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Quantitative HPTLC analysis of Diosgenin from methanol extract of Moringa oleifera Lam

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ABSTRACT

Purpose: Quantitative analysis of steroidal saponin 'Diosgenin' from methanol extract of Moringa oleifera Lam (Drumstick) by HPTLC technique.

Approach: The approach of the study was to find out the presence of Diosgenin from Moringa and then its quantity.

Method: HPTLC analysis was performed with methonolic extract of seeds. Standard Diosgenin curve was used to detect the availability of the steroidal saponin Diosgenin.

Results: 20 µL of sample was applied which showed 159.4084 ng of Diosgenin. About 0.797 % w/v of Diosgenin was estimated form seeds of Moringa in methonolic extract. It was compared with the standard Diosgenin curve.

Conclusions: Moringa oleifera Lam seeds are the source of steroidal saponin- Diosgenin which can be used as female contraceptive to control human population.

Keywords: Contraceptive, Diosgenin, HPTLC and Moringa oleifera Lam.

1. INTRODUCTION

Some plants are traditionally used as contraceptive. Herbal contraceptive is as alternative for women living in the rural areas in developing nations with very high population like India, Africa and Bangladesh [1].Targeting the traditional contraceptive plants it is an urgent need to formulate the herbal contraceptive drug. *Moringa oleifera* (Linn) is a medicinally important plant, belonging to family Moringaceae. The plant is also well recognized in India, Pakistan, Bangladesh and Afghanistan as a folkloric medicine. *Moringa oleifera* is a small or medium sized tree up to 10 m tall, with thick, soft, corky, deeply fissured bark, growing mainly in semiarid, tropical and subtropical areas [2]. Different parts of the tree have been used in the traditional system of medicine. The work revealed that *Moringa oleifera* stem bark is being used traditionally as an abortifacint [3]. The root of *Moringa oleifera* was shown to possess antifertility and abortifacient activity [4, 5].

The contraceptive and abortifacient activity may be due to the presence of saponins present in *Moringa*. It was reported that *M. oleifera* leaves have saponins content equivalent diosgenin [6, 7]. A saponin molecule consists of an aglycone (or sapogenin) and one or two sugar molecules. According to the structures of the aglycone, saponins can be classified into two types: triterpenoidal and steroidal [8, 9]. Diosgenin is a bioactive steroidal sapogenin of great interest to the pharmaceutical industry [10]. It serves as an important starting material for the production of steroidal drugs and hormones such as testosterone, glucocorticoids, progesterone, corticosteroids, and oral contraceptives [11, 12]. Diosgenin and related steroidal saponins were commercially obtained from only few plants like *Dioscorea, Yucca* and *Trigonella* [13] and thus, there is an anthropogenic pressure on these plants. Therefore, it is essential to discover a new and alternate source of these compounds due to decreasing plant resources as well as increasing demand. There are many of studies on analysis of diosgenin content in different plants like *Trigonella* (fenugreek), Dioscorea (wild yam), Yucca, etc but as per our knowledge till date no study has been done to estimate diosgenin content in *Moringa*. Hence, the objective of this work, therefore, was to evaluate *Moringa oleifera* Lam. seeds for saponin i.e.diosgenin content, which are commercially used as a raw material for preparing contraceptive pills.

2. MATERIAL AND METHODS

2.1 Collection of Plant Material: Fresh Seeds of *Moringa oleifera* Lam. were harvested from their natural habitat and were collected from various areas of Ahmednagar District, India. Selected plant material was identified from Botanical Survey of India, Western Circle, Pune. The voucher specimens were deposited in the Herbarium, BSI, Pune as well as Herbarium of Department of Botany, New Arts, Commerce and Science College, Ahmednagar. Voucher specimen number was ABK 002.

2.2 Extraction of Plant Material: Collected seeds were cleaned, dried, and finely powdered in a grinding machine. 250 gm of seed samples were powdered and was extracted in methanol using Soxhlet extraction or hot continuous extraction [14, 15]. In this method, finely ground sample was placed in a porous bag and placed in thimble chamber of the Soxhlet apparatus. Methanol solvent was heated in the bottom flask, vaporizes into the sample thimble, and condenses in the condenser and drip back. It is continuous process. Once the process has finished, the solvent was evaporated using a rotary evaporator, leaving a small yield of extracted plant

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material (about 10 to 15 ml) in the glass bottom flask. The advantage of this method is that large amounts of drug can be extracted with a much smaller quantity of solvent. The extract was preserved at 4^0 C till the analysis.

