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HPTLC Technique: Determination of flavonoid from *Clerodendrum viscosum vent* roots

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ABSTRACT

A simple and rapid high performance liquid chromatography (HPTLC) technique has been developed for the determination of flavonoids in Clerodendrum viscosum vent roots. The successive solvent extraction of clerodendrum vicosum vent roots was carried out using petroleum ether, ethyl acetate, chloroform and ethanol by maceration process. The Clerodendrum viscosum root powders were subjected to preliminary phytochemical analysis. Based on phytochemical studies, ethyl acetate extract of Clerodendrum viscosum roots was subjected to column chromatography and eluted successively with chloroform and methanol mixture. The fractions obtained were subjected to HPTLC analysis to identify flavanoids in the collected fractions. HPTLC was performed with silica gel 60F_{254} plates with solvent system chloroform: methanol (11.8:0.2 v/v) as mobile phase. Detection of flavonoid compound was performed by scanning the developed plate at 366 nm. The results indicated that fractions obtained with mixture of 90% and 80 % chloroform in methanol mixture showed single band of flavonoid compound with R_f value 0.25. Therefore, HPTLC is simple and rapid technique and can be used to determine the bioactive compounds in the plant extracts.

Key Words: Flavonoid, *Clerodendrum viscosum vent* roots, HPTLC

INTRODUCTION

Clerodendrum viscosum Vent. (Verbenaceae) is a small shrub and found abundantly in India, Malaysia and Pakisthan. Leaves and roots are commonly used in Indian medical traditional system. The most important constituents of *Clerodendrum viscosum* are alkaloids, flavonoids, saponin, cleodendroside and beta-sitosterol. *Clerodendrum viscosum vent* has been used as an anti-inflammatory, antipyretic, antiseptic and vermifuge, [1]. The *Clerodendrum viscosum* roots are externally used for tumors and skin diseases as paste. Thus *Clerodendrum viscosum vent root* extracts may have potential pharmacological activities [2]. An extensive search of the literature indicates that the characterization of biological constituents of *Clerodendrum viscosum roots* by HPTLC technique has not been scientifically evaluated so far. High performance thin-layer chromatography (HPTLC) is a rapid, precise and cost-effective method and this method is widely used for the determination of biological compounds from medicinal plants. Thus, HPTLC method has been developed for the determination of flavonoid in *Clerodendrum viscosum roots*.

MATERIALS AND METHODS

Plant Materials

Clerodendrum viscosum vent roots were collected from Pallakad district Kerala in the month of September and October 2010. The roots were inspected to be healthy and botanically identified and authenticated by Dr. G.V.S. Moorthy, Plant Biotechnologist, botanical survey of India, Coimbatore. The herbarium *Clerodendrum viscosum Vent* roots were deposited in botanical survey of India (BSI) against voucher no. BSI/SRC/5/23/10-11/Tech1152. The *Clerodendrum viscosum Vent* roots after collection were dried at room temperature (27-30º C) for 40-50 days. After complete drying, the dried root materials were grounded into coarse powder using domestic electric grinder and used for extraction.

Extraction, phytochemical evaluations and isolation of flavonoid

The dried coarse powders of *clerodendrum vicosum vent* root (30 g) were subjected to successive maceration with 300 ml of petroleum ether (60-80º C), ethyl acetate, chloroform and ethanol in a shaker system at room temperature. Then each extracts were filtered. The filtrate was subjected to evaporation under reduced pressure to obtain dries extract. The percentage yield of each dried root extracts was calculated [Table 1]. Each dried extract of *Clerodendrum vicosum vent* root was subjected to the qualitative chemical test for the identification of various bioactive constituents [3, 4]. Based on phytochemical evaluation (Table 2), a solid was obtained from ethyl acetate extract of *clerodendrum vicosum vent* root was subjected to column chromatography using silica gel (60-120 mesh) and column eluted successively with chloroform and methanol mixture (90, 80, 70…….10%). Each collected fraction was tested for the presences of flavonoids by folin- Ciocalteu reagent test.

