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IDENTIFICATION OF THE EFFECT OF EXTRACTION SOLVENT ON PHYTOCONSTITUENTS OF *CINNAMOMUM VERUM* BY USING HPTLC

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ABSTRACT

The aim of the study was to identify the effect of extraction solvents on phytoconstituents of *Cinnamomum verum* by HPTLC. The *Cinnamomum verum* leaves were successfully extracted with various solvents according to their increasing order of polarity. The phytochemicals present in the extracts were identified by qualitative phytochemical screening, which reveals the presence of fats in petroleum ether extract; tannins, terpenes in chloroform extract; terpenes, flavonoids in ethyl acetate extract; saponin, flavonoids in water extract; and terpenes, phenolics, tannins and flavonoids in the alcoholic extract of *Cinnamomum verum* leaves respectively. Hptlc densitogram of each extract was carried out and their Rf value was compared with the reference standards and identified. This method point out that which extraction solvent suitable to extraction of phytoconstituents of *Cinnamomum verum*.

KEYWORDS: *Cinnamomum verum*, HPTLC, Densitogram, Extraction solvents.

INTRODUCTION

Cinnamomum verum, called true cinnamon tree or Ceylon cinnamon tree is a small evergreen tree belonging to the family lauraceae, native to Sri Lanka.[1] Among other species, its inner bark is used to make cinnamon. *Cinnamomum verum* leaves consists of eugenol, caryophyllene, linalool, flavonoids.[2] As a results of its aroma and medicinal properties these are used as a spice in culinary purpose, it is also used in various dessert recipes, such as apple pie, doughnuts and cinnamon buns in addition to tea, and liquors. It is used to treat problems with digestive system. Cinnamon contains a number of antioxidant compounds which can effectively scavenge reactive oxygen species including superoxide anions and hydroxyl radicals as other free radicals.[3] Antimicrobial activity of cinnamon bark and oil described against many bacterial and fungal strains. The cinnamon essential oils have been proved to inhibit the growth of molds, and bacteria.[4]

MATERIALS AND METHODS

Plant Material

Cinnamomum verum leaves were collected from various place of Malappuram during the month of May-June. Authentication of the plant was done BY Mr. A.K Pradeep,from Calicut University Herbarium which was certified that the given specimen no-88430 belonged to *Cinnamomum verum* Presl [Lauraceae]. A voucher specimen was deposited in the Department of Pharmacognosy of Al Shifa College of Pharmacy.

Extraction

Freshly collected leaves after cleaning were left for shade-drying on the floor above the newspaper for 10-15 days. After that, the leaves were dried in hot air oven at 40^0C for an hour just before starting the extraction process to remove the equilibrium moisture content.The extraction of dried and coarsely powdered leaves of *Cinnamomum verum* Presel was carried out by continuous soxhlet extraction using petroleum ether, chloroform, ethyl acetate, ethanol, and water in the order of increasing polarity. The percentage yield of extract was calculated. The extracts were then concentrated to dryness under reduced pressure and they were preserved in a refrigerator.

Phytochemical Screening

The extracts of Cinnamomum verum leaves were subjected to preliminary phytochemical evaluation.[5,6]

Chromatographic Conditions for Hptlc

Leaf extract of *Cinnamomum verum* were spotted in the form of bands (6mm width) with a Camag microlitre syringe on precoated silica gel HPTLC plate $60F_{254}$ (10× 10 cm with 250 µm thickness E. Merck, Darmstadt, Germany) using a Camag Linomat V (Switzerland). The plates were pre-washed by methanol and activated at 60º C for 5 min prior to chromatography. The sample loaded plate was kept in TLC twin trough developing chamber with respective mobile phase and the plate was developed in the respective mobile phase up to 90 mm. The Toluene-ethyl acetate (7:3) was employed as mobile

phase. Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber saturated with the mobile phase. The developed plate was air dried and scanned at a wavelength of 254 nm, 550nm after derivatising with Fast Blue Salt reagent in CAMAG TLC SCANNER 3 with winCATS software.

