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Identification and Quantification of Pinitol in Selected Anti-Diabetic Medicinal Plants by an Optimized HPTLC Method

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ABSTRACT

A high performance thin layer chromatography method was validated for the quantification of insulinomimetic pinitol in the extracts of anti diabetic plants. The alcoholic extract of selected anti diabetic plants was chromatographed on silica gel 60 F254 plates with CHCl3 :MeOH:H2O, 6:3.5:0.5 as mobile phase. Detection and quantification was performed by densitometry scanning at λ=500 nm. The method provides a good resolution of pinitol from the ethanolic extract of dried leaves of selected plants. Pinitol was identified in ten indigenous medicinal plants

Keywords : HPTLC, anti diabetic, Pinitol

Introduction:
Plants are an immediate source of medicines. In view of the large number of active principles produced by them one can only wonder at the incredibly vast reserves of ingredients that are still largely untapped. Numerous biomarkers are available for quantification of plant extracts which are potential candidates of herbal formulations. Pinitol is an anti diabetic biomarker. Its pharmacological significance is highly remarkable and there are a volly of reports [1-13] on its use in medicinal formulations.

In view of the pharmacological significance of pinitol especially as an anti diabetic bio marker, an optimized HPTLC method of identifying pinitol in proven anti diabetic plant extracts was targeted.

MATERIALS AND METHODS
Collection of Plant Materials
Plant materials needed for the study were identified and collected from local areas, air-dried and pulverized. .

Extraction of plant materials
The dried plant material (10 g) was extracted with ethanol (75 ml) by heating over a water bath for 1 hour. The extract was filtered and concentrated. The extraction procedure was repeated again with fresh alcohol. The residues from ethanol extracts were weighed. The residual plant material was extracted with water (60 ml) for 1 hour. The extract was filtered and concentrated to give a residue which was weighed. The above extraction process was carried out for each of the 18 samples of plant material taken up for the present study. The percentage yield of residue was calculated. Table1 gives the details of plants chosen and the plant part used for the study.

TLC examination of the extracts
The ethanol extracted residues of 18 plant samples were analysed by an optimized TLC method. This study was done to identify the presence of the anti diabetic molecule pinitol in the plant samples. Silica gel 60 F254 pre-coated chromatographic plates (6cmx10cm) were used for the TLC study. The sample was dissolved in ethanol and spotted on the TLC plate. All the samples were similarly spotted. The plate was developed in chloroform: methanol: water (6: 3.5: 0.5) solvent system. After development, the TLC chromatogram was examined under UV light and then sprayed with ammoniacal silver nitrate solution. It was then placed in an oven for half an hour. Development of an orange brown spot for pinitol was noted and its R_f was recorded.

Preparation of spray reagent - Ammoniacal silver nitrate solution:
A equal amounts of Tollen's reagent I and II were mixed together till a black precipitate appeared. Ammonia solution was added to this mixture until the black precipitate disappeared. The resulting solution was used on the spray reagent.

Identification and quantification of pinitol in the extracts by HPTLC
HPTLC is an improved method of TLC which utilizes the conventional technique of TLC in a more optimized way. The ethanol extract of ten chosen plant materials namely *Bougainvillea spectabilis leaves (BS*L*), Eugenia brazillensis leaves (EB*L*), Mangifera indica leaves (*MIL*), Mimosa pudica leaves (MP*L*), Musa paradisiacal flower (MPA*F*), Mirabilis jalapa leaves (MJ*L*), Ocimum sanctum leaves (OS*L*), Pithecellobium dulce fruit peel (PD*F*), Pisonia grandis leaves (PG*L*), Psidium gujava leaves (PGU*L*)* was analysed by HPTLC. These plant samples were chosen based on identification of pinitol in their extracts by TLC analysis.

Procedure for HPTLC Analysis
Test solution preparation
The samples were dissolved in 200µl of dimethyl sulfoxide (DMSO) and centrifuged at 3000 rpm for 2min. These solutions were used as test solution for HPTLC analysis.

Sample application
The above test solution (2µl) were loaded as 5mm band length on Silica gel F_{254} TLC plate using Hamilton syringe.