High-Performance Thin-Layer Chromatography

Chromatography was performed on silica gel $60F_{254}$ (10 cm× 10 cm; 0.25 mm layer thickness; Merck). Ethyl acetate extract of *Clerodendrum vicosum vent* root (10mg/ml) and collected fractions residue (1mg/ml) was subjected to HPTLC (CAMAG, Switzerland) analysis. Extract and each fractions were spotted on a silica gel $60F_{254}$ (Merck, Darmstadt, Germany) TLC plate. The plate was air dried and then developed by using the solvent system chloroform: methanol (11.8:0.2 v/v) as mobile phase in a CAMAG- twin-trough glass chamber previously saturated with mobile phase vapor for 20 min. After developing the plate, it was dried at 105ºC for 15 min and then it was scanned using Scanner 3 (CAMAG, Switzerland) at 366 nm using WinCATS 4 software.

RESULTS AND DISCUSSION

The percentage yield of *Clerodendrum vicosum vent* root in various solvents were shown in table 1. Petroleum ether (60-80º C), ethyl acetate, chloroform and ethanol extract of *Clerodendrum vicosum vent* root were subjected to preliminary phytochemical analysis. Table 2 showed that presence of flavonoid in ethyl acetate extract and alkaloid, glycoside in ethanol extract.

Table 1: Percentage yield of *Clerodendrum vicosum vent* root in various solvents (%W/W)

Plant	Pet.Ether	$CHCl_3$	Ethyl acetate	Ethanol
Clerodendrum vicosum vent root	0.5	1.2	1.5	2.3

Table 2: Preliminary phytochemical analysis of *Clerodendrum vicosum vent* root

Test	Pet.Ether (60-80º C)	Chloroform	Ethanol	Ethyl acetate
Flavonoids	---	--	---	+++
Steroids	---	---	---	---
Alkaloids	---	---	+	---
Tannins	---	---	---	---
Glycosides	---	---	+	---

Negative (--), Positive (+)

Based on the preliminary phytochemical analysis, the ethyl acetate extract of *Clerodendrum vicosum vent* root was taken and flavonoid compound was determined by HPTLC technique. In column chromatography, the fractions obtained with 90% and 80% of chloroform in methanol mixture showed positive test with folin- Ciocalteu reagent. Figure 1 shows the HPTLC profiles of ethyl acetate extract of *Clerodendrum vicosum vent* root and collected fractions residue from column chromatography.

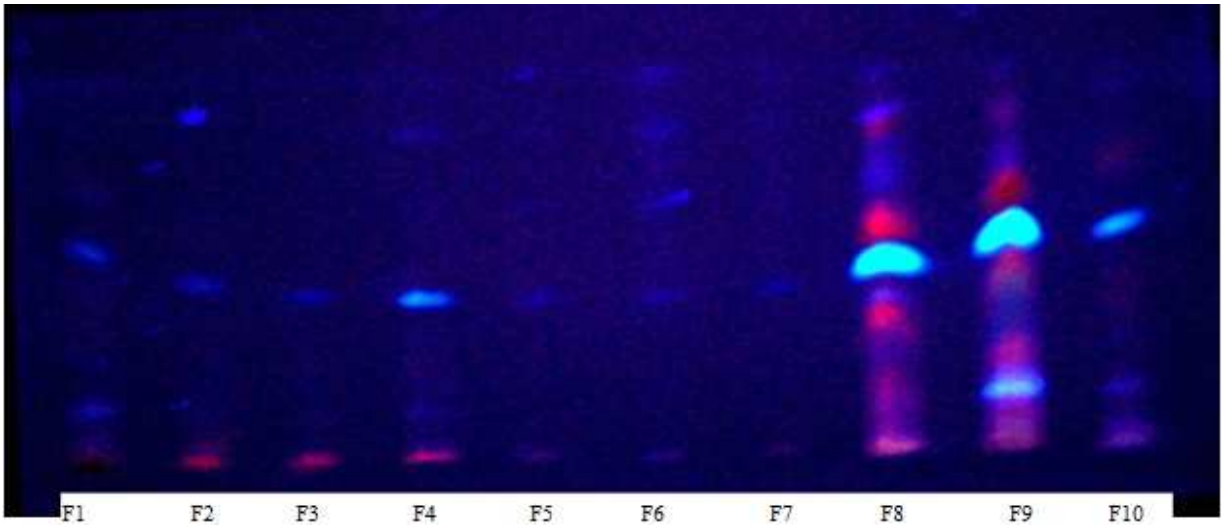

Figure 1: F1: Ethyl acetate extract of *Clerodendrum vicosum vent* root (Crude extract); F2: 90% chloroform in methanol column fraction; F3: 80% chloroform in methanol column fraction; F4: 70% chloroform in methanol column fraction; F5: 60% chloroform in methanol column fraction; F6: 50% chloroform in methanol column fraction; F7: 40% chloroform in methanol column fraction; F8: 30% chloroform in methanol column fraction; F9: 20% chloroform in methanol column fraction; F10: 10% chloroform in methanol column fraction.

