45 Rf	25.2 AU	6.42 %	0.48 Rf	18.0 AU	1076.4 AU	11.66 %
5	0.49 Rf	18.1 AU	0.51 Rf	28.2 AU	7.17 %	0.52 Rf	26.5 AU	652.9 AU	7.07 %
6	0.62 Rf	24.4 AU	0.65 Rf	36.9 AU	9.38 %	0.69 Rf	25.4 AU	1633.8 AU	17.70 %
7	0.75 Rf	19.9 AU	0.76 Rf	58.6 AU	14.91 %	0.79 Rf	30.6 AU	1610.0 AU	17.44 %
8	0.81 Rf	30.1 AU	0.82 Rf	110.1 AU	28.01 %	0.85 Rf	37.3 AU	1791.0 AU	19.40 %
9	0.86 Rf	34.2 AU	0.86 Rf	40.5 AU	10.30 %	0.89 Rf	20.1 AU	812.0 AU	8.80 %
10	0.90 Rf	18.2 AU	0.91 Rf	30.8 AU	7.84 %	0.95 Rf	11.2 AU	668.3 AU	7.24 %
11	0.95 Rf	11.5 AU	0.96 Rf	13.2 AU	3.36 %	0.98 Rf	8.0 AU	262.5 AU	2.84 %

Track 2:

Hydrolyzed *Trigonella foenum-graecum* seeds extract

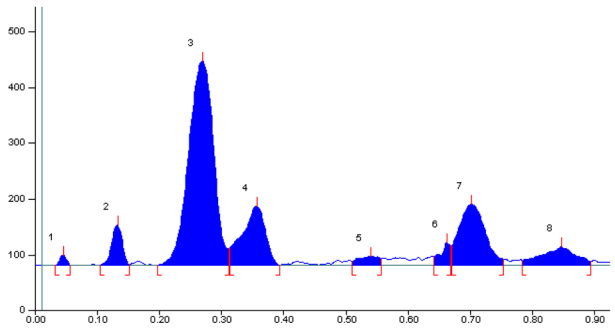


Fig. 4 HPTLC fingerprint profiles of *Trigonella foenum-graecum* seeds extract, coded ayurvedic formulation extract and Diosgenin reference standard at UV 254 nm

HPTLC fingerprint profile

The 7 µl each of the test solutions of hydrolysed *Trigonella foenum-graecum* seeds, *Trigonella foenum-graecum* seeds, hydrolysed coded formulation, coded formulation extract, and 2 µl Diosgenin standard solution was applied on a precoated silica gel 60 F_{254} TLC plate (E. Merck) of 0.2 mm thickness by using automatic TLC sample applicator (ATS-4). The plate was developed in a suitable solvent system of toluene/ethylacetate (9:1; v/v) in a saturated TLC chamber till the solvent rises to a distance of 8 cm.

The developed plate was dried and observed through CAMAG TLC Visualizer under UV at 254 and 366 nm, and photographs were documented. Derivatized with anisaldehyde sulphuric acid reagent and heated in a hot air oven at 105 °C until the colour of the spots appeared and the photograph was documented under white light. Before derivatization, the plate was scanned using CAMAG TLC Scanner with WINCATS software at a wavelength of UV 254 and 366 nm using deuterium lamps. After derivatization, the plate was scanned at 540 nm using a tungsten lamp.

HPLC analysis

HPLC instrumentation and chromatographic conditions

HPLC analysis was performed on an Agilent 1200 series high-performance liquid chromatographic system equipped with a quaternary pump, manual sample

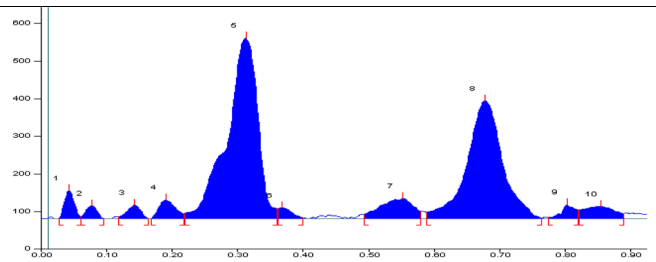
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.0 AU	0.04 Rf	18.4 AU	2.42 %	0.06 Rf	0.3 AU	181.4 AU	0.71 %
2	0.11 Rf	0.5 AU	0.13 Rf	72.0 AU	9.44 %	0.15 Rf	1.5 AU	1142.9 AU	4.46 %
3	0.20 Rf	0.1 AU	0.27 Rf	366.1 AU	48.00 %	0.31 Rf	29.7 AU	13597.0 AU	53.06 %
4	0.31 Rf	30.7 AU	0.36 Rf	106.5 AU	13.97 %	0.39 Rf	0.0 AU	3640.8 AU	14.21 %
5	0.51 Rf	6.9 AU	0.54 Rf	16.7 AU	2.19 %	0.56 Rf	12.4 AU	502.8 AU	1.96 %
6	0.64 Rf	16.4 AU	0.66 Rf	41.0 AU	5.37 %	0.67 Rf	35.8 AU	612.5 AU	2.39 %
7	0.67 Rf	36.0 AU	0.70 Rf	109.0 AU	14.30 %	0.75 Rf	10.7 AU	4263.4 AU	16.64 %
8	0.78 Rf	9.5 AU	0.85 Rf	32.9 AU	4.31 %	0.89 Rf	7.3 AU	1685.1 AU	6.58 %

