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Der Pharmacia Lettre, 2015, 7 (5):236-244
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ISSN 0975-5071
USA CODEN: DPLEB4

Quantitative analysis for "Diosgenin" content in *Elephantopus scaber* (Linn.) by HPTLC using successive solvent extraction method

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ABSTRACT

Elephantopus scaber Linn is an important medicinal plant. The whole plant is a good source of various bioactive phytochemicals. The High Performance Thin Layer Chromatography was carried out for the estimation of diosgenin content in Elephantopus scaber. HPTLC analysis was performed using Toluene: ethyl acetate: formic acid [6: 5: 1 v/v/v] as a mobile phase. Detection and quantification were done densitometrically. HPTLC plate was scanned at 366 nm after derivatization showing good partition and banding. The diosgenin content in pet ether, chloroform, ethyl acetate, ethanol, methanol and aqueous extract was estimated by successive solvent (soxhlet) extracts, Standard diosgenin was used for calibration. The quantity of this important phytoconstituent in Elephantopus scaber was high (69.37 µg /ml) in aqueous extract and remarkably low (11.04 µg /ml) in methanolic extract. The saponins and diosgenin are used as alternate natural sources in the synthesis of various pharmaceutical products such as oral contraceptives, sex hormones and other steroids. In present investigation, the quantitative assay was performed for saponin and diosgenin content and the result obtained are discussed.

Keywords: *Elephantopus scaber*, HPTLC, Diosgenin, steroidal saponin.

INTRODUCTION

Elephantopus scaber Linn belong to the family Asteraceae (tribe Vernonieae) and is an erect herb with 15-38 cm height. The leaves are mostly radical and they form a spreading rosette on the ground. Inflorescence is large, flat at the top and terminal nearly 2.5 cm across. *Elephantopus scaber* L. (Asteraceae), distributed all over the world, especially in America. It is found in Tropical Africa, Eastern Asia, Indian Subcontinent, Southeast Asia, and Australia. Its natural habitat is subtropical or tropical moist mountain forests. *Elephantopus scaber* is popularly known as Elephant's foot (English), Gojivha (Sanskrit), Eddumalikechettu (Telugu), and Nayi nalige (Kannada). It is used in Colombia and Brazil as a tonic, febrifuge, and diaphoretic against cough, bronchitis, and asthma. In south China, the whole plant is used for the treatment of hepatitis, bronchitis, fever, cough associated with pneumonia [1]. In Malaysia, decoction of *E. scaber* root has been used to accelerate contraction of abdominal area and prevent inflammation after childbirth. Besides, whole *E. scaber* is also boiled with red bean to remove flatulence, of the 32 species, only one species, namely, *Elephantopus scaber* is known to grow in India[2].

The whole plant of *Elephantopus scaber* contains biologically important compounds such as Germacranolide, sesquiterpene dilactones like Elephantopin, Deoxyelephantopin, Isodeoxyelephantopin, 11, 13-dihydrodeoxyelephantopin and Germacranolide sesquiterpene lactones like Scabertopin, flavonoids, triterpenoids, sterols and flavonoid esters. The plant parts (leaf, bark and root) are medicinal, because of the presence of epifriedelinol, lupeol and stigmasterol [3,4]. The phytoconstituents in this plant, deoxyelephantopin and

isodeoxyelephantopin are tumor inhibitors [5,6]. Previous phytochemical investigations on *Elephantopus* have also resulted in the isolation of flavonoids [7], triterpe-noids, caffeic acids. Among these compounds, sesquiterpene lactone is a chemotaxonomic marker for the genus *Elephantopus*. It is reported that the species is also used for its antitumor, hepatoprotective, wound healing and anti-inflammatory activity [8]. Deoxyelephantopin from *Elephantopus scaber* L. induces cell-cycle arrest and apoptosis in the human nasopharyngeal cancer CNE cells. Tumor suppression effect of Deoxyelephantopin was investigated on mammary adenocarcinoma, the results provides an evidence of the anti tumor activity of the compound.

Among the various secondary metabolites saponins have an enormous significance in pharmaceutical industry. A saponin molecule consists of an aglycone (or sapogenin) and one or two sugar moieties. According to the structures of the aglycones, saponins can be classified into two types: triterpenoid and steroidal [9]. They have been reported to have a variety of beneficial health effects. The therapeutic effects of a large number of folk medicines considered to be associated with their saponin content [10].