2.3 Preparation of standard solution: The standard stock solution for diosgenin was prepared by dissolving 5 mg in 5 ml of methanol by means of sonicated for 15 min. From this stock (1 mg/ml), six different concentrations (100-600 µg/ml) of diosgenin standard were prepared for presentation curve.

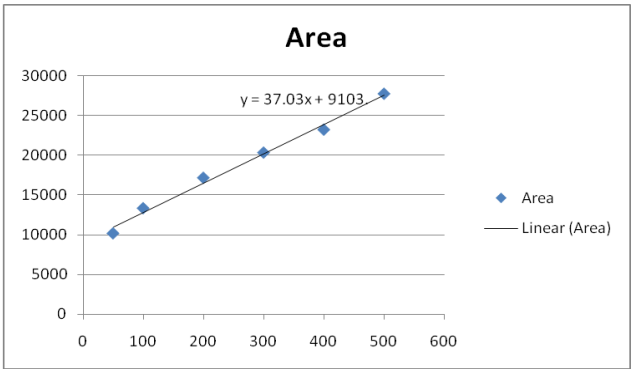

Fig.1. HPTLC calibration curve for standard Diosgenin

2.4 Instrumentation and Chromatographic conditions: The samples were spotted in the form of bands of width of 6 mm with space between bands of 8.2 mm, with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on percolated silica gel aluminum plate 60 F_{254} (5 cm ×10 cm) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 5 mm × 0.45 mm and scanning speed of 20 mm/sec was employed.

The linear ascending development was carried out in 10 cm×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using Toluene: Ethyl Acetate: Formic Acid (5 : 4 : 1 v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 15 min. The length of chromatogram run was 8 cm and development time was approximately 40 min. TLC plates were dried in a current of air with the help of a hair drier. After development plate was dipped in Anisaldehyde-Sulphuric Acid Reagent. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 450 nm for all developments operated by WINCATS software version 1.4.2.

2.5 Quantification of diosgenin: The methanolic extract (20 µl) was applied in triplicate and densitograms were obtained under same conditions as that of the standard. Area under the peak corresponding to that of the standard was recorded and content of the same was calculated.

3. RESULT AND DISCUSSIONS

After derivatization at 450 nm, standard diosgenin with various concentrations showed the various peaks accordingly with Rf values. The track 2^{nd} and 3^{rd} of TLC showed Rf values 0.79 for both with retention area 10147.8 and 13324.6 AU respectively (Table 1. And Fig. 2and 3). However, an intense peak of sample (Track No.8) coincided to standard diosgenin (Track No. 3) with Rf value 0.79 and peak area 13324.6 AU. It was observed when the sample was spotted in high quantity 20 ul. The plant sample has showed maximum 0.80 Rf with peak area 15005.3 AU. The Rf values were similar to the standard at 50 and 100 µl.

Table1. Rf values with retention area of standard Diosgenin and Methanolic plant extract

Track No	Start Position	Start Height	Max Position	Max Height	End Position	End Height	Area
1	--	--	--	--	--	--	--
2	0.70 Rf	0.7 AU	0.79 Rf	315.4 AU	0.85 Rf	0.1 AU	10147.8 AU
3	0.69 Rf	9.1 AU	0.79 Rf	393.8 AU	0.83 Rf	8.2 AU	13324.6 AU
4	0.69 Rf	23.7 AU	0.78 Rf	490.3 AU	0.86 Rf	3.9 AU	17164.9 AU
5	0.69 Rf	23.9 AU	0.78 Rf	506.5 AU	0.86 Rf	1.8 AU	20348.4 AU
6	0.69 Rf	10.1 AU	0.78 Rf	521.3 AU	0.86 Rf	0.0 AU	23250.0 AU
7	0.69 Rf	10.0 AU	0.78 Rf	553.8 AU	0.84 Rf	9.9 AU	27786.5 AU
8	0.71 Rf	1.0 AU	0.80 Rf	464.2 AU	0.86 Rf	0.2 AU	15005.3 AU

Previous reports revealed that *M. oleifera* leaves had negligible tannins; saponins content (5.0% as diosgenin equivalent) was similar to that present in soyabean meal, and trypsin inhibitors and lectins were not detected [6]. Total saponin content was found to be 3.022 mg/g equivalent to diosgenin [7]. The amount of diosgenin in methanolic and ethanolic leaves extract of *Abutilon indicum* was found to be 0.43% (w/w) and 0.10% (w/w), respectively [16]. Our results are in good agreement with the results reported earlier.