Spot development
The sample loaded plate was kept in TLC twin trough developing chamber (after saturating with solvent vapor) and developed in the respective mobile phase up to 90mm.

Photo-documentation
The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in the photo-documentation chamber (CAMAG REPROSTAR 3) and the images captured in white light and UV light (254nm and 366nm).

Derivatization
The developed plate was sprayed with respective spray reagent and dried at 100°C in hot air oven. The plate was photo-documented in day light mode using photo-documentation (CAMAG REPROSTAR 3) chamber.

Scanning
After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done in white light at 500nm. The peak table, peak display and peak densitogram were noted.

ANALYSIS DETAILS
Mobile phase-Chloroform-Methanol-Water (6: 3.5: 0.5)

Spray reagent-Ammoniacal silver nitrate reagent

Detection
Yellowish brown colored zones were present corresponding to the standard track of all sample tracks when the plate was observed in day light mode after derivatization.

RESULTS AND DISCUSSION
The present study was undertaken with the main aim of analyzing extracts of 18 chosen plant materials from 12 anti diabetic plants for the presence of the bioactive insulin mimetic molecule pinitol in them by TLC and High performance TLC (HPTLC). The TLC method was optimized for the developing solvent system and the spray reagent used. The results are presented below.

Extraction Analysis
All the 18 plant materials (10 g each) were first extracted with ethanol for 1 hour followed by a second extraction of the residual plant material with ethanol for 1hour. Then the residual plant material was extracted with water for 1 hour (Heating over a water bath for ethanol extraction and direct heating for water extraction). The extraction strategy revealed that most of the plants gave a higher yield of residue in the first ethanol extraction. The percentage yield of residue obtained is given in Table 2. Four plant samples (Leaves of *Mimosa pudica, Pisona grandis, Cissus quadrangularis and Annona squamosa,)* gave a maximum yield of residue in the range 18-33% from only 10 g of plant material by extraction for one hour. This is a notable aspect of the extraction analysis. However three plants (*Pithecellobium dulce fruit peel, Pisonia grandis, Musa paradasica flower*) gave a lower yield of residue in the range 2-5%. Samples EBL, MIL and OSL gave a higher yield of ethanol residue in the second extraction contrary to the trend in the other plant samples.

In the aqueous extraction, plant samples AQL, BSL, CQL,MPL, MJL,OSL, PDL and PDL gave a high yield of residue (10% to 29%) with AQL,CQL, BSL and PDL giving more than 20% yield from 10 g of plant material.The same plant samples (AQL, BSL, CQL, MJL, OSL, PDL and PDL) except MPL also gave a higher yield of residue in aqueous extraction than in ethanol extraction indicating probably the presence of a higher concentration of more polar constituents in them.

TLC analysis
TLC analysis of 18 plant samples was done to identify the anti diabetic molecule pinitol in their ethanol extract by an optimized method using chloroform: methanol: water (6: 3.5: 0.5) solvent system. TLC of the plant samples was compared with that of standard pinitol. Pinitol was identified in ten plant samples, namely *Bougainvillea spectabilis (BS*L*), Eugenia brazillensis(leaf) (EB*L*), Mangifera indica(*leaf*) (*MIL*), Mimosa pudica(leaf) (MP*L*), Musa paradasica (flower) (MPA*L*), Mirabilis jalapa (*leaf*) (MJ*L*), Ocimum sanctum(leaf) (OS*L*), Pithecellobium dulce(fruit peel) (PD*F*), Pisonia grandis(leaf) (PG*L*) and Psidium gujava(leaf)(PGU*L*)*. These samples were chosen for HPTLC analysis to ascertain the presence of pinitol in them.

Identification and Quantification of Pinitol by HPTLC
HPTLC method was adopted to quantify the amount of pinitol in the ethanol extracts of ten chosen plants which were identified to have pinitol or pinitol-like compounds. The amount of pinitol in the extracts was calculated from the respective peak area using the following formula.

$$\% \text{ of the component} = \frac{\text{Area of the sample}}{\text{Area of the standard}} \times \frac{\text{Concentration of the standard}}{\text{Concentration of the sample}} \times \frac{\text{Applied vol of the standard}}{\text{Applied vol of the sample}} \times \text{Purity of std}$$

This is a relative quantification only.
Table 3 provides details of relative factor (R_f) peak number and peak area obtained from the HPTLC analysis of the chosen samples.