Diosgenin is a bioactive steroidal sapogenin, and of great interest to the pharmaceutical industry [11,12]. It is an important material for the production of corticosteroids, oral contraceptives as well as other steroidal drugs [13,14] and possesses estrogenin and antitumour properties. Diosgenin and related steroidal saponins are commercially obtained from only few plants like *Dioscorea*, *Trigonella*, gokshur, *Anredera cordifolia* etc [15,16]. *Trigonella foenum-graecum*, *Yucca* and *A. indicum* are also known to contain diosgenin. The reports on diosgenin in *Elephantopus scaber* are scanty and, thus, there is an anthropogenic pressure on these plants. Therefore, it is essential to discover an alternate source for these compounds due to decreasing plant resources as well as increasing demand.

The present study is aimed to determine diosgenin content in the dried leaves of *Elephantopus scaber* using High performance thin layer chromatography (HPTLC) which is a precious quality assessment tool for the evaluation of bioactive compounds efficiently and cost effectively. Pet ether, chloroform, ethyl acetate, ethanol, methanol and water were used as a solvents and the analysis performance was further extended using successive solvent extraction method in studied experimental system.

MATERIALS AND METHODS

Plant material

The plant *Elephantopus scaber* was used as an a experimental plant system and the leaves were used as experimental material to prepare the samples for analysis. The plants were grown in the Botanical garden of The Institute of Science, Mumbai and taxonomically authenticated from Department of Botany, Blatter Herbarium, St. Xavier's College, Mumbai. The leaves were shade dried and grounded to fine powder.

Preparation of plant extracts for HPTLC

The leaves of plants were collected and dried under shade. These dried materials were mechanically powdered sheaved using 80 meshes. 25g of leaf powder was taken in 300 ml of pet ether, chloroform, ethyl acetate, ethanol, ethanol and aqueous extracted by soxhtlet apparatus (successive soxhtlet extraction method) for 8 hrs. All the plant extract were analyzed independently. From this extract 10 mg of samples was dissolved in concern solvent to make final volume of 1 mg/ml. For HPTLC analysis, 5 µl extract of each stock was used.

Preparation of standard solution and linearity

Standard stock solution for diosgenin was prepared by dissolving 5 mg diosgenin in 5 ml of methanol and sonicated for 15 minutes. From this stock (1mg/ml), six different concentrations (100-600µg/ml) of diosgenin standard were prepared. The linearity of diosgenin was determine by applying standard solution of different concentrations ranging from 0.5 -3.0 µg/spot. The diosgenin standard was purchased from Sigma Aldrich scientific chemicals. All the solvents used in analysis were HPLC grade.

Chromatographic conditions

Chromatography was performed on preactivated (at 1100 ^{0}C) silica gel 60 F_{254} HPTLC plates (10x10 cm). Both, sample and standard (5 µl each) compounds were applied to the layer as 6.0 mm wide bands, positioned 8.0 mm from the bottom of the plate, using an automated CAMAG LINOMAT-5, TLC applicator instrument with nitrogen flow providing the delivery by 100 µl Hamilton syringe.

Detection and quantification of compounds

TLC was perform with toluene: ethyl acetate: formic acid [6:5:1 v/v/v] on 10x10 cm HPTLC plates using sample applicator. The detector response for diosgenin was measured for each band at wavelength of 254 nm and 366 nm, using Camag TLC scanner & WinCAT software. The compounds were investigated according to their R_F values with the corresponding spot of standards. Calculations for percentage were done considering standard and sample R_F, AUC and dilution factor. For validation of the method, calibration curve was obtained by plotting the peak area against concentration of diosgenin. The spectrum obtained from the samples was correlated to the standard compound used. The percentage of diosgenin present in pet ether, chloroform, ethyl acetate, ethanol, methanol and aqueous extract was calculated by comparison of the areas measured for standard solution (Fig. 3 to 8). The peak areas of diosgenin were obtained by plotting a graph of peak vs applied concentration of diosgenin (µg) [17,18].

Chromatogram development

Sample loaded TLC plate was place in glass twin-trough developing chambers (10 mm × 10 mm, with metal lid) previously saturated with solvent vapor with mobile phase, for 30 min, at room temperature (24 ± 1^0C).

Photo-documentation

The developed plate was dried by hot air dryer to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG Reprostar-3) and the images were captured at UV- 254 nm, UV-366 nm and daylight mode.

Derivatization

The developed plate was sprayed with spraying reagent anisaldehyde - sulphuric acid reagent [12,15,19] and TLC plate was dried at 110 ^{0}C for 10 min on hot plate. Immediately after drying, the plate was photo-documented in visible light, UV-254 nm, UV-366 nm and daylight mode using CAMAG-TLC equipment.