Track 3:
Diosgenin

Spots are not visible at 254 nm

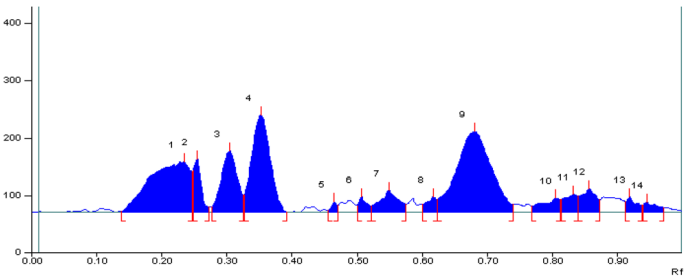
Track: 4

Hydrolysed
Codded
Formulation
Tablet Extract



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.9 AU	0.04 Rf	73.9 AU	6.51 %	0.06 Rf	5.5 AU	1010.7 AU	2.25 %
2	0.06 Rf	5.6 AU	0.08 Rf	33.9 AU	2.99 %	0.10 Rf	0.4 AU	521.2 AU	1.16 %
3	0.12 Rf	5.4 AU	0.14 Rf	35.2 AU	3.10 %	0.16 Rf	0.3 AU	683.6 AU	1.52 %
4	0.17 Rf	0.6 AU	0.19 Rf	49.1 AU	4.32 %	0.22 Rf	12.6 AU	1148.4 AU	2.56 %
5	0.22 Rf	12.9 AU	0.31 Rf	480.0 AU	42.29 %	0.36 Rf	27.2 AU	20866.6 AU	46.45 %
6	0.36 Rf	27.1 AU	0.37 Rf	28.6 AU	2.52 %	0.40 Rf	2.5 AU	548.5 AU	1.22 %
7	0.49 Rf	10.9 AU	0.55 Rf	53.7 AU	4.73 %	0.58 Rf	18.9 AU	2456.3 AU	5.47 %
8	0.59 Rf	16.7 AU	0.68 Rf	312.8 AU	27.56 %	0.76 Rf	1.7 AU	15597.3 AU	34.72 %
9	0.78 Rf	2.9 AU	0.80 Rf	36.3 AU	3.20 %	0.82 Rf	22.6 AU	695.4 AU	1.55 %
10	0.82 Rf	22.7 AU	0.85 Rf	31.5 AU	2.78 %	0.89 Rf	12.9 AU	1398.2 AU	3.11 %

Track: 5
Coded
ayurvedic
formulation
Extract

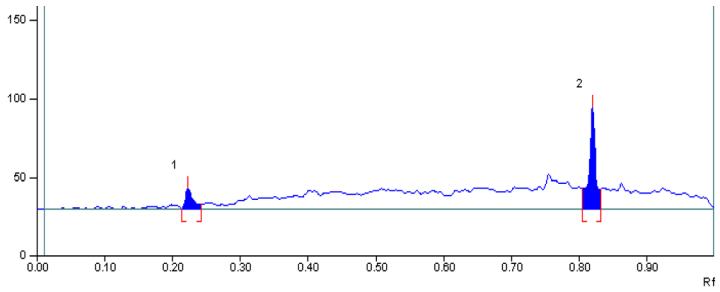


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.14 Rf	0.8 AU	0.23 Rf	88.4 AU	10.43 %	0.25 Rf	71.4 AU	5033.0 AU	20.58 %
2	0.25 Rf	72.4 AU	0.25 Rf	93.1 AU	10.98 %	0.27 Rf	9.8 AU	1034.1 AU	4.23 %
3	0.28 Rf	8.6 AU	0.30 Rf	106.2 AU	12.53 %	0.33 Rf	30.1 AU	2419.9 AU	9.90 %
4	0.33 Rf	30.4 AU	0.35 Rf	168.9 AU	19.93 %	0.39 Rf	0.4 AU	4419.4 AU	18.07 %
5	0.46 Rf	2.6 AU	0.47 Rf	18.1 AU	2.13 %	0.47 Rf	12.2 AU	143.6 AU	0.59 %
6	0.50 Rf	11.4 AU	0.51 Rf	26.8 AU	3.16 %	0.52 Rf	11.5 AU	314.9 AU	1.29 %
7	0.52 Rf	11.7 AU	0.55 Rf	38.2 AU	4.51 %	0.57 Rf	14.1 AU	979.5 AU	4.01 %
8	0.60 Rf	13.7 AU	0.62 Rf	26.8 AU	3.16 %	0.62 Rf	21.7 AU	379.2 AU	1.55 %
9	0.62 Rf	22.1 AU	0.68 Rf	140.8 AU	16.61 %	0.74 Rf	13.8 AU	6971.9 AU	28.51 %
10	0.77 Rf	10.7 AU	0.81 Rf	24.3 AU	2.87 %	0.81 Rf	21.6 AU	620.5 AU	2.54 %
11	0.81 Rf	21.9 AU	0.83 Rf	31.9 AU	3.76 %	0.84 Rf	28.2 AU	569.4 AU	2.33 %
12	0.84 Rf	28.4 AU	0.86 Rf	40.5 AU	4.77 %	0.87 Rf	22.2 AU	848.3 AU	3.47 %
13	0.91 Rf	18.4 AU	0.92 Rf	26.5 AU	3.12 %	0.94 Rf	12.1 AU	379.5 AU	1.55 %
14	0.94 Rf	12.5 AU	0.94 Rf	17.3 AU	2.04 %	0.97 Rf	8.4 AU	338.9 AU	1.39 %