Figure 1 represents the HPTLC chromatogram of the chosen samples PGL, MIL, MPL, MJL, EBL, BSL, PGUL, MPAF, OSL and PDF. Peak densitogram display of standard Pinitol and the samples (Scanned at 500nm) are illustrated in Figures (2-12)

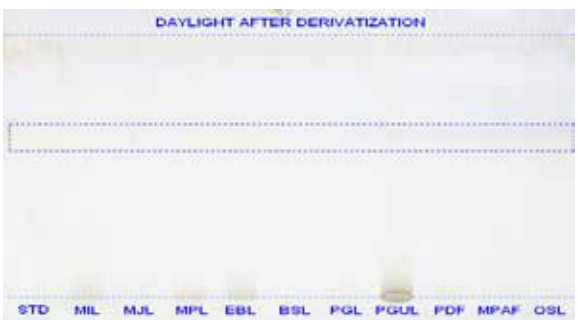

Figure 1

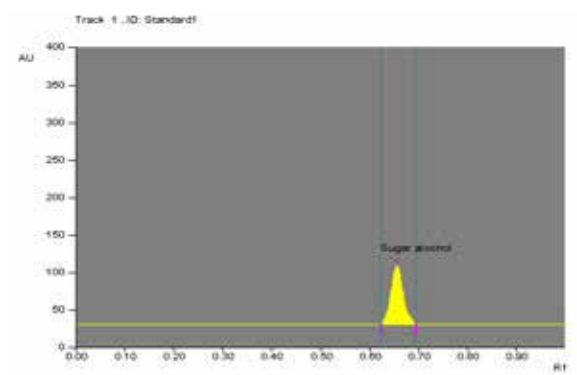

Fig .2 Peak Densitogram of Standard

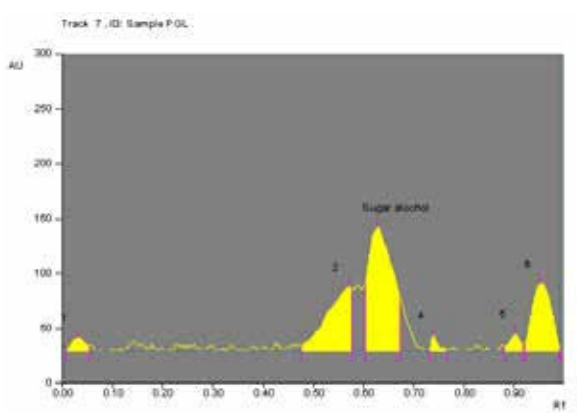

Fig .3 Peak Densitogram of PGL

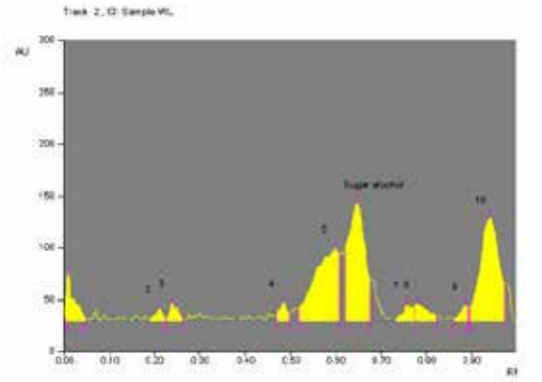

Fig .4 Peak Densitogram of MIL

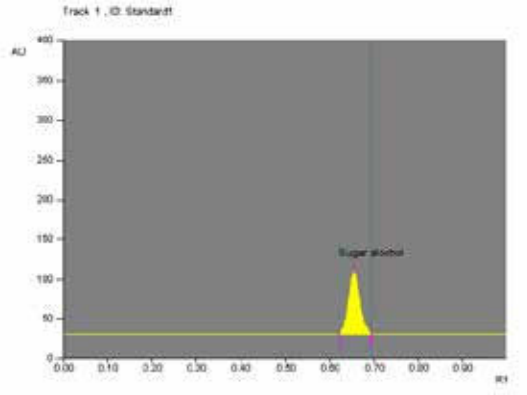

Fig .2 Peak Densitogram of Standard

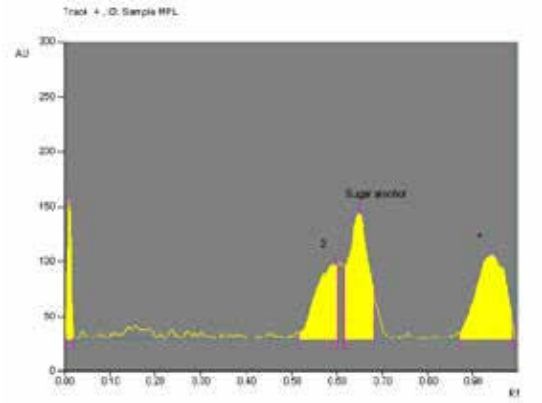

Fig .5 Peak Densitogram of MPL

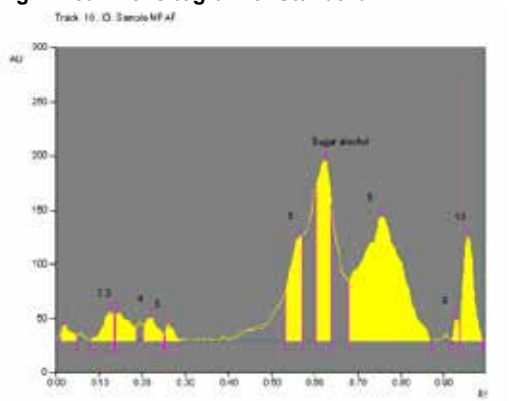

Fig .8 Peak Densitogram of OSL