RESULTS AND DISCUSSION

Content of Diosgenin in plant extract by HPTLC Analysis

The calibration curve of diosgenin was obtained by spotting diosgenin on HPTLC plate. After derivatization the plate was scanned densitometrically at 366 nm, diosgenin showed single peak in HPTLC chromatogram at 0.77 R_F (Fig. - 2). The calibration curve was prepared by plotting the concentration of diosgenin versus average area of the peak and it was linear in the range of 100 - 600 µg /ml for diosgenin (Fig. -1). The correlation coefficient was found to be 0.968. The experiment was perform in triplicate for reproducibility, accuracy and was found correct. The obtained data was analyzed statistically. The results obtained of diosgenin content in the plant leaf extract in different solvents by soxhlet extraction (successive) are discussed below.

HPTLC analysis of pet ether, chloroform, ethyl acetate, ethanol, methanol and aqueous successive solvent extracts of *Elephantopus scaber* indicated with good estimates occurrence of diosgenin. The highest amount (69.37 µg /ml) of diosgenin was recorded in aqueous extract followed by ethanolic extract (33.02 µg /ml) and chloroform extract (32.72 µg /ml) was observed and lowest (11.04 µg /ml) was observed for methanolic extract (Table- 1). The plant leaf extract in various solvents indicated marked variation in diosgenin content. (Fig. - 9).

The R_F value and retention area of diosgenin was found to be 0.77 and area 3047 (Fig.- 2). The pet ether extract of *E. scaber* showed 9 peaks and the 5^{th} peak with R_F value 0.76 and retention area 353.7 (Fig.- 3) was homologous to the standard diosgenin. While the chloroform extract of *E. scaber* showed 12 peaks and 11^{th} peak with R_F value 0.77 and retention area 419.2 (Fig.- 4) indicated exact homology to the diosgenin standard. The ethyl acetate extract of *E. scaber* showed total of 14 peaks and 9^{th} one with R_F value 0.73 and retention area 262.1 (Fig.- 5) was coinciding with standard diosgenin. The ethanolic extract of *E. scaber* showed 13 peaks and 10^{th} peak with R_F value 0.77 and retention area 423.8 (Fig. - 6) was homology to the diosgenin standard. The methanolic extract of *E. scaber* showed 12 peaks and 10^{th} one with R_F value 0.73 and retention area 141.5 was (Fig. - 7) exhibits homology with diosgenin standard. While aqueous extract of *E. scaber* showed total of 9 peaks and 6^{th} one with R_F value 0.72 and retention area 888.7 (Fig. - 8) coincides with diosgenin standard. HPTLC densitogram of all plant extract exhibited the presence of total 14 types of saponins when scanned at 366 nm with R_F values ranging from 0.02 to 0.93 (Figures- 3 to 8). After derivatisation with anisaldehyde-sulphuric acid reagent, the plate showed blue, brown, yellow and purple colored spots at day light mode, confirming the presence of diosgenin saponin in the analysed samples when

compared with standard. The appearance of brown color spot at $R_{F=}$ 0.77 confirms the presence of diosgenin in the samples. (Photoplate -1, 2 and 3).

Figure 1: Calibration curve of standard diosgenin for hptlc analysis

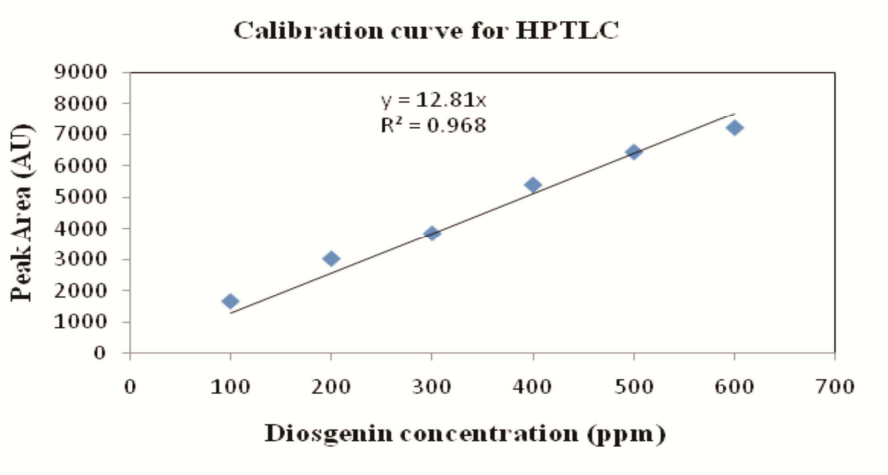


Table 1: Diosgenin content in *E. scaber* leaf extract by hptlc using successive solvents extraction method (µg/ ml)

Sr.No.	Solvents	Diosgenin content (µg/ ml)
1.	Pet ether	27.61
2.	Chloroform	32.72
3.	Ethyl acetate	20.46
4.	Ethanol	33.02
5.	Methanol	11.04
6.	Aqueous	69.37

Figure 2: HPTLC densitogram for diosgenin standard.