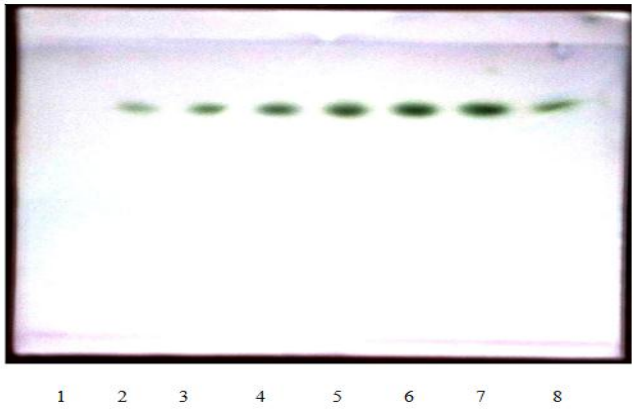

Fig.2. HPTLC profile of standard diosgenin and plant sample at 450 nm after derivatization (Seen under Visible light)

Track 1: Blank (Methanol), Track 2: Diosgenin in Methanol (50 ng/band), Track 3: Diosgenin in Methanol (100 ng/band), Track 4: Diosgenin in Methanol (200 ng/band), Track 5: Diosgenin in Methanol (300 ng/band), Track 6: Diosgenin in Methanol (400 ng/band), Track 7: Diosgenin in Methanol (500 ng/band), Track 8: Methanol extract of Moringa seeds (20 µL).

3.1 Quantification of steroidal Saponin 'Diosgenin' from M. oleifera Lam

A quantitative HPTLC method that gives dense and compact spots with significant *Rf* values for simultaneous

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determination ion of diosgenin in *M. oleifera* Lam seeds was developed. The six point linear calibration curves of diosgenin were linear in the range of 50–600 ng for diosgenin, which indicates that the method has good sensitivity. Specificity of the proposed method was evaluated by comparing the Rf values acquired from standards and the plant sample separated under same condition. (Fig.1- 4). 20 µL of plant sample applied. It contains 159.4084 ng of Diosgenin. Therefore 01 ml of extract contains - 159.4084 ng * 50 = 7970.42 ng = 7.970 µg of Diosgenin. Therefore 100 ml of extract contains - 7.970 µg *100 = 797 µg of Diosgenin = 0.797 mg of Diosgenin = 0.797 % w/v of Diosgenin.

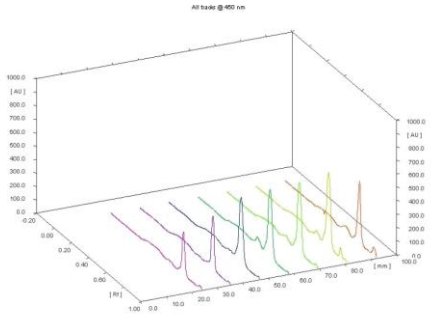

Fig.3. 2D Densitogram showing tracks of Standard Diosgenin and the last track of plant sample

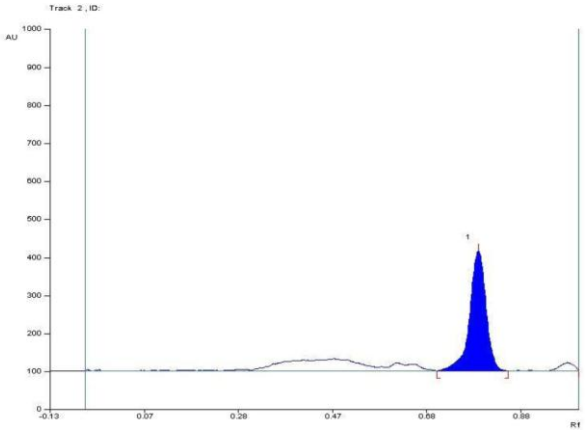

Fig.4. Densitogram of track 3 (Standard Diosgenin, 100 ng/band)

4. CONCLUSION

The amount of Diosgenin in methenolic extract of seed sample was found to be 0.797 % w/v. Our results are very good as compared to the previous reports of various plants other than Dioscoria. The results obtained in the present work conclude that the *M. oleifera* Lam seeds contain measurable amount of steroidal saponin "Diosgenin".

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