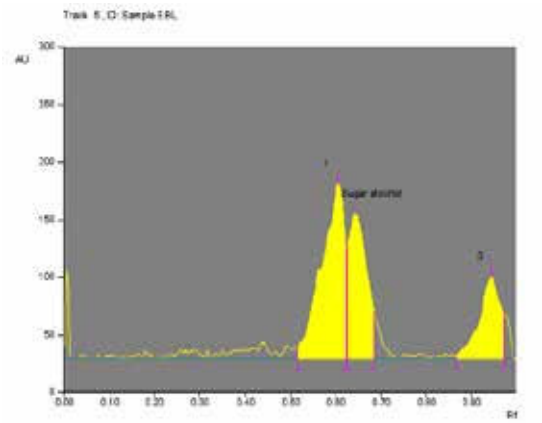

Fig.6 Peak Densitogram of EBL

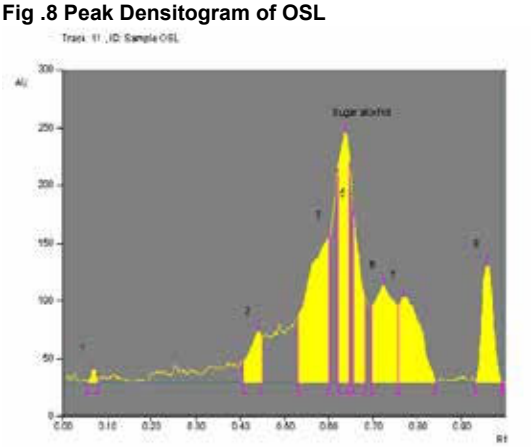

Fig .9 Peak Densitogram of MPAF

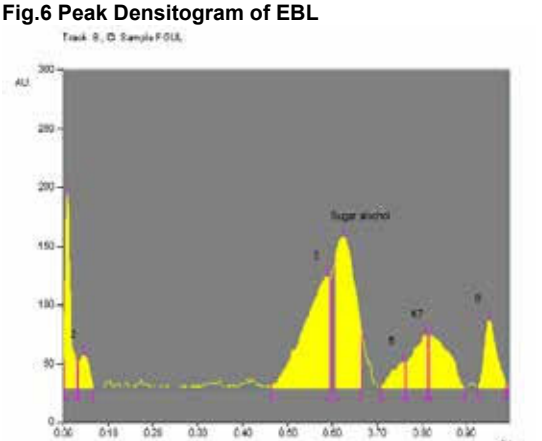

Fig.7 Peak Densitogram of PGUL

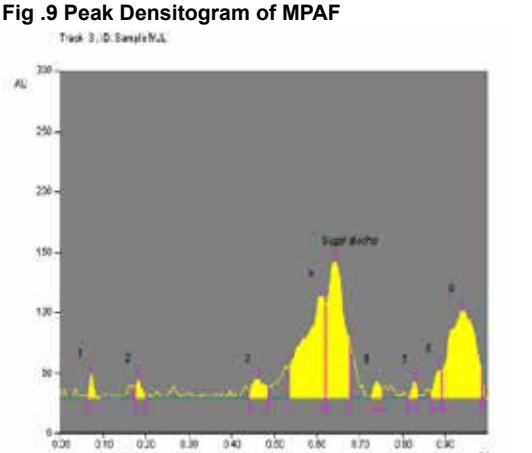

Fig .10 Peak Densitogram of MJL

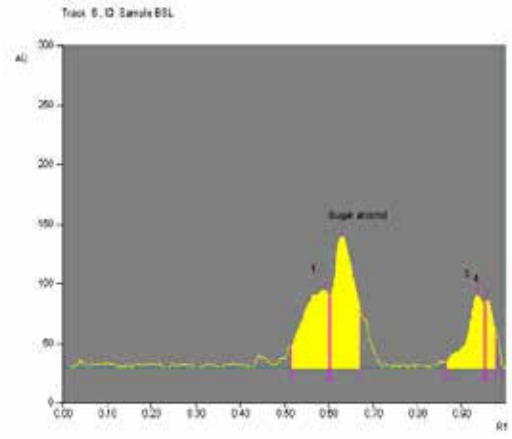

Fig.11 Peak Densitogram of BSL

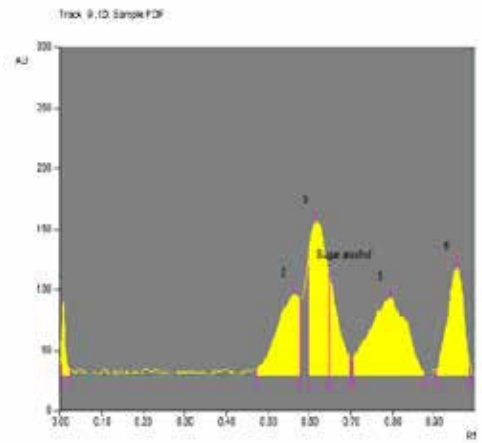

Fig.12 Peak Densitogram of PDF

Table 1: Details of Plants Chosen and the Plant Part Used for the Study

S.No	Plant Name	Family Name	Tamil Name	English Name	Plant Part Used	Sample Code
1	Annona squamosa	Annonaceae	Seetha Maram	Custard	Leaves	AQL
2	Bougainvillea spectabilis	Nyctaginaceae	Kagitha poo Maram	Paper plant	Leaves	BSL
3	Cissus quadrangularis	Vitaceae	Pirandai	Veld grape	Leaves	CQL
					Fleshy part	CQF
4	Eugenia brazillensis	Myrtaceae	Naval Maram	Jambulona	Leaves	EBL
5	Mangifera indica	Anacardiaceae	Maa Maram	Mango tree	Leaves	MIL
6	Mimosa pudica	Leguminosae	Thotta Chinungi	Touch me not plant	Leaves	MPL
7	Musa paradasiaca	Musaceae	Valai Maram	Plantain tree	Flowers	MPAF
					Leaves	MPAL
					Pistil	MPAP
8	Mirabilis jalapa	Nyctaginaceae	Anthimandarai	4"O"clock plant	Leaves	MJL
9	Ocimum sanctum	Lamiaceae	Thulasi	Tulsi	Leaves	OSL
10	Pithecellobium dulce	Leguminosae	Kodukkapuli	Manila tamarind tree	Leaves	PDL
					Fruit Peel	PDF
11	Pisonia grandis(R.Br)	Nyctaginaceae	Leechaikottai Maram	Lettuce tree	Leaves	PGL
					Stem	PGS
					Roots	PGR
12	Psidium gujava	Myrtaceae,	Koiya Maram	Guava	Leaves	PGUL

