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Phytochemical characterization of *Plumeria acuminata* (L) using High Performance Thin Layer Chromatography (HPTLC)

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Abstract

Plumeria acuminata is a small laticiferous tree, a native of tropical America, which is commonly used in several traditional medicines to cure various diseases. The objective of this study was to develop high performance thin layer chromatography (HPTLC) finger print profile of flavonoid in the flowers of *P. acuminata* . Preliminary phytochemical results showed the presence of alkaloids, flavonoids, steroids, glycosides, reducing sugar, coumarins and triterpenoids. The results showed the presence of flavonoid, quercetin at 1.7264%w/w. The proposed HPTLC method provides a good resolution of quercetin from other constituents present in the ethanolic extract of dried flowers of *P. acuminata*. The method is rapid, simple and precise.

Keywords: *Plumeria acuminata*, HPTLC analysis, quercetin, aspirin.

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INTRODUCTION

Phytomedicines are great importance in the primary healthcare in many countries. According to World Health Organization (WHO) about 80% of the world's population depends on plant-based drugs. In traditional medicine, a large number of medicinal plants are used for the treatment of human diseases (Valsaraj *et al.*,1997). The plants occupied a unique place in human life it gives more information about the use of plants as medicine (Saikia, 2006). Plant-based medicines have paid great attention because it is easily available, less expensive and also have no side effects (Cathrine and Prabavathi, 2011). Plants synthesize a wide variety of phytochemical compounds as secondary metabolites. Many of the phytochemicals have been used effectively to treat the various ailments of mankind. Attempts have been made to identify all medicinal plants used globally and listed. Most of the medicinal plants are used as crude drug and they possess variety of medicinal properties (Mahesh and Sathish, 2008). Plants have a great potential for developing new drugs that could be used to treat infectious diseases (Panda *et al.*, 2009). In the recent years, there is an increasing awareness about the importance of medicinal plants, which needs thorough elucidation of chemical components and active principles of the plants using sophisticate and sensitive tools and techniques.

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High-performance Thin Layer Chromatography (HPTLC) is an efficient instrumentation technique, and the optimised quantitative densitometric evaluation can produce results analogous to those obtained with gas chromatography (GC) and high performance liquid chromatography (HPLC) (Wagner, 2001; Medic-Saric *et al.*, 2008). Thus, HPTLC fingerprint analysis may be an effective tool for the quality control of raw plant material and analyse the crude plant extracts. It is an important technique, over the conventional TLC, HPTLC and also an analytical technique whereby special plates and instrumental resources for sampling are used and the quantitative separations is aided by densitometry (Nile and Park, 2014).

Plumeria acuminata is used as medicinal plant native to Mexico, Central America, the Caribbean and south America (Tropical America), and spread throughout the tropics. They are commonly known as "Temple tree" or "Champa" in India. It is an evergreen or partly deciduous tree upto 6-7 meters height; stem smooth and shinning succulent with abundant white latex, The leaves are light green in colout, elliptical in shape and reaching a length of 40cm and a width of 7cm. The colour of the flowers varies from white to yellow and 5-6cm long. The flowers are bisexual, fragrant, the upper portion whitish, while the inner lower portion yellow and 5-6cm long. The fruits are inner oblong or ellipsoid follicles. They are brownish black in colour and seeds are oblong (Khare, 2007; Nandkarni, 1976). In the traditional medicine system different parts of the plant have been mentioned to be used against a variety of diseases such as diarrhoea and itches, and used as purgative. The milky juice is