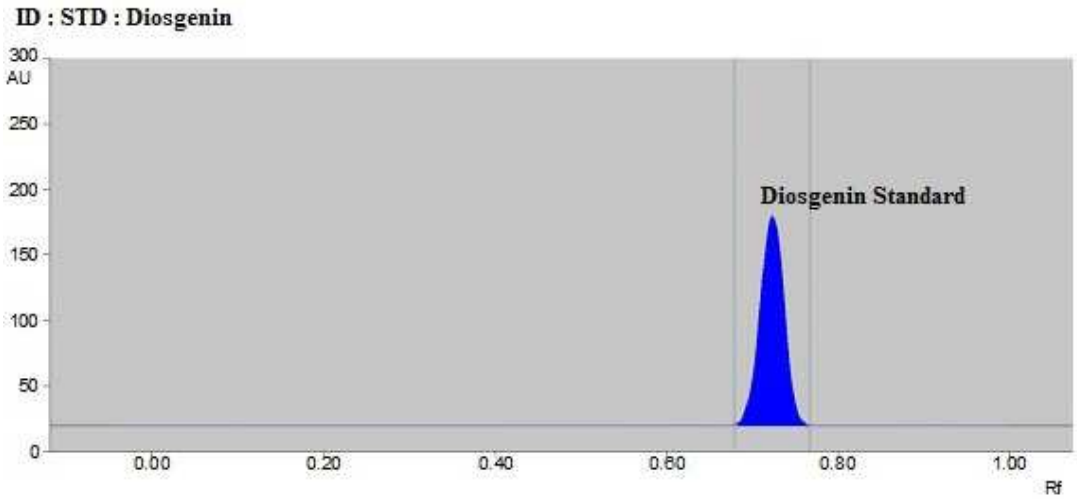


Figure 3: HPTLC densitogram of pet ether extract for *E. scaber* and diosgenin present in plant extract

