

HPTLC ANALYSIS OF AQUEOUS EXTRACT OF *Annona reticulata* LEAVESK.Mouliya^a and R.Venkatesh^b^a Assistant Professor, Department of Biochemistry, Sri Ramakrishna College of Arts & Science for Women, Coimbatore^b Assistant Professor, Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore.**ABSTRACT**

Plants are used as a major source of medicinal drugs, is considered to be effective in many diseases as well as environmentally friendly and inexpensive. Several active compounds have been identified in plant foods, including phenols, alkaloids, flavonoids and terpenoids which are widely distributed in the plant kingdom as secondary metabolites of photosynthesis. This study aims to investigate the HPTLC analysis of aqueous extract of *Annona reticulata* leaves.

Keywords: *Annona reticulata*, HPTLC analysis, Medicinal plants

INTRODUCTION

Currently, plants and plant-based treatments are encouraging choices for the treatment of life-threatening diseases (Al-Rimawi *et al.*, 2019). Medicinal plants are valuable natural sources and regarded as potential and safe drugs. *Annona reticulata* Linn., is a semi-evergreen and tiny deciduous tree belonging to the plant family *Annonaceae*. commonly known as Ramphal, Bullock's heart and Custard apple and it is native to India. It is a highly apparent plant in ayurvedic system of medicine for the treatment of various ailments include antioxidant, anticancer, analgesic and CNS depressant properties as well as antimalarial properties and effectiveness in syphilis treatment. It also has antifertility and abortifacient properties (Karmarkar *et al.*, 2021).

Annona reticulata is one of the most popular nutritional and medicinal plant which is rich in variety of secondary metabolites including polyphenols. Various phytochemicals present include glycosides, phytosterols, proteins, carbohydrates, saponins, tannins, alkaloids, phenols, flavonoids, peptides and acetogenins. These compounds contribute to numerous bioactivities including antidiabetic, hepatoprotective, antimicrobial, and lipid-lowering effects. Different parts like leaves, roots, seeds, fruits and bark of this plant are used for various medicinal purposes. *Annona reticulata* leaves are potential cost-effective ingredients

for nutraceutical, medicinal and food applications. The phytochemical and pharmacological activities of the plant make them an indispensable and essential component for natural medicine, immunity boosters and health-promoting ingredients (Jamkhande *et al.*, 2016).

MATERIALS AND METHODS

COLLECTION AND AUTHENTICATION OF PLANT

The whole plant of *Annona reticulata* Linn. leaves belonging to the plant family *Annonaceae* was collected from Coimbatore District, Tamil Nādu, India. The plant sample was identified and authenticated by Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore.

PREPARATION OF THE PLANT EXTRACT

The leaves were washed, shade dried and pulverized into powder. About 20 gm of the powdered sample was weighed and extracted with 200 ml of aqueous using Soxhlet apparatus separately and the extract was filtered through Whatman No.1 filter paper. The filtrate was evaporated under reduced pressure and restricted temperature in a rotary evaporator. The concentrated extract of the plant using aqueous was stored at -20°C until used for further studies.

HPTLC FINGERPRINTING ANALYSIS OF AQUEOUS EXTRACT OF *Annona reticulata* LEAVES (Reich and Schibili, 2007)

Descriptive analysis of bioactive compounds includes phenols and terpenoids were carried out using High performance thin layer chromatography.

Principle

HPTLC is an augmented form of thin layer chromatography. Solute and solvent molecule compete for its sites on adsorbent of the plate, the solute molecule displaces solvent molecule. The molecules with polar functional groups and molecules with hydrogen bonding capability will have a strong affinity towards adsorbent surface.

Procedure

Preparation of test solution

Weighed 25 mg of aqueous leaf extract was dissolved in 250 µl of methanol and centrifuged at 3000 rpm for 5 minutes. The supernatant was used as a sample for HPTLC analysis.

Application of sample

3 µl of the test solution was loaded as 5mm band length in the 3x10 silica gel 60F254

TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

Spot development

The plates with loaded samples were kept in TLC twin trough developing chamber (after saturated with solvent vapor) with respective mobile phase (phenols and terpenoids) and the plate was developed in the respective mobile phase up to 90 mm.

Photo-documentation of chromatographic plates

Hot air was applied on the developed plate to evaporate solvents from the plate. The plate was placed in photo-documentation chamber (CAMAG REPROSTAR 3) and the images were captured in visible light- UV 254 nm and UV 366 nm.