Fig. 4 continued

Track 1:

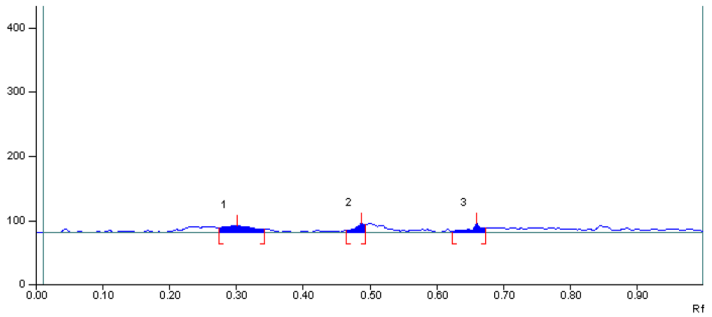
Trigonella foenum-graecum seeds extract



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.21 Rf	0.0 AU	0.22 Rf	13.0 AU	16.71 %	0.24 Rf	2.9 AU	139.9 AU	19.40 %
2	0.81 Rf	13.0 AU	0.82 Rf	65.1 AU	83.29 %	0.83 Rf	12.3 AU	581.1 AU	80.60 %

Track 2:

Hydrolysed *Trigonella foenum-graecum*-seeds extract



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.27 Rf	6.7 AU	0.30 Rf	10.6 AU	27.06 %	0.34 Rf	4.7 AU	453.9 AU	52.55 %
2	0.47 Rf	2.8 AU	0.49 Rf	13.8 AU	35.20 %	0.49 Rf	12.7 AU	185.2 AU	21.44 %
3	0.62 Rf	0.7 AU	0.66 Rf	14.8 AU	37.74 %	0.67 Rf	6.8 AU	224.7 AU	26.01 %

Track 3:

Diosgenin

Spots are not visible at 366 nm

Fig. 5 HPTLC fingerprint profiles of *Trigonella foenum-graecum* seeds extract, coded ayurvedic formulation extract and Diosgenin reference standard at UV 366 nm

injector using Chemstation HPLC software. All samples and standards were filtered through 0.22 µm filters. Separation was achieved on C_{18} Eclipse, XDB, 4.6 mm × 50 mm, 5 µm particle size Agilent Column. The mobile phase has consisted of acetonitrile/water (95:5) (v/ v) in an isocratic elution with a flow rate of 2 ml/min, and 10 µl of the test sample (triplicate) was injected into the HPLC system. The column temperature was kept at 32 °C. The detection of analytes at 210 nm was carried out using Variable Wavelength Detector (VWD) [26, 27].

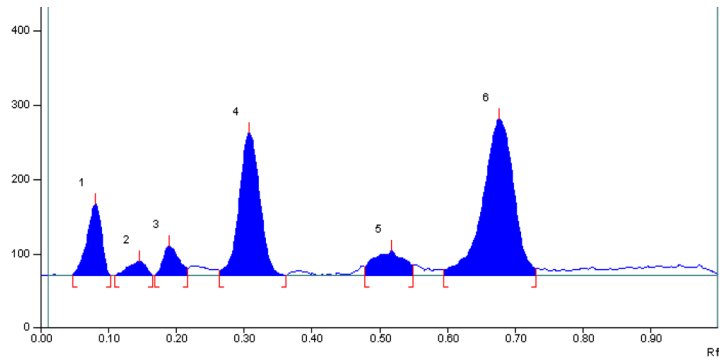
Solution A: Acetonitrile.

Solution B: Water

Good separations and suitable retention time of Diosgenin were obtained in isocratic elution using the following optimized chromatographic conditions: Column: C_{18} Eclipse, XDB, 4.6 mm 150 mm, 5 µm particle size. Detection: 227 nm wavelength. Detector: VWD Detector. Column temperature: 32 °C. Mobile phase: Acetonitrile: Water (95:5) (v/v). Flow rate: 2 ml/min. Injection volume: 10 µl. Mode of Operation: Isocratic elution. Retention Time: 5.278 Min. Run time: 10 min.