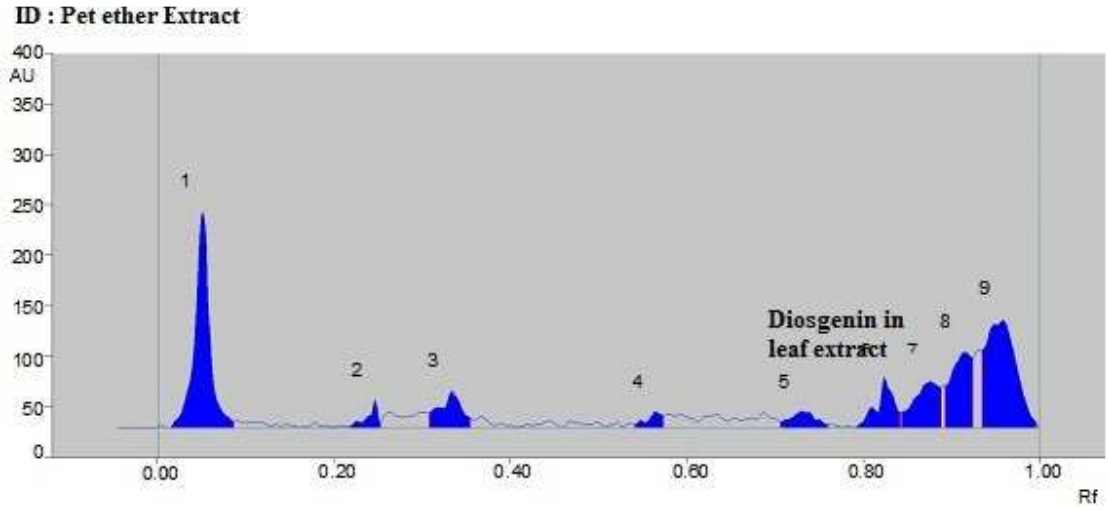


Figure 4: HPTLC densitogram for diosgenin in chloroform extract of *E. scaber*

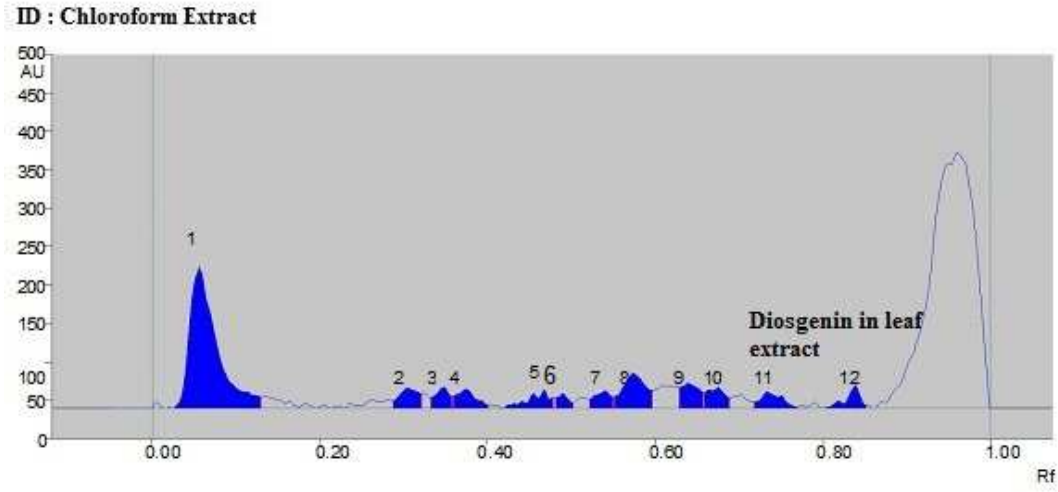


Figure 5: HPTLC densitogram for diosgenin in ethyl acetate extract of *E. scaber*