Derivatization

The spray reagent was sprayed on the developed plates (phenols and terpenoids) and dried at 100°C in hot air oven. The plate was photo-documented in visible light and UV at 366 nm mode using photo-documentation (CAMAG REPROSTAR 3) chamber.

Peak analysis

Before derivatization, the plate was fixed on scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 254 nm. The peak display, peak table and peak densitogram were marked. Win CATS 1.3.4 version software was used for analysis.

Standards used for analysis

- Phenols: Quercetin
- Terpenoids: Lupeol

Analysis details for Phenols

- Mobile phase: Chloroform: Formic acid: Ethyl acetate (5:1:4)
- Spray reagent: Sodium carbonate solution and Folin- Ciocalteu reagent

Analysis details for terpenoids

- Mobile phase: n-Butanol: Isopropyl alcohol (5:5)
- Spray reagent: Anisaldehyde sulphuric acid reagent

RESULTS & DISCUSSION

HPTLC FINGERPRINTING ANALYSIS OF AQUEOUS EXTRACT OF *Annona reticulata* LEAVES

High-performance thin-layer chromatography (HPTLC) is a versatile and flexible technique for the analysis of secondary metabolites present in the medicinal plants. Standardization of plant materials was essential for their identification and assessment of quality in herbal medicines. HPTLC fingerprinting analysis represents as an important

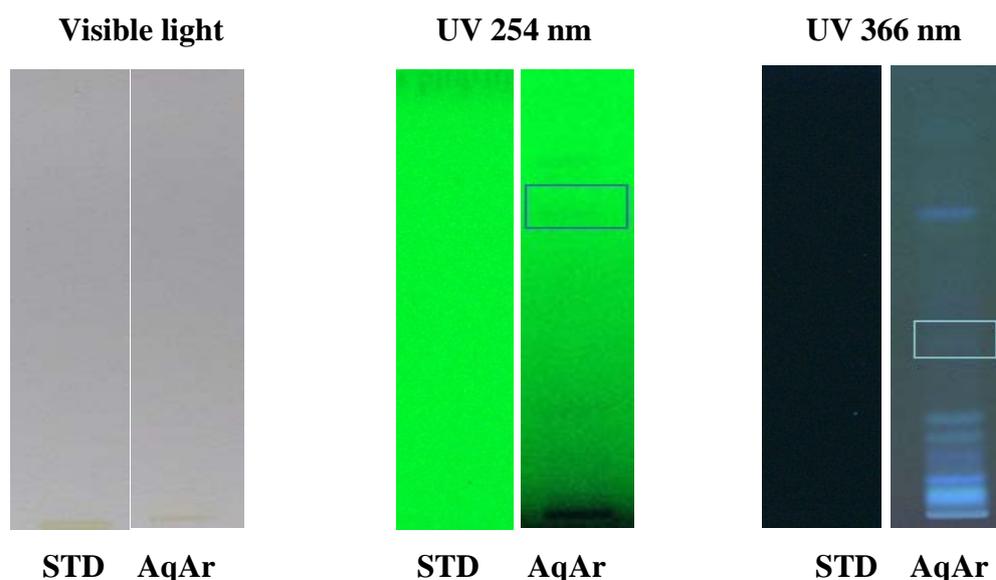
analytical tool for, qualitative, quantitative and semiquantitative estimation of phytochemicals. HPTLC fingerprint analysis plays a crucial role in ensuring the quality of medicinal plants and the products that are made from them since it is straightforward and reliable and the results can be readily checked visually. Among the wide variety of bioactive compounds, polysaccharides, alkaloids, flavonoids, saponins, and phenols are particularly significant. There are various techniques for separating plant components, but the chromatographic approach is the most popular amongst other techniques because to its simplicity and reliability (Singh *et al.*, 2021).

1. HPTLC FINGERPRINTING ANALYSIS OF PHENOLS OF *Annona reticulata* LEAVES

Phenolic compounds are considered as secondary metabolites widely distributed in various medicinal plants which is composed of two important amino acids include phenylalanine and tyrosine. This compound falls under several categories and the major class among these are the flavonoids which have potent antioxidant activities (Bora *et al.*, 2019).

Plate 1, Table 1 and Figure 1 showed the phenols of aqueous extract of *Annona reticulata* leaves. The quercetin was used as a standard which developed with the Rf value 0.72.