Track 4:

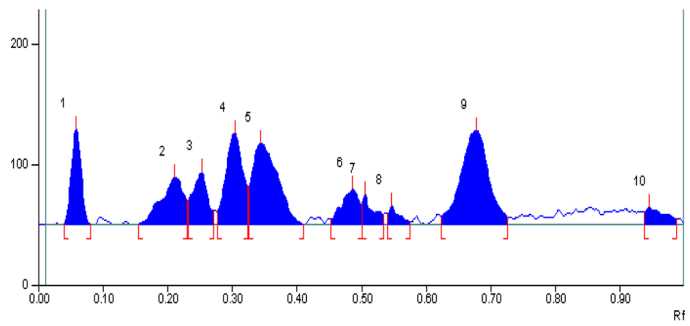
Hydrolyzed Coded ayurvedic formulation Extract



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	0.3 AU	0.08 Rf	95.4 AU	16.20 %	0.10 Rf	0.2 AU	1875.2 AU	10.05 %
2	0.11 Rf	0.1 AU	0.14 Rf	19.1 AU	3.24 %	0.17 Rf	0.2 AU	458.0 AU	2.45 %
3	0.17 Rf	0.3 AU	0.19 Rf	39.2 AU	6.66 %	0.22 Rf	9.9 AU	841.8 AU	4.51 %
4	0.26 Rf	6.6 AU	0.31 Rf	191.4 AU	32.51 %	0.36 Rf	0.2 AU	5329.2 AU	28.55 %
5	0.48 Rf	11.7 AU	0.52 Rf	33.1 AU	5.62 %	0.55 Rf	13.9 AU	1356.1 AU	7.27 %
6	0.59 Rf	8.0 AU	0.68 Rf	210.5 AU	35.76 %	0.73 Rf	8.8 AU	8805.2 AU	47.17 %

Track 5:

Coded ayurvedic formulation Extract



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.8 AU	0.06 Rf	79.3 AU	17.01 %	0.08 Rf	0.3 AU	1127.7 AU	9.10 %
2	0.15 Rf	0.0 AU	0.21 Rf	39.2 AU	8.40 %	0.23 Rf	19.8 AU	1251.8 AU	10.10 %
3	0.23 Rf	20.1 AU	0.25 Rf	43.1 AU	9.24 %	0.27 Rf	11.3 AU	909.6 AU	7.34 %
4	0.28 Rf	10.8 AU	0.30 Rf	75.4 AU	16.17 %	0.32 Rf	32.0 AU	1835.3 AU	14.81 %
5	0.33 Rf	32.3 AU	0.34 Rf	66.8 AU	14.33 %	0.41 Rf	0.1 AU	2445.9 AU	19.73 %
6	0.45 Rf	4.8 AU	0.49 Rf	29.9 AU	6.42 %	0.50 Rf	16.2 AU	734.8 AU	5.93 %
7	0.50 Rf	17.6 AU	0.51 Rf	24.9 AU	5.34 %	0.53 Rf	8.6 AU	361.8 AU	2.92 %
8	0.54 Rf	9.2 AU	0.55 Rf	15.6 AU	3.35 %	0.57 Rf	1.9 AU	227.9 AU	1.84 %
9	0.62 Rf	6.5 AU	0.68 Rf	77.5 AU	16.62 %	0.73 Rf	6.1 AU	3108.0 AU	25.08 %
10	0.94 Rf	10.3 AU	0.94 Rf	14.5 AU	3.11 %	0.99 Rf	4.5 AU	391.0 AU	3.15 %

Fig. 5 continued

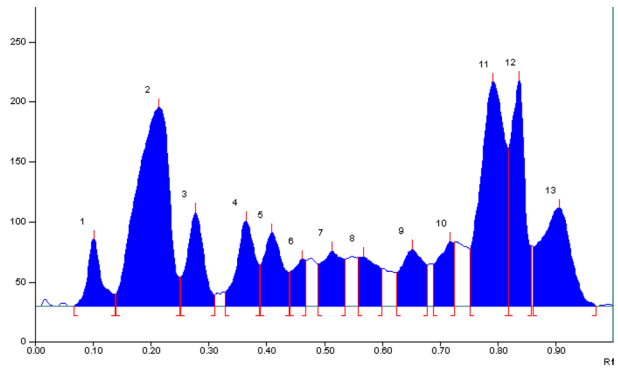
Quantification by HPLC

From 0.52 mg/ml Diosgenin stock solution was appropriately further diluted five different concentrations as 0.26, 0.13, 0.065, 0.0325 mg/ml of working concentrations. Each of the standard solutions was run through the HPLC system and recorded the respective peak areas. The standard calibration curve was established for peak area Vs. The concentration of Diosgenin was applied.