Table 2: Yield of Residue Obtained

S.No	Sample	Residue weight (g) in ethanol extraction		Yield (%) in ethanol extraction		Residue weight (g) in aqueous extraction	Yield (%) in aqueous extraction
		First Extraction	Second Extraction	First Extraction	Second Extraction		
1	AQL	1.8	0.5	18.0	5.0	2.7	27.0
2	BSL	0.8	0.4	8.0	4.0	2.02	20.2
3	CQL	2.0	0.3	20.0	3.0	2.93	29.3
4	CQF	0.7	0.5	7.0	5.0	1.1	11.0
5	EBL	1.0	1.8	10.0	18.0	0.49	4.9
6	MIL	0.9	1.2	9.0	12.0	0.8	8.0
7	MPL	2.5	0.6	25.0	6.0	1.08	10.8
8	MPAF	0.48	0.1	4.8	1.0	0.1	1.0
9	MPAL	0.6	0.3	6.0	3.0	0.23	2.3
10	MPAP	0.5	0.1	5.0	1.0	0.88	8.8
11	MJL	0.6	0.4	6.0	4.0	1.81	18.1
12	OSL	0.7	1.6	7.0	16.0	1.84	18.4
13	PDL	1.0	0.6	10.0	6.0	2.1	21.0
14	PDF	0.2	0.1	2.0	1.0	0.55	5.5
15	PGL	3.3	0.6	33.0	6.0	1.76	17.6
16	PGS	0.4	0.2	4.0	2.0	0.49	4.9
17	PGR	0.6	0.4	6.0	4.0	0.41	4.1
18	PGU	0.8	0.7	8.0	7.0	0.57	5.7

Table 3: HPTLC Analysis Data

Sample code	Sample Name	R_f	Peak No	Peak Area	% of Pinitol
Standard	Sigma Standard	0.65	1	5121.5	94.0
MIL	Mangifero indica Leaves	0.65	6	3749.8	77.12
MJL	Mirabilis jalapa Leaves	0.65	5	3896.3	80.83
MPL	Mimosa pudica Leaves	0.65	3	4338.3	82.80
EBL	Eugenia brazillensis Leaves	0.65	2	4516.2	74.82
BSL	Bougainvillea spectabilis Leaves	0.64	2	4682.4	79.22
PGL	Pisonia grandis Leaves	0.64	3	4879.1	91.81
PGUL	Psidium gujava Leaves	0.64	4	4976.6	90.45
PDF	Pithecellobium dulce Fruit peel	0.65	4	1724.9	31.67
MPAF	Musa paradisiacal Flower	0.64	7	4450.0	92.28
OSL	Ocimum sanctum Leaves	0.64	4	4292.6	92.96

CONCLUSION

Pinitol possesses immense potential as an anti diabetic molecule. It is one of the recently discovered anti diabetic natural products which has no side effect at all. Its presence has been identified in many plants mostly from pine, soyabean, *Bougainvillea* and carob extracts. In addition to its anti diabetic activity, pinitol also has anti inflammatory activity and improves glucose transport and finds use in the treatment of polycystic ovarian syndrome (PCOS). Pinitol supplements are available in the market. Pinitol is reported to have a variety of roles in plant biology such as drought stress, radical scavenging and more recently, enzyme stability. In view of the enormous pharmacological potential bestowed with this molecule, the present work was undertaken to ascertain the presence of anti diabetic principle pinitol in 18 chosen indigenous plant samples. Though many plants of the *Leguminosae* family are found to elaborate pinitol, ascertaining its presence and quantifying it by modern analytical techniques will facilitate the development of anti diabetic formulations containing pinitol. This study revealed the presence of pinitol in ten plant samples. However the revelation will be further ascertained by HPLC and GC-MS analysis.

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