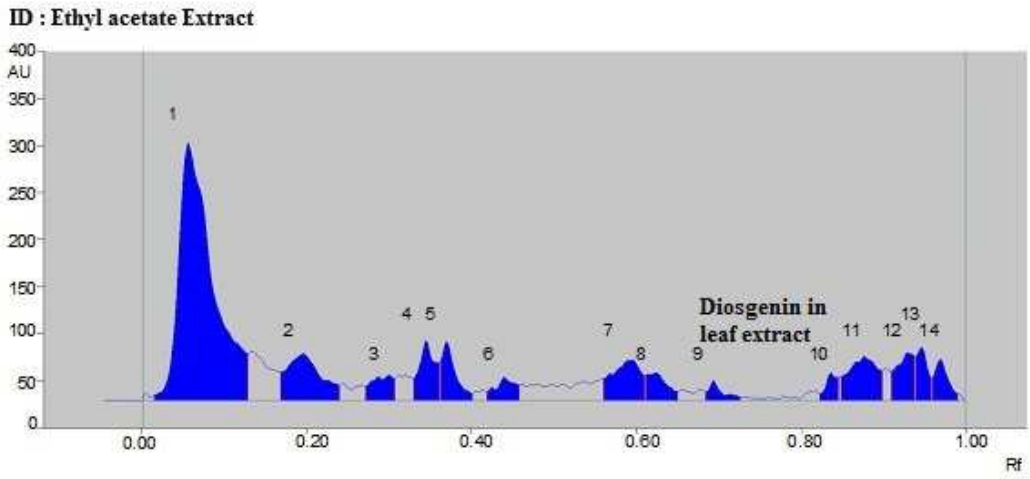


Figure 6: HPTLC densitogram for diosgenin in ethanolic extract of *E. scaber*

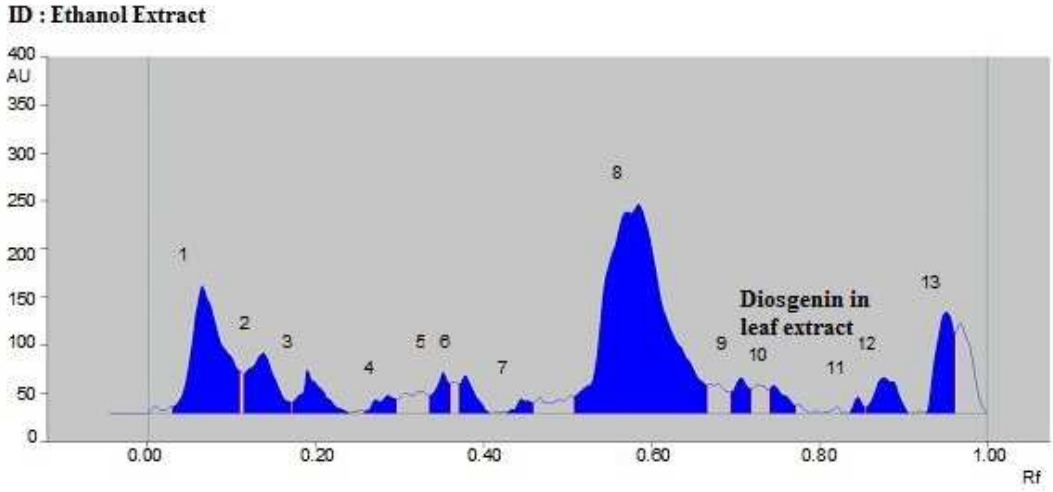


Figure 7: HPTLC densitogram for diosgenin in methanolic extract of *E. scaber*

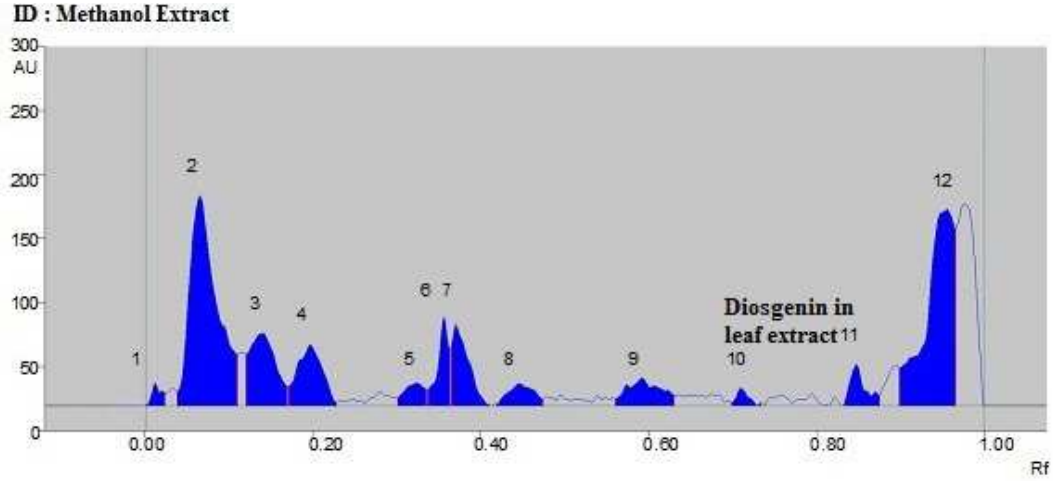