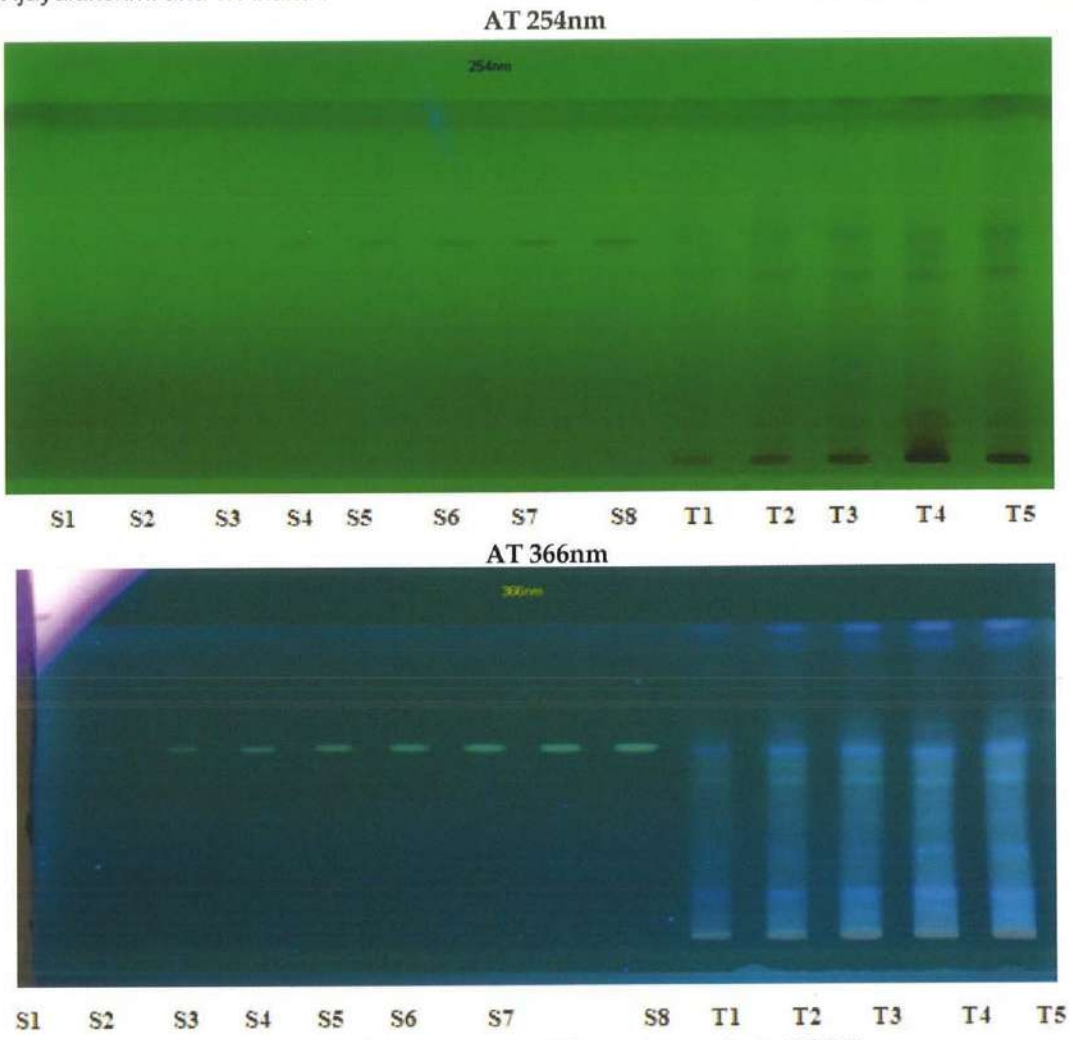

Fig 1. HPTLC finger printing of *Plumeria acuminata* AT 254nm

used in the treatment of inflammation and rheumatism. The bark has been applied as a plaster over inflammation and hard tumours. The leaves are reported to have anti-inflammatory, rubefacient in rheumatism and have strong purgative effect. Its branches are used like those of '*chitraka*' to produce abortion. In this context the present articles deals with the effectiveness of HPTLC over the conventional TLC and HPLC in developing fingerprint profile of the chemical constituents especially active principles present in *P.acuminata*.

MATERIALS AND METHODS

Collection of plant materials

The fresh flowers of *Plumeria acuminata* were collected from Mannargudi, Thiruvarur district, Tamilnadu, India. The collected materials were cleaned, shade dried and coarsely powdered and used for further studies.

Preparation of ethanol extract

The dried flowers were powdered using grinder, 100 gm of the powdered plant materials was subjected to extraction with 500ml of ethanol. After extraction, the solvent was drained off and the extracts were concentrated on water bath and kept in a dessicator The crude extract was used for further studies.

Qualitative analysis of phytochemical and screening

Ethanolic extract of the flowers of *Plumeria acuminata* were subjected to preliminary screening of phytochemical constituents. They were analyzed qualitatively by the standard method (Sofowora, 1993)

High Performance Thin Layer Chromatography (HPTLC)

Standard preparation

About 20 mg of quercetin standard was dissolved in 10 ml of ethanol. 1ml of the stock solution was diluted to 10 ml with ethanol. Spot of 1µl to 4.5µl containing the concentration in the range of 200ng-800ng was made from the solution.