A 10 µl test solution of coded ayurvedic formulation, *Trigonella foenum-graecum* seeds, hydrolysed *Trigonella foenum-graecum,* and hydrolysed coded ayurvedic formulation extracts was injected in triplicate to HPLC

Track 1:

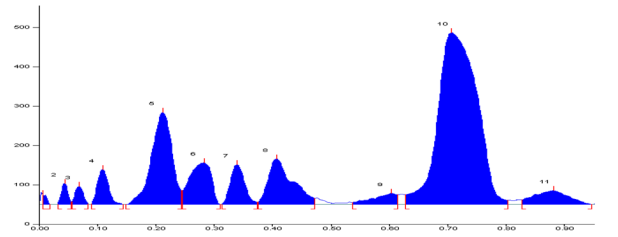
Trigonella foenum-graecum- extract



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.07 Rf	0.0 AU	0.10 Rf	55.7 AU	4.98 %	0.14 Rf	9.8 AU	1220.8 AU	3.26 %
2	0.14 Rf	9.9 AU	0.21 Rf	165.6 AU	14.81 %	0.25 Rf	24.0 AU	8508.5 AU	22.75 %
3	0.25 Rf	24.2 AU	0.28 Rf	78.1 AU	6.99 %	0.31 Rf	9.6 AU	2042.3 AU	5.46 %
4	0.33 Rf	11.7 AU	0.36 Rf	71.3 AU	6.38 %	0.39 Rf	34.5 AU	2023.1 AU	5.41 %
5	0.39 Rf	34.6 AU	0.41 Rf	61.3 AU	5.48 %	0.44 Rf	28.6 AU	1819.2 AU	4.86 %
6	0.44 Rf	28.7 AU	0.46 Rf	39.6 AU	3.54 %	0.47 Rf	38.2 AU	794.3 AU	2.12 %
7	0.49 Rf	35.4 AU	0.51 Rf	46.0 AU	4.12 %	0.54 Rf	39.3 AU	1541.5 AU	4.12 %
8	0.56 Rf	40.8 AU	0.57 Rf	41.2 AU	3.69 %	0.60 Rf	31.2 AU	1226.0 AU	3.28 %
9	0.63 Rf	28.5 AU	0.65 Rf	47.6 AU	4.26 %	0.68 Rf	34.7 AU	1683.9 AU	4.50 %
10	0.69 Rf	35.6 AU	0.72 Rf	54.0 AU	4.83 %	0.72 Rf	53.2 AU	1373.2 AU	3.67 %
11	0.75 Rf	47.7 AU	0.79 Rf	187.0 AU	16.73 %	0.82 Rf	31.2 AU	7150.0 AU	19.12 %
12	0.82 Rf	131.3 AU	0.84 Rf	188.3 AU	16.85 %	0.86 Rf	50.3 AU	4132.6 AU	11.05 %
13	0.86 Rf	49.2 AU	0.91 Rf	81.8 AU	7.32 %	0.97 Rf	0.0 AU	3888.3 AU	10.40 %

Track 2:

Hydrolyzed *Trigonella foenum-graecum-* seeds extract



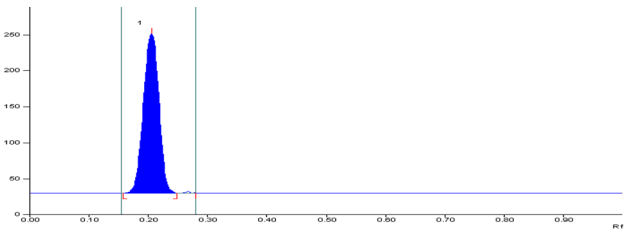
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	24.4 AU	0.01 Rf	24.4 AU	1.92 %	0.02 Rf	0.1 AU	157.0 AU	0.31 %
2	0.03 Rf	0.5 AU	0.04 Rf	53.3 AU	4.21 %	0.06 Rf	5.3 AU	543.6 AU	1.07 %
3	0.06 Rf	6.6 AU	0.07 Rf	45.9 AU	3.62 %	0.08 Rf	1.0 AU	585.0 AU	1.15 %
4	0.09 Rf	0.8 AU	0.11 Rf	88.3 AU	6.96 %	0.14 Rf	0.2 AU	1534.7 AU	3.02 %
5	0.15 Rf	0.0 AU	0.21 Rf	234.7 AU	18.51 %	0.24 Rf	35.5 AU	7570.7 AU	14.88 %
6	0.25 Rf	35.6 AU	0.28 Rf	105.4 AU	8.32 %	0.31 Rf	0.1 AU	3639.1 AU	7.15 %
7	0.31 Rf	0.3 AU	0.34 Rf	100.9 AU	7.96 %	0.37 Rf	5.8 AU	2255.6 AU	4.43 %
8	0.38 Rf	6.1 AU	0.41 Rf	115.6 AU	9.12 %	0.47 Rf	15.6 AU	4462.3 AU	8.77 %
9	0.54 Rf	4.8 AU	0.60 Rf	27.9 AU	2.20 %	0.61 Rf	24.7 AU	1046.6 AU	2.06 %
10	0.63 Rf	24.6 AU	0.71 Rf	436.2 AU	34.41 %	0.80 Rf	10.4 AU	27154.5 AU	53.37 %
11	0.83 Rf	12.1 AU	0.88 Rf	34.9 AU	2.75 %	0.95 Rf	0.2 AU	1935.3 AU	3.80 %

Fig. 6 HPTLC Fingerprint profiles of *Trigonella foenum-graecum* seeds extract, coded ayurvedic formulation extract, and Diosgenin reference standard at 540 nm

system. Recorded the chromatogram and determined the area of the peak of the test solution corresponding to that of Diosgenin as described above from the calibration curve. The amount of Diosgenin present in the residues of coded ayurvedic formulation and *Trigonella foenum-graecum* seeds extract was calculated.