Plate 1. HPTLC chromatogram of phenols of aqueous extract of *Annona reticulata* leaves



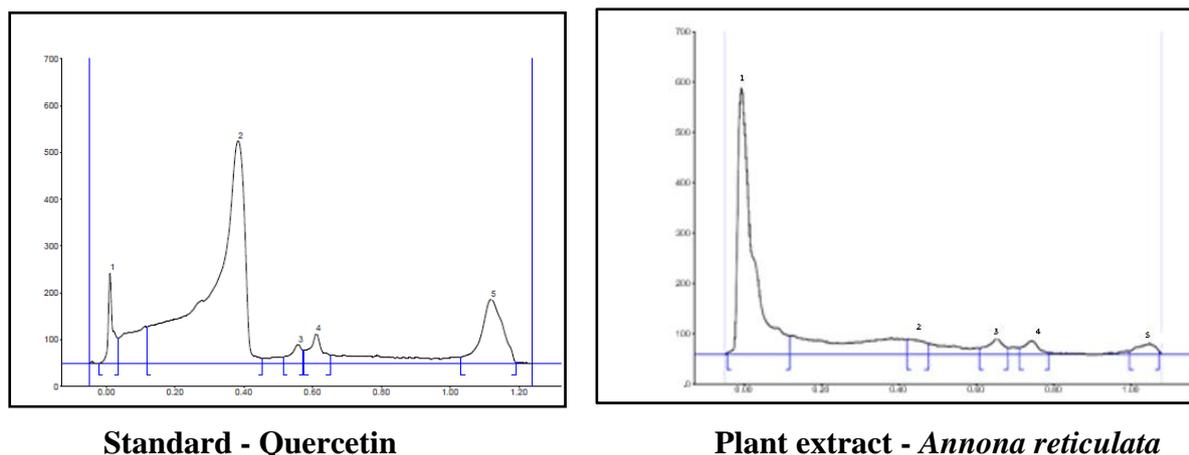


Figure 1. Peak densitogram display of phenols of aqueous extract of *Annona reticulata* leaves (Scanned at 254 nm)

Table 1. Effect of *Annona reticulata* leaves on HPTLC analysis for Phenols

Track	Peak	Rf value	Height	Area	Assigned substance
Standard	1	0.72	303.1	9304.9	Quercetin
Sample	1	0.00	527.2	14520.4	Phenolic compound 1
Sample	2	0.43	30.8	953.7	Phenolic compound 2
Sample	3	0.65	31.5	934.1	Unknown
Sample	4	0.75	26.6	740.0	Phenolic compound 3
Sample	5	1.05	21.5	735.9	Phenolic compound 4

The Rf value of the different compounds present in the plant extract was found to be 0.00, 0.43, 0.65, 0.75 and 1.05 of peak 1,2,3,4 and 5 whereas the Rf value of standard Quercetin was found to be 0.72 at peak 1. Comparing Rf values and peak regions of both leaf extract and standard showed the presence of four phenolic compounds at the peak region 1,2,4 and 5 in the aqueous extract of *Annona reticulata* leaves.

Phenolic compounds present in the plants are reported to be the major phytochemicals responsible for antioxidant activity. The extracts also contained unknown compounds as evident from the HPTLC data whose characterization would serve to further evaluate the beneficial properties of this plant.

Polyphenolic compounds regulate various biochemical and physiological processes,

namely enzymatic activity, cell proliferation, signal transduction pathways and cellular redox potential to fight against chronic pathologies (Kumar *et al.*, 2021).

The results of the present study directly coincided with the previous observations of Narayanan *et al.*, (2016) who reported the maximum number of phenolics has been observed in the ethanolic extract of *C.crinita*. The phenolic band with Rf value 0.75 confirmed the presence of quercetin.

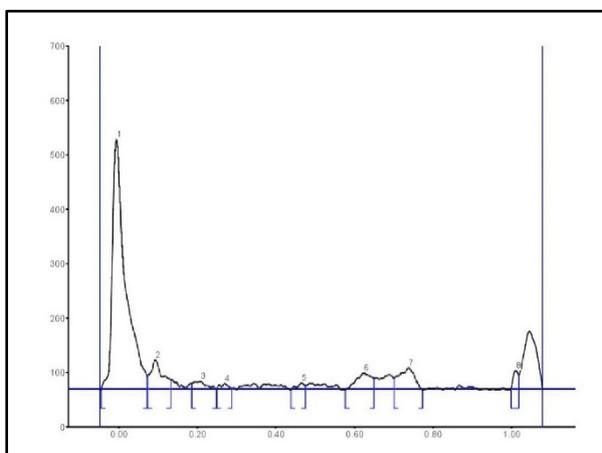
