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# Identification of Pinitol in plants extracts by HPTLC

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## ABSTRACT

*Pinitol is a very common cyclitol present in many leguminous plants and it is the mono methyl ether of d – inositol. The present study was designed to identify Pinitol, a proven anti-diabetic insulommetic drug in plants extracts by High Performance Thin Layer Chromatography (HPTLC). The TLC procedure was optimized with mobile phase chloroform: methanol: water (6:3.5:0.5) and ammoniacal silver nitrate as a derivatizing agent. The proposed HPTLC method was found to be simple, faster and specific laboratory method of identification of pinitol in plants extracts.*

**Keywords:** Pinitol, insulommetic drug, *Pisonia grandis* and HPTLC.

## INTRODUCTION

Plants represent the eternal kindness of nature by all means which is expressed in varied human culture from time immemorial. The history of the use of plants as a primary source of medicine can be traced back several millennia to the ancient written documents of early civilization of China, India and the Middle East. Alkaloids, flavonoids, steroids, terpenoids, tannins, glycosides, saponins and sugar alcohols are economically important secondary metabolites elaborated by plants. Polyhydroxylated cyclohexane derivatives are called cyclitols [1]. Cyclitol, their derivatives and analogs have continued to attract the attention of chemists and biologists owing to the potential pharmacological significance bestowed with them [2-5].

Pinitol is a very common cyclitol present in many leguminous plants and it is the mono methyl ether of d- inositol [6, 7]. It is a normal component of human diet and has been shown not to add any calories when consumed and critically it has been demonstrated to have hypoglycaemic effect. Pinitol is a commercially very important anti-diabetic molecule proven to have insulinomimetic action and is non-toxic with no side effects [8-11]. Chromatographic techniques such as LCMS, GCMS and HPLC are used to identify pinitol [12-17] but the cost per analysis is

expensive. HPTLC is the most simple and readily available chromatographic technique used for fast screening of plant extracts [18-22] with moderate cost of analysis and in the present study it was endeavored to establish a basic method of identification of pinitol in plants extracts by HPTLC.

## EXPERIMENTAL SECTION

### Plant Material

Fully mature leaves of *Pisonia grandis* (R.Br.) were collected from local areas of Coimbatore district of TamilNadu, India and authenticated by the taxonomist, Dr. C. Kunhikannan, Scientist D, Biodiversity Division, Institute of Forest Genetics & Tree Breading, Coimbatore.

### Processing of plant samples

Leaves of *Pisonia grandis* were cleaned and dried in shade. The shade dried leaves were cut into small pieces and then used for the study.

### Preparation of leaf extract

Air dried pieces of leaves of *Pisonia grandis* were extracted with 100% ethanol at reflux temperature for 6 hours. The extract was filtered; the filtrate was evaporated to one tenth volume under reduced pressure to get a greenish black pasty solid (PGEE).

### Analytical Procedure

### Chemicals

Silica gel 60 $F_{254}$ TLC aluminium sheets (20x20), chloroform, methanol, di-methylsulphoxide, liquid ammonia, sodium hydroxide and silver nitrate were purchased from Merck. Standard D-Pinitol was purchased from Sigma Aldrich.

### Preparation of test solutions

A known quantity of Sigma (D-Pinitol), Pinitol (PG1) isolated from the leaves of Pisonia *grandis* [23](Patent Pending 3805/CHE/2010) and the concentrated ethanol extract (PGEE) was dissolved in a known volume of dimethyl sulfoxide (DMSO) and centrifuged at 3000rpm for 2min. These solutions were used as test solutions for HPTLC analysis.

### TLC analysis of PGEE

TLC of PGEE was run on a pre-coated silica gel plate along with PG1 and standard D-Pinitol. The plate was developed with mobile phase chloroform: methanol: water (6:3.5:0.5). After the development the plate was sprayed with ammoniacal silver nitrate solution. Brown spots developed after heating the plates in an oven at 100°C for 15 minutes. The TLC procedure was optimized in the above conditions.

### HPTLC analysis

HPTLC was performed on silica gel 60 $F_{254}$ TLC aluminium sheet under laboratory conditions. 5 µl of sigma, 1 µl of PG1 and 0.6 µl of PGEE were applied as 5 mm band using Hamilton syringe with Camag Linomat automated TLC applicator. After the sample application, the plate was developed in a Camag Twin trough glass tank pre saturated with mobile phase chloroform: methanol: water (6:3.5:0.5) up to 90 mm. After the development, the plate was dried in hot air and the images captured in photo-documentation chamber in white light and in UV (254nm and UV366nm). The developed plate was sprayed with ammoniacal silver nitrate [24-26] and dried at 100 ° C for 10 minutes and documented again in day light and in UV. The retention factor ($R_f$) and % area were calculated using Wincats software.

**Preliminary phytochemical screening**

The ethanolic extract of *Pisonia grandis* (R.Br) was subjected to preliminary phytochemical screening for the presence of alkaloids, flavonoids, steroids, triterpenoids, tannins and glycosides.