Sample preparation

5g of dried powder was refluxed with 25ml of ethanol on a water bath for 25 minutes and consecutively three times and filtered to remove the solvent under reduced pressure. The ethanolic extraction in 100ml of water

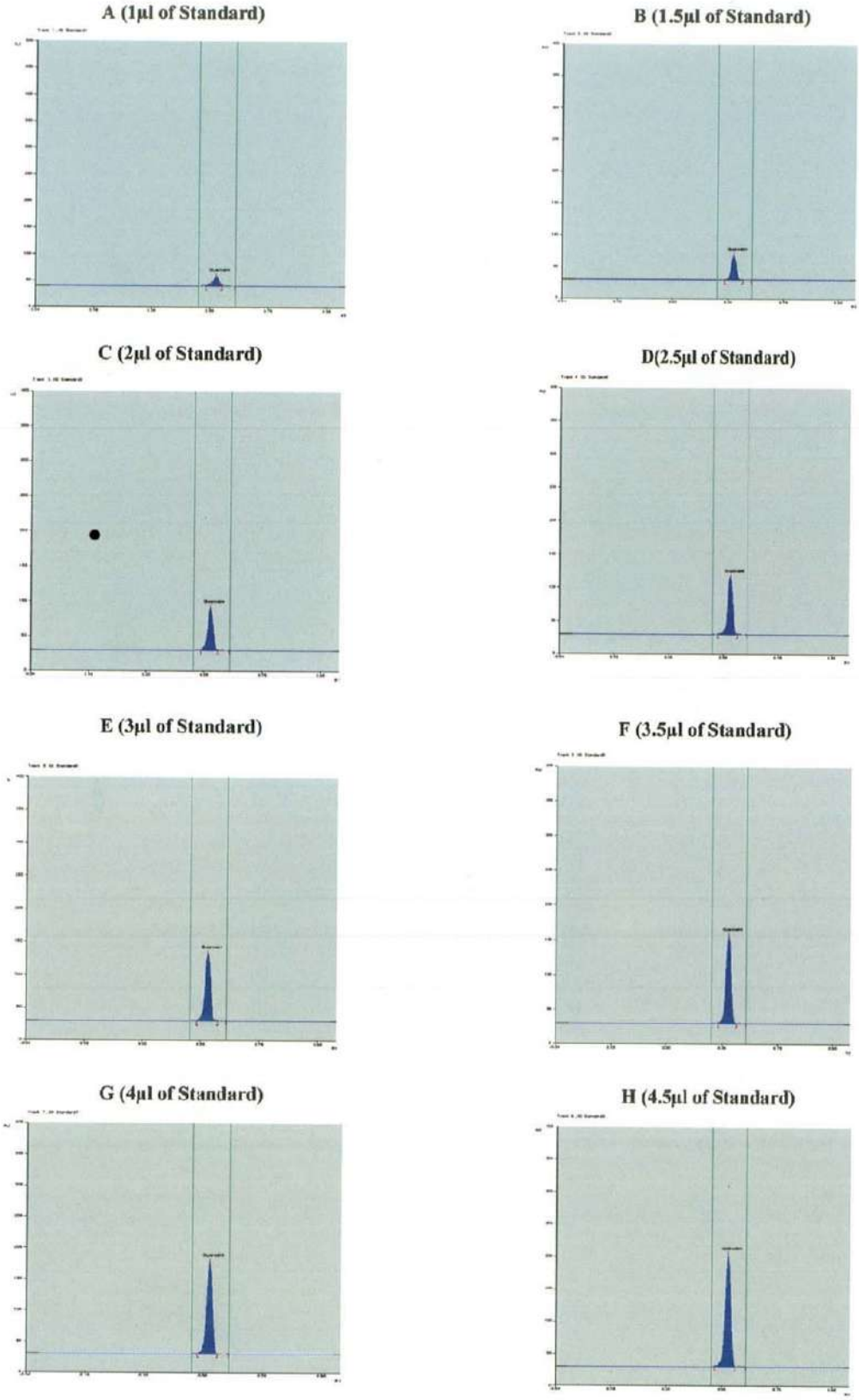