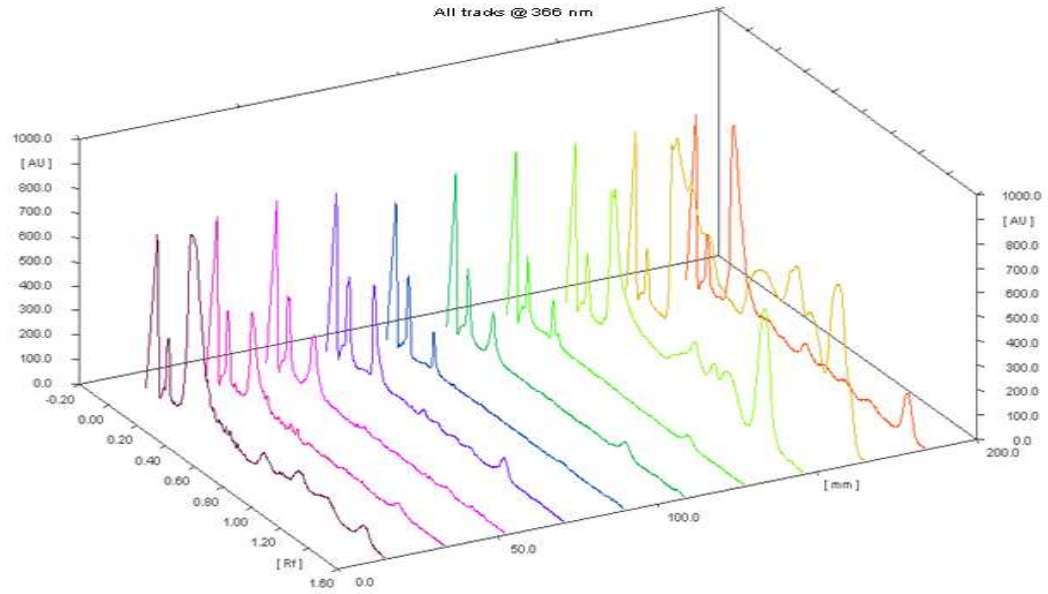

Figure 2: F1: Ethyl acetate extract of *Clerodendrum vicosum vent* root (Crude extract); F2: 90% chloroform in methanol column fraction; F3: 80% chloroform in methanol column fraction; F4: 70% chloroform in methanol column fraction; F5: 60% chloroform in methanol column fraction; F6: 50% chloroform in methanol column fraction; F7: 40% chloroform in methanol column fraction; F8: 30% chloroform in methanol column fraction; F9: 20% chloroform in methanol column fraction; F10: 10% chloroform in methanol column fraction.

In HPTLC echniques, the flavonoid from *Clerodendrum vicosum vent* root was determined by using solvent system chloroform: methanol (11.8:0.2 v/v) as mobile phase. The fractions obtained with 90%, and 80% of chloroform in methanol mixture showed a single band of flavonoid, which gave peak at R_f 0.25 for flavonoid (Figure 2). Thus HPTLC techniques could be considered as a accurate and precise method for the determination of flavonoid in *Clerodendrum vicosum vent* root samples.

CONCLUSION

The HPTLC technique expressed for the determination of flavonoid from *Clerodendrum vicosum vent* root is simple, precise and can be used for standardization of biological compounds in the plant extracts. This HPLC technique is highly adaptable, because of the precision and repeatability of compound analysis in plant extracts. Therefore, HPTLC technique can be used for determination of biological compounds in other plant materials. The structural characterizations (FTIR, ESI-MS, NMR studies) of isolated flavonoid from *Clerodendrum vicosum vent* root are in progress.

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