Figure 8: HPTLC densitogram for diosgenin in water extract of *E. scaber*

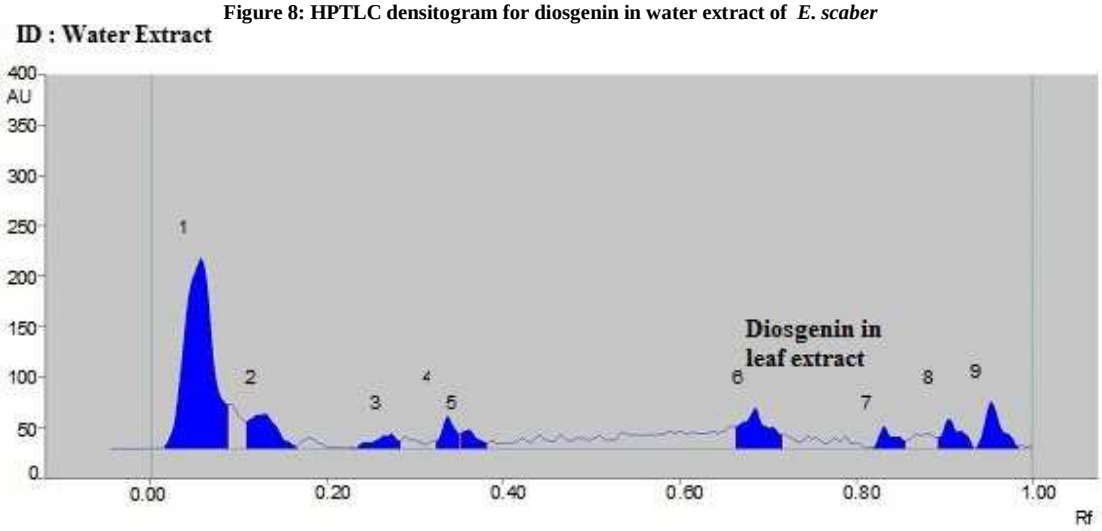
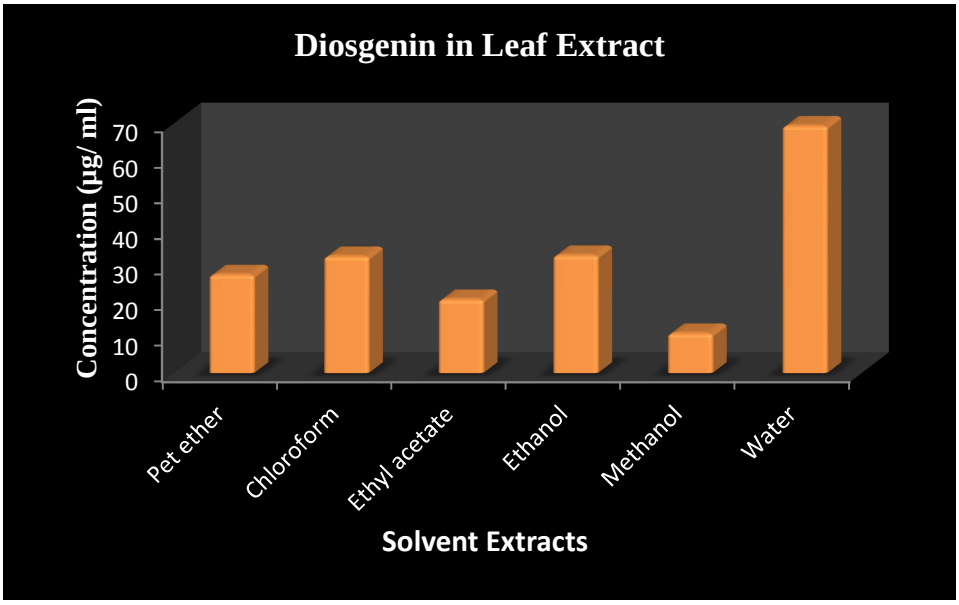
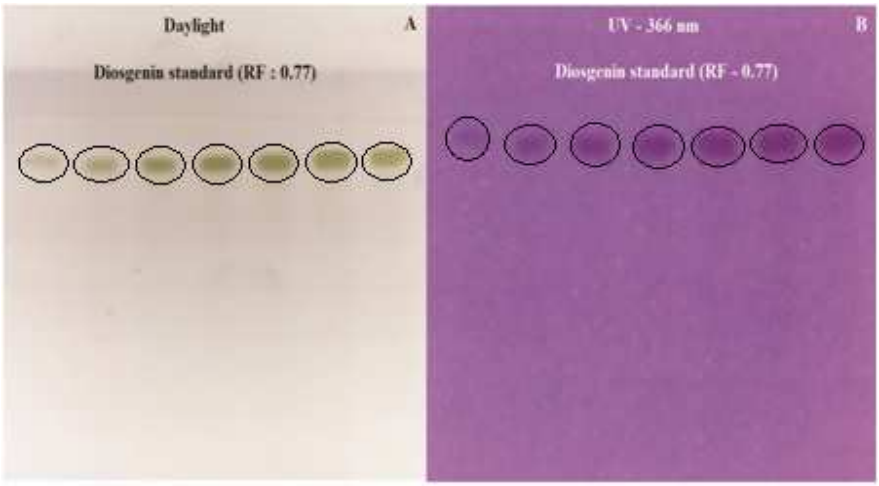


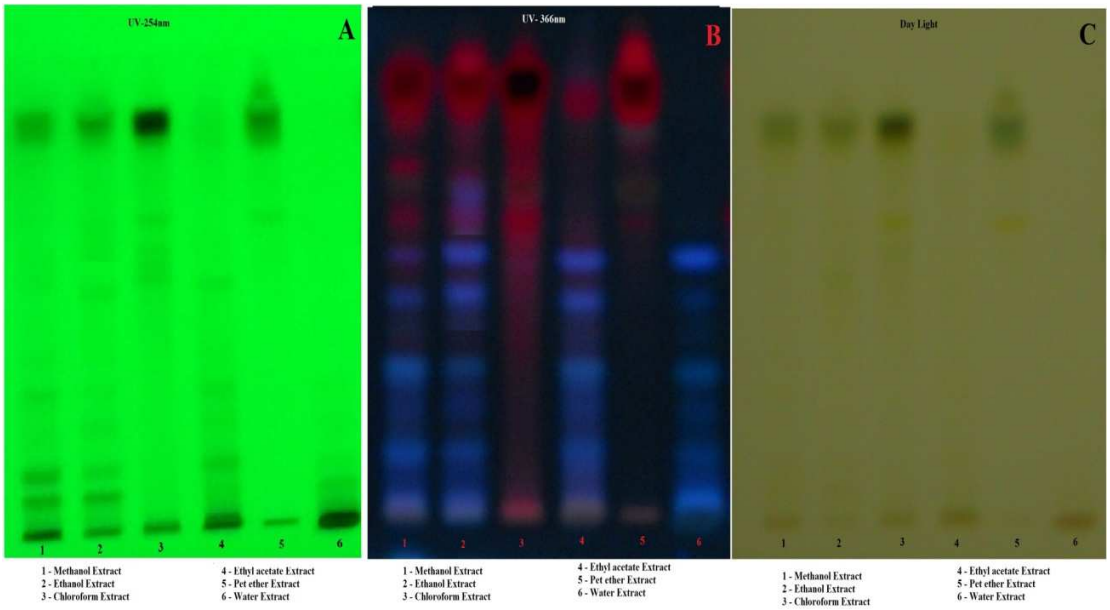
Figure 9: Diosgenin content in different solvents in plant extract