## RESULTS AND DISCUSSION

Preliminary phytochemical analysis of ethanolic extract of the leaves of *Pisonia grandis* revealed the presence of alkaloids, amino acids, phenols, sterols, terpenoids, carbohydrates, flavonoids, saponins and tannins. The TLC chromatogram run for PGEE along with standard pinitol obtained from Sigma Aldrich and pinitol isolated from the same plant is shown in Fig.1. HPTLC chromatograms run for the ethanol extract of leaves of *Pisonia grandis* along with standard pinitol obtained from Sigma Aldrich and pinitol isolated from the same plant are shown in Fig.2 and Fig.3. The peak densitograms of standard pinitol (Fig.4), PG1 (Fig.5) and PGEE (Fig.6) are also shown below. The results of the HPTLC analysis are presented in table 1.

**Table 1: Retention factor ($R_f$), height and area of peaks of standard D-Pinitol, PG1 and PGEE**

| Track | Peak | Rf | Height | Area | Assigned substance |
|---|---|---|---|---|---|
| SIG (Standard) | 1 | 0.70 | 269.1 | 14804.9 | D-Pinitol |
| PG1 | 1 | 0.66 | 311.1 | 18085.1 | Standard |
| PGEE | 1 | 0.05 | 30.1 | 455.8 | Unknown |
| PGEE | 2 | 0.14 | 31.7 | 615.7 | Unknown |
| PGEE | 3 | 0.19 | 38.1 | 473.2 | Unknown |
| PGEE | 4 | 0.50 | 123.3 | 4404.4 | Unknown |
| PGEE | 5 | 0.62 | 487.1 | 16821.4 | Unknown |
| PGEE | 6 | 0.65 | 496.7 | 23883.1 | Standard |
| PGEE | 7 | 0.76 | 248.5 | 13670.4 | Unknown |
| PGEE | 8 | 0.85 | 66.2 | 1480.4 | Unknown |
| PGEE | 9 | 0.89 | 189.0 | 6937.6 | Unknown |
| PGEE | 10 | 0.94 | 153.0 | 3874.8 | Unknown |


**Fig.1: Photograph of TLC chromatogram in day light after derivatization**


**Fig.2: Photograph of HPTLC chromatogram in day light after derivatization**


**Fig.3: Photograph of HPTLC chromatogram in UV 254 nm after derivatization**

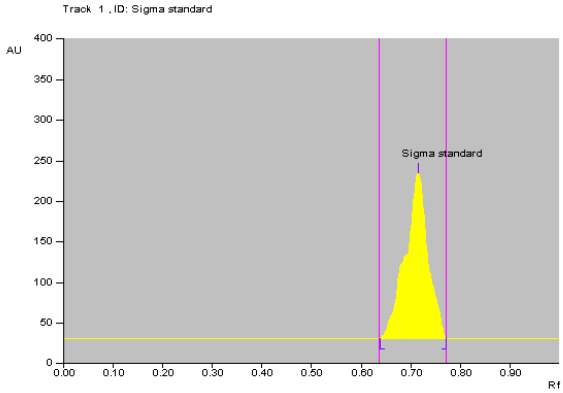


**Fig.4: Peak densitogam of standard Pinitol**

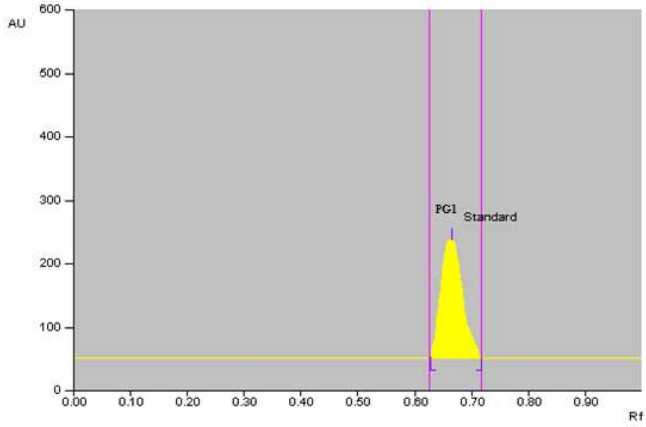


**Fig.5: Peak densitogram of PG1**

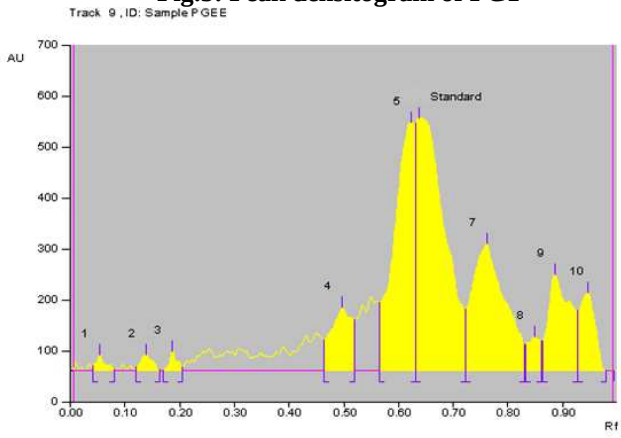


**Fig.6: Peak densitogram of PGEE**

## CONCLUSION

The commonly known plant *Pisonia grandis* (R.Br.) is rich in popular phytoconstituents such as alkaloids, flavonoids, steroids, triterpenoids, tannins and glycosides. This HPTLC analysis using the mobile phase chloroform: methanol: water and involving derivatization using ammoniacal silver nitrate can evolve as a specific laboratory method for identification of cyclitol like pinitol in plants extracts.

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