Track 3:

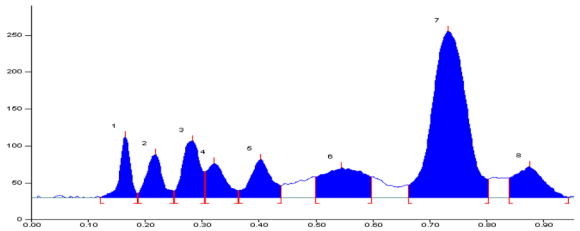
Diosgenin



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.16 Rf	0.0 AU	0.20 Rf	221.8 AU	100.00 %	0.25 Rf	0.0 AU	5360.9 AU	100.00 %

Track 4:

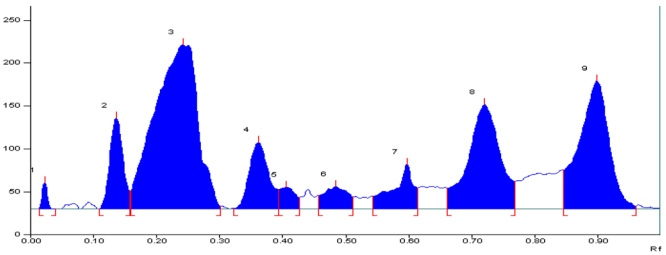
Hydrolysed Codded Formulation Tablet Extract



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.12 Rf	0.9 AU	0.17 Rf	82.1 AU	13.14 %	0.19 Rf	6.0 AU	1375.9 AU	5.44 %
2	0.19 Rf	6.1 AU	0.22 Rf	58.7 AU	9.39 %	0.25 Rf	9.3 AU	1473.4 AU	5.83 %
3	0.25 Rf	9.3 AU	0.28 Rf	77.1 AU	12.34 %	0.30 Rf	35.3 AU	2139.2 AU	8.46 %
4	0.30 Rf	35.3 AU	0.32 Rf	46.7 AU	7.48 %	0.36 Rf	9.5 AU	1398.2 AU	5.53 %
5	0.36 Rf	9.6 AU	0.40 Rf	51.6 AU	8.27 %	0.44 Rf	16.5 AU	1730.5 AU	6.85 %
6	0.50 Rf	28.1 AU	0.55 Rf	40.8 AU	6.53 %	0.60 Rf	28.5 AU	2773.6 AU	10.97 %
7	0.66 Rf	17.4 AU	0.73 Rf	225.4 AU	36.09 %	0.80 Rf	25.0 AU	12386.1 AU	48.99 %
8	0.84 Rf	26.9 AU	0.88 Rf	42.3 AU	6.77 %	0.94 Rf	0.4 AU	2003.6 AU	7.93 %

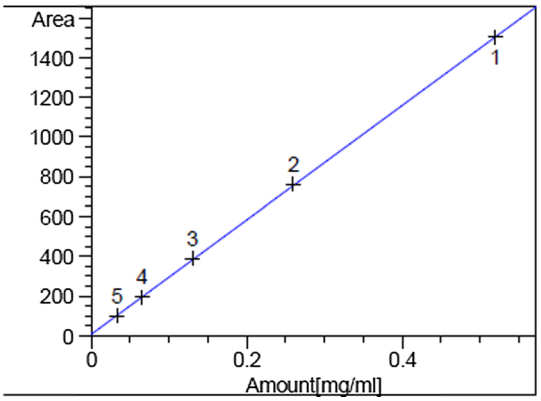
Track 5:

Coded ayurvedic formulation Extract



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.1 AU	0.02 Rf	30.4 AU	3.91 %	0.04 Rf	0.0 AU	223.5 AU	0.70 %
2	0.11 Rf	0.2 AU	0.14 Rf	105.8 AU	13.58 %	0.16 Rf	20.9 AU	2040.2 AU	6.43 %
3	0.16 Rf	21.0 AU	0.24 Rf	191.5 AU	24.58 %	0.30 Rf	3.0 AU	12112.4 AU	38.17 %
4	0.32 Rf	0.7 AU	0.36 Rf	77.4 AU	9.93 %	0.39 Rf	22.3 AU	2191.4 AU	6.91 %
5	0.39 Rf	22.4 AU	0.41 Rf	25.1 AU	3.22 %	0.43 Rf	13.6 AU	578.4 AU	1.82 %
6	0.46 Rf	15.4 AU	0.48 Rf	26.2 AU	3.36 %	0.51 Rf	13.8 AU	909.1 AU	2.86 %
7	0.54 Rf	14.9 AU	0.60 Rf	52.1 AU	6.68 %	0.61 Rf	24.7 AU	1516.5 AU	4.78 %
8	0.66 Rf	24.3 AU	0.72 Rf	121.6 AU	15.61 %	0.77 Rf	31.9 AU	5652.8 AU	17.81 %
9	0.84 Rf	45.2 AU	0.90 Rf	149.1 AU	19.13 %	0.96 Rf	3.4 AU	6509.5 AU	20.51 %

Fig. 6 continued




```
Diosgenin at exp. RT: 5.278
VWD1 A, Wavelength=210 nm
Correlation:            0.99996
Residual Std. Dev.:     5.95041
Formula: y = mx + b
      m:   2884.54176
      b:      7.82120
      x: Amount
      y: Area
```

Fig. 7 Calibration curve of Diosgenin peak area Vs concentration of Diosgenin

Results

HPTLC chromatogram gives a clear picture of standard biomarker whether it is present in ayurvedic formulations and their ingredients. R_F values were calculated, and photograph documentation is recorded in Fig. 3 and Table 1. The fingerprint profile data were recorded by WIN CATS software. Details of HPTLC fingerprint profiling are given in Figs. 4, 5 and 6.