Fig. 2. (A-H) HPTLC chromatogram of Quercetin standard

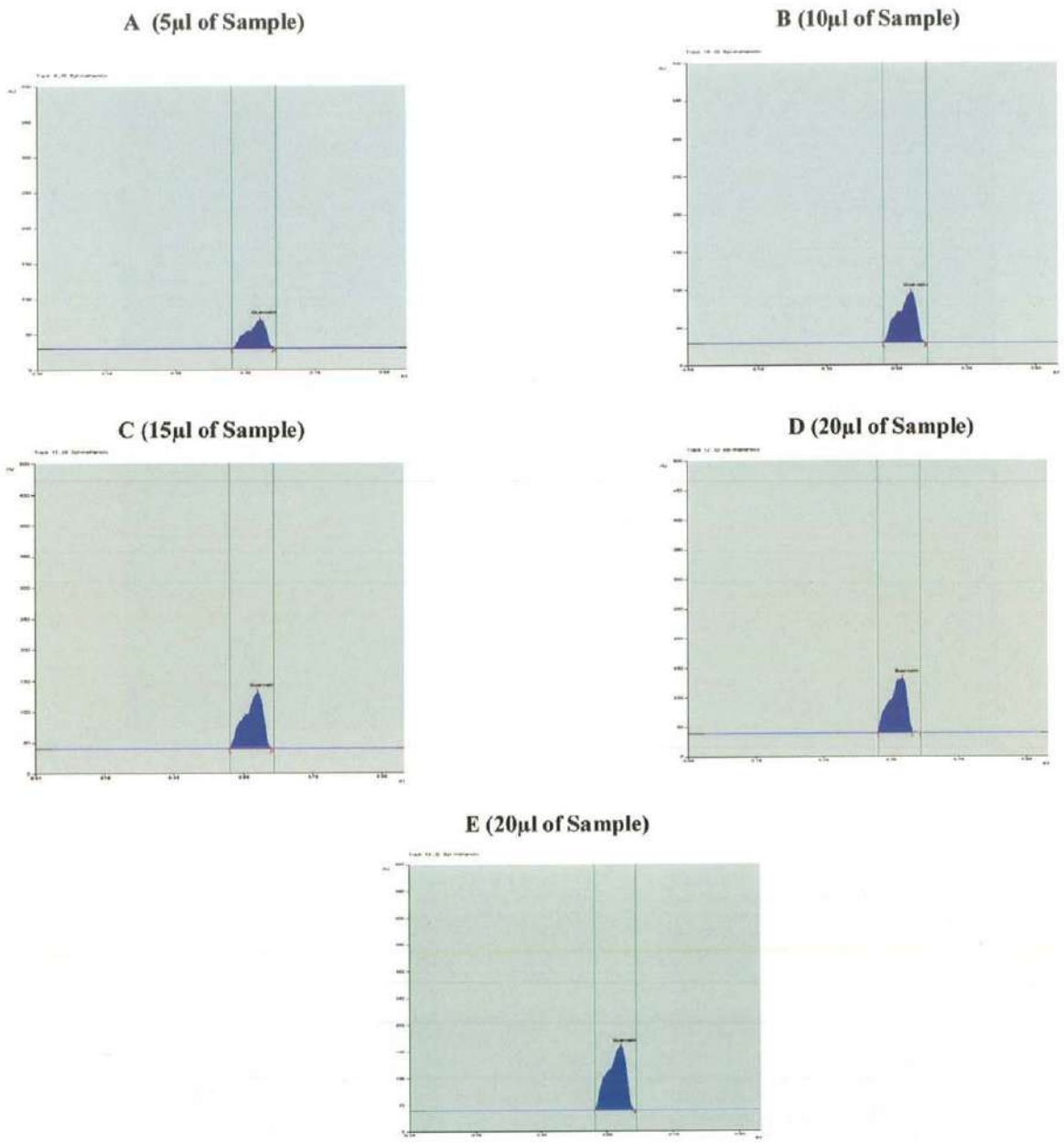

Fig. 3. (A-E) HPTLC chromatogram of flower extract of P.acuminata

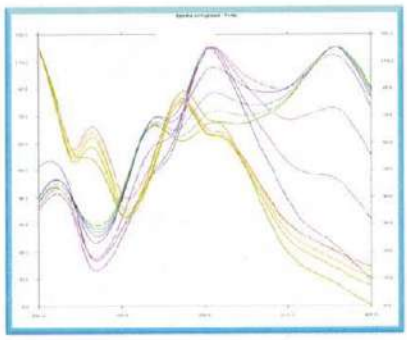

Fig. 4. Spectral comparison of purity of sample Tracks with standard at selected wavelength