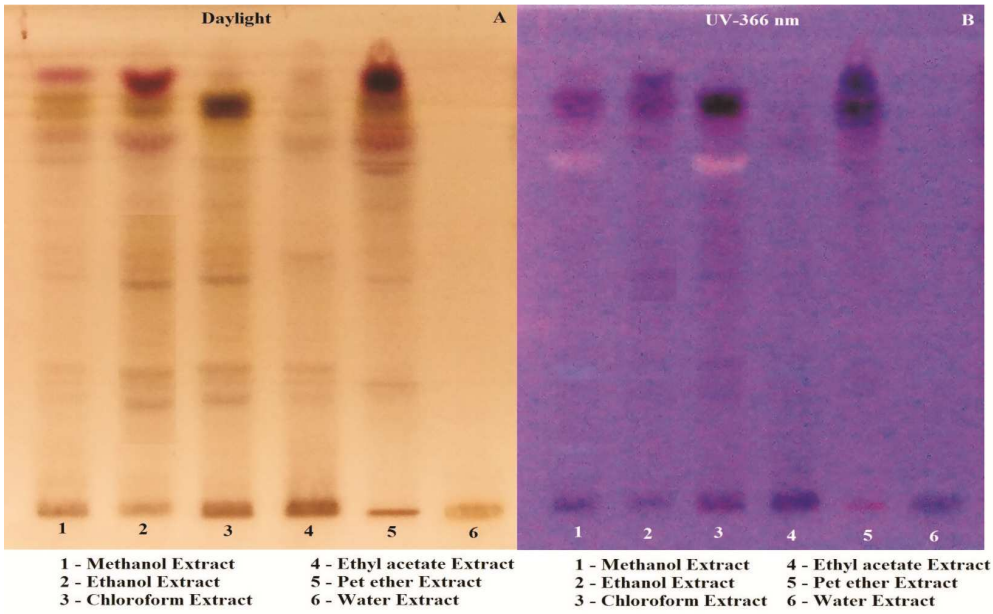
Photoplate 1: HPTLC photoplate of diosgenin standard
A. Daylight After derivatization.
B. UV-366 nm After derivatization.



Photoplate 2: HPTLC photoplate of plant leaf extracts
A. 254 nm
B. UV-366 nm and
C. Daylight After derivatization.



Photoplate 3: HPTLC photoplate of plant leaf extracts
A. Daylight
B. UV-366 nm After Derivatization.



CONCLUSION

The presence of saponin compounds is determined using different solvent methods by HPTLC technique in *Elephantopus scaber*. *E. scaber* leaves are good sources for saponin. This is isolated for variety of commercial uses. Among all the studied solvents, we observed that the aqueous extract exhibited high content of diosgenin (69.37 µg /ml) in *Elephantopus scaber*. The next highest range of of the diosgenin content was recorded for ethanolic extract (33.02 µg /ml) followed by chloroform extract (32.72 µg /ml) and the low estimate was recorded (11.04 µg /ml) in methanolic extract. Diosgenin constituent is potentially used in ethnomedicine as anti-cancer, antibiotics, antidiabetic, antifungal, anti-inflammatory, and antioxidant, antiprotozoal, anti-ulcer and antiviral agents. Saponins

were used as oral contraceptives drug formulation and sex hormones. Hence the present analysis has commercial as well as pharmaceutical and clinical importance and provides good scope to the researcher to formulate the drugs that are potentially used for various ailments.

Acknowledgment
The authors are thankful to the Director and Head, Department of Botany, The Institute of Science, Mumbai for providing Analytical facilities.

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