Quantification by HPLC

Calibration curve

A linear regression analysis has an equation of the form y = mx + b. The standard calibration curve was established for peak area Vs concentration of Diosgenin applied is shown in Fig. 7. Diosgenin at exp. RT: 5.278 Min, Detector: VWD, Wavelength: 210 nm, Correlation: 0.99996, Residual Std. Dev.: 5.95041 Formula: y = mx + b; m: 2884.54176; b: 7.82120 x: Amount, y: Area.

Quantitative analysis

Recorded the chromatogram and determined the area of the peak of the test solution corresponding to that of Diosgenin as described above from the calibration curve given in Fig. 8. The amount of Diosgenin present in the residues of coded ayurvedic formulation and *Trigonella foenum-graecum* seeds extract was calculated and tabulated in Table 2.

HPLC chromatogram of standard Diosgenin and calibration curve, Ayush D tab, and *Trigonella foenum-graecum* seeds are given in Figs. 7 and 8.

Discussion

In the current study, qualitative Diosgenin identification was carried out using the HPTLC method, and quantitative estimation of specific biologically active Diosgenin compound was conducted in the ayurvedic coded formulation and its ingredient *Trigonella foenum-graecum* seed using the HPLC method [14, 15]. The HPTLC fingerprinting method is an accurate, simple, and specific method for quantifiable bioactive markers [20, 21]. HPTLC chromatogram gives a clear picture of standard biomarker whether it is present in ayurvedic formulations and their ingredients.

The HPTLC study evaluated that [11], a band in wavelength 540 nm (Green, R_F 0.26) corresponding to Diosgenin is visible in both standard and test solution tracks of *Trigonella foenum-graecum* seeds and coded ayurvedic formulation extract.

Estimated standard Diosgenin content in coded ayurvedic formulation and *Trigonella foenum-graecum* seeds extracts was performed using the HPLC method. All the samples showed characteristic peaks of Diosgenin at the same retention time as that of standard Diosgenin.

The results obtained from HPLC analysis show that the *Trigonella foenum-graecum* seed, hydrolysed *Trigonella foenum-graecum* seeds, coded ayurvedic formulation, and hydrolysed coded ayurvedic formulation extracts contain 0.0504%, 0.1676%, 0.01620% and 0.0224% Diosgenin biomarker compound, respectively.

All the samples showed characteristic peaks of Diosgenin at the same retention time as that of standard Diosgenin. Retention time is 5.728 min detected at 210 nm. The results of HPLC showed that standard Diosgenin are present in *Trigonella foenum-graecum* seeds are 0.0109 mg/ml, hydrolysed *Trigonella foenum-graecum* 0.433 mg/ml, Ayush D (0.0716 mg/ml) hydrolysed coded ayurvedic formulation (0.0892 mg/ml). The amount of standard Diosgenin is tabulated in Table 2.

Conclusions

Traditional medicine plays a vital role in the healing of many ailments. Interest in herbal resources is growing day by day due to their efficacy and lesser