RESULTS AND DISCUSSION

The experimental yield of petroleum ether, chloroform, ethyl acetate, ethanol, and water extracts of *Cinnamomum verum* leaves were found to be 2.23%, 5.41%, 6.10%, 9.43% and 8.01% respectively (Table. 1). Preliminary phytochemical screening indicate the presence or absence of phytochemical constituents in different extracts of Cinnamomum verum leaves (Table.2). Alkalloids were found to be absent in all the five extracts. Carbohydrates, saponins, flavonoids, terpenes, tannins and phenols were found to be present in ethanol extracts. The chloroform extracts and ethyl acetate extracts contains only flavonoids. The petroleum ether extracts contains only tannins whereas the water extracts contains flavonoids, terpenes, tannins, and phenols.

Table 1: Percentage yield of different extracts.

Sr.No	Extracts	% Yield Value
1.	Petroleum ether	2.23
2.	Chloroform	5.41
3.	Ethyl acetate	6.10
4.	Ethanol	9.43
5.	Water	8.01

PHYTOCHEMICAL SCREENING

Table No. 2: Phytochemical screening of extracts of *Cinnamomum verum* leaves.

Sr. No	Phytochemicals	PEE	CHE	EAE	ETL	WTR
1.	Alkaloids					
2.	Glycosides					
3.	Carbohydrates				+	
4.	Saponins				++	++
5.	Flavones and Flavonoids		+	+	++	+
6.	Terpenes				++	++
7.	Tannins	+			+	
8.	Phenols				++	+
9.	Proteins and aminoacids					

PEE - Petroleum ether extract, CHE - Chloroform extract, EAE - Ethyl acetate extract, ETL-Ethanolic extract, WTE-Water extract

HPTLC analysis

Standard quercetin showed single peak at 550nm with Rf 0.5- 0.9 in HPTLC (Fig.1). From HPTLC, it was reported that in chloroform extract (Fig.2) 4 spots were obtained with Rf values 0.1,0.32,0.59,0.75respectively.Here spots with Rf values 0.1 and 0.75 have more peak area. In Ethanolic extract (Fig.3) 4 spots were obtained with Rf values 0.19,0.38,0.58,0.872. Here, spot with Rf value 0.872 have more area. In water extract (Fig.4) 3 spots were obtained with Rf values 0.19, 0.48, 0.79.Here, spot with Rf value 0.79 have more peak area. In ethyl acetate extract(Fig.5)4 spots with Rf values 0.16,0.39,0.58,0.87.Here,spots with Rf values 0.16,0.39,0.58 shows broader peak area.

On reference analysis, it was found that the Standard quercetin were having Rf value range between 0.5-0.9. Here, chloroform extract, ethanolic extract and water extract of *Cinnamomum verum* leaves showed Rf values of 0.75, 0.872 and 0.79 with a broader peak area respectively, indicating the presence of some flavonoid constituent in it.

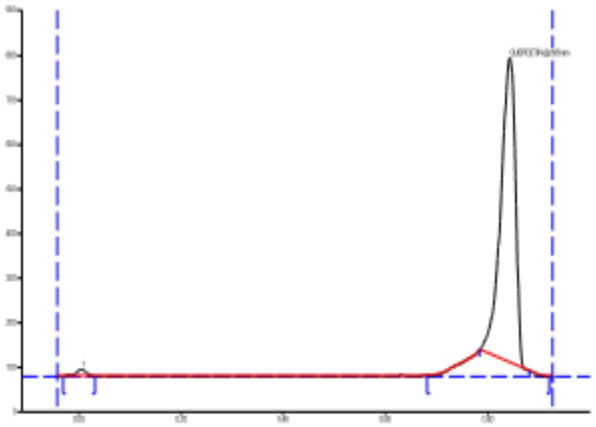

Fig. 1: Densitometric scanning of standard quercetin at 550nm.