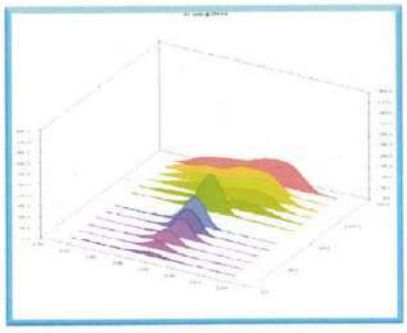

Fig. 5. HPTLC-3D display *Plumeria acuminata*

resulted in dark semi solid matter which was then fractionate with petroleum ether and chloroform successively. The chloroform fraction was concentrated to dryness. The dried residue was dissolved in ethanol for TLC analysis.

HPTLC was performed on silica gel 60 f_{254}, 20X10 cm HPTLC plates, Toluene : Ethyl Acetate : Formic acid (5:4:1v/v) as a mobile phase. The standard (Quercetin) solutions (1.0µl to 4.5µl) were applied over the plates with CAMAG- REPROSTAR. The plate was developed in the solvent system to a distance of 8cm. The plate was then scanned at 254nm and 366 nm using TLC scanner.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

The present study was carried out the plant *Plumeria acuminate* L. and the flower extract indicated the presence of secondary metabolites as summarized in the Table 1. The flower extract showed the presence of phytochemicals such as alkaloids, flavonoids, steroids, triterpenoids, coumarins, glycoside, and reducing sugar and absence of phenols, tannins and saponins.

High Performance Thin Layer Chromatography (HPTLC) analysis

Under chromatographic conditions, a densitometry HPTLC analysis was performed for the development of characteristic finger print profile for ethanolic flower extract of *P.acuminata* (Fig 1) which may be used as markers for quality evaluations and standardization of the drug. The densitometric quantification obtained for standard and sample exhibited the same Rf value as shown in Table 2. The chromatograms of standard quercetin are shown in Fig 2 (A-H) and that of quercetin in *P.acuminata* is shown in Fig 3 (A-E). Spectral comparison of purity for quercetin reference standard with quercetin in samples is shown in Fig 4. The 3D spectra of all tracks scanned at 366nm are shown in Fig 5. From the regression equation, y= -502.663x 3.38, a good linear relationship (r = 0.99988) with respective to height and peak area, respectively) was observed between the concentration ranges 200-800ng. The use of standard ensures the concentration and ratio of the test compound in the flowers. The concentration of the test sample was estimated to be about 1.7264% w/w.

Quercetin, a bioflavonoid, is a well known antioxidant which brings about the formation of considerably less reactive species from the highly reactive free radicals by its reactivity (Coskum, 2005). It is known to exert shielding effect on damaged â-cells in STZ induced diabetic rats (Oyaizu, 1986). The inhibition of aldose reductase enzyme by quercetin prevents glucose conversion to sorbitol which might be one of the ways of restoring the normal glycemic condition in diabetic rats. Occurrence of quercetin in the extracts obtained from different parts of the plant supplements the bioactivity of the plant in regulating the diverse factors responsible for ageing and cellular damages.

Quercitin in *Cassia auriculata* L. using HPTLC fingerprint profile has been reported (Jyothi*et al.*, 2013). It was first of its kind to report the HPTLC fingerprint of ethyl acetate and methanol extracts of *C. fistula* leaves showing maximum number of components 16 and 15 respectively at 400nm with solvent system of Toluene: Ethyl acetate: Formic acid in the ratio of 5:4:1. In the present HPTLCanalysis, it was found that ethyl acetate and methanol extracts contain a mixture of terpenoids, steroids and saponins. This densitometric HPTLC fingerprint profile may be used as marker for quality evaluation and standardization of the drug. Thus, HPTLC fingerprint profile along with their Rf values were recorded, which would serve as a reference standard for the scientists engaged in research on the medicinal properties of plant.

On the basis of HPTLC data, quercetin was identified in the ethanol extract of the flowers of *P.acuminata* (1.7264%w/w). It has been reported that, quercetin has lot of biological activities to prevent organs from various ailments. Studies on *P. acuminata* prove that it could be used as good pharmaceutical and therapeutic agent.

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