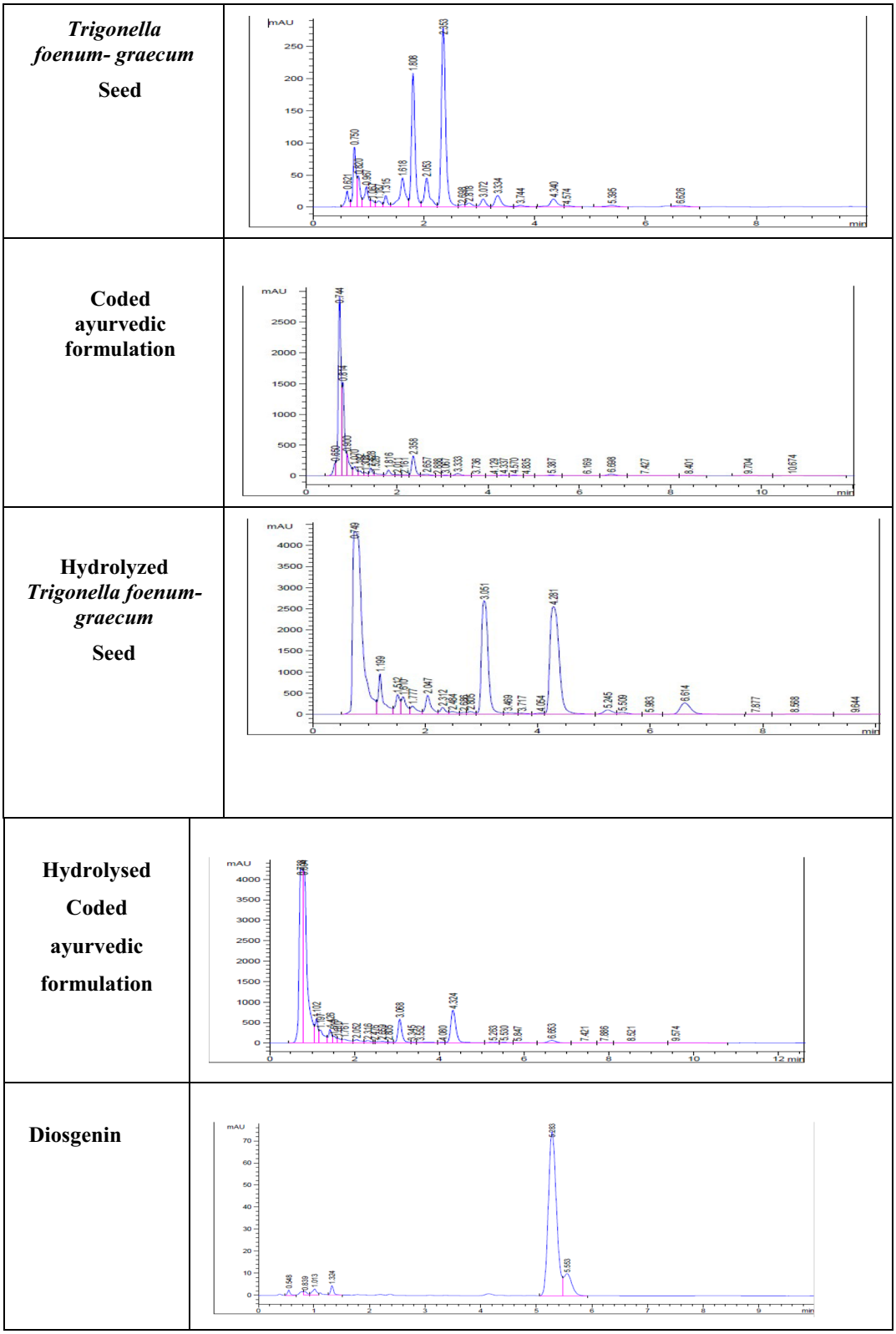


Fig. 8 HPLC chromatogram of Diosgenin biomarker compound in coded formulation tablet and *Trigonella foenum-graecum* seeds

Table 2 Estimation of Diosgenin biomarker compound in coded ayurvedic formulation and *Trigonella foenum-graecum* seed

S. no.	Sample name	Weight of Extract used for HPLC	Amount of Diosgenin present in samples	Percentage of Diosgenin present in the sample	
		mg/10 ml	mg/ml	Results	Mean
1	*Trigonella foenum-graecum* seeds	45	0.0109	0.0504	0.0504
2	Hydrolysed *Trigonella foenum-graecum* seeds	538	0.433	0.1676	0.1676
3	Coded ayurvedic formulation	555	0.0716	0.0162	0.0162
4	Hydrolysed coded ayurvedic formulation	500	0.0892	0.0224	0.0224

side effects. *Trigonella foenum-graecum,* a leguminous plant, is botanically known as *Trigonella foenum-graecum* Linn, belong to the family Fabaceae. *Trigonella foenum-graecum i*s used for various health conditions, including digestive problems, bronchitis, tuberculosis, fevers, sore throats, wounds, arthritis, abscesses, swollen glands, skin irritations, diabetes and loss of appetite, ulcers, and menopausal symptoms as well as in the treatment of cancer. Proper identification and quality control of the drug provide standard HPTLC and HPLC profiles with the selected solvent system. Major phytoconstituents are reported to be extracted in methanol and visualized under 254 nm and 366 nm. They were derivatized with the anisaldehyde sulphuric acid reagent. The R_f value of Diosgenin observed after derivatization is 0.22. The HPTLC profile can also be used to refer the proper identification/authentication of the drug. HPLC chromatogram concluded that all the samples showed characteristic peaks of Diosgenin at the same retention time as that of standard Diosgenin. The present study concludes that hydrolysed *Trigonella foenum-graecum* seeds and coded ayurvedic formulation give better results than the unhydrolysed form of the same with standard Diosgenin. Further studies are required to elucidate the mechanism of various phytoconstituents with the human body in treating illness.

Abbreviations

HPLC: High-performance liquid chromatography; HPTLC: High-performance thin-layer chromatography; R_T: Retention time; R_f: Retention factor; TLC: Thin-layer chromatography; VWD: Variable wavelength detector; UV: Ultraviolet rays; WHO: World Health Organization.

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Authors' contributions

AKM and SKN have performed the experimental work. AKM, RI, NS, and AS worked in experimental design. AKM has written the manuscript, VB also helped in manuscript writing. All authors read and approved the final manuscript.

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Availability of data and materials

All data and material are available upon request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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