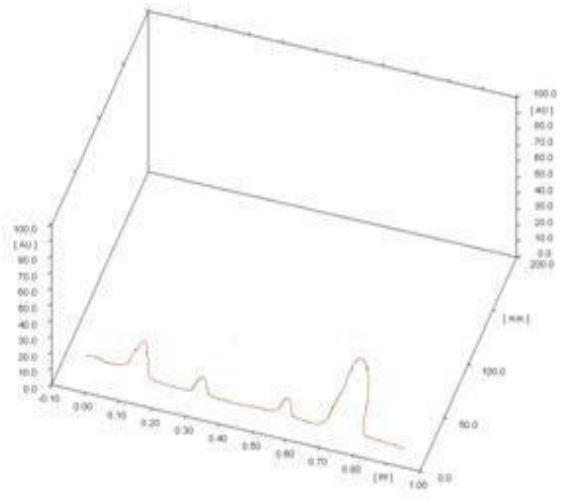

Fig. 2: Densitometric scanning of Chloroform Extract at 550nm.

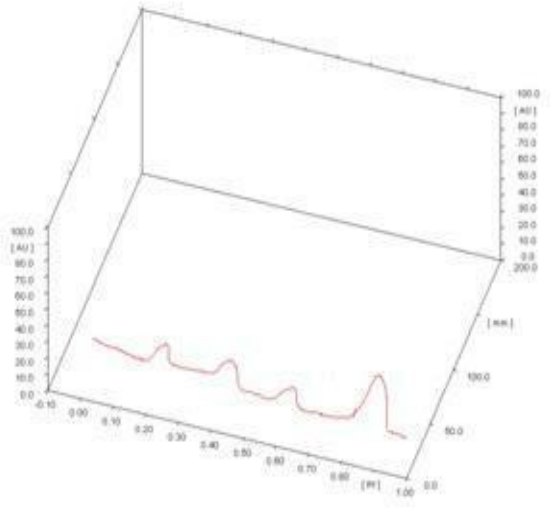

Fig. 3: Densitometric scanning of Ethanoilc Extract at 550nm.

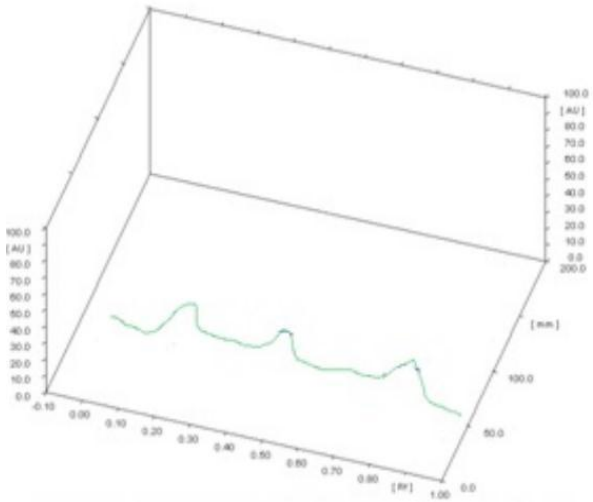

Fig.4: Densitometric scanning of Water Extract at 550nm.

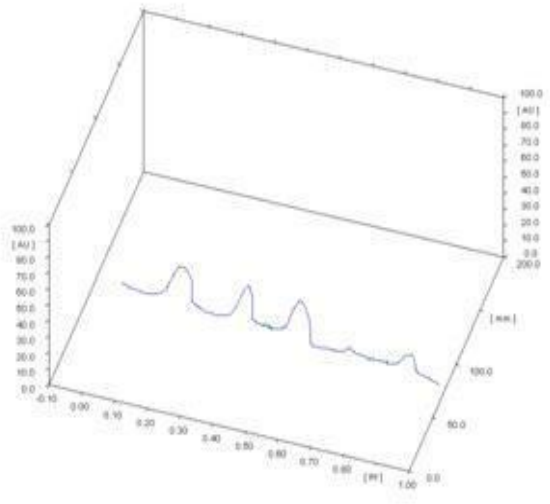

Fig.5: Densitometric scanning of Ethyl acetate Extract at 550nm.

CONCLUSION

This developed HPTLC technique is suitable for flavonoid determination in different extracts of *Cinnamomum verum.* This phytochemical screening of this plant revealed the presence of tannins, terpenes, flavonoids, saponins and glycosides in different extracts of the leaves of Cinnamomum verum. These results shows that leaves of Cinnamomum verum contain a number of chemical ingredients, which may be responsible for various pharmacological actions. It has been observed that most active principles present in the leaves are flavonoid, saponins, tannins, terpenes and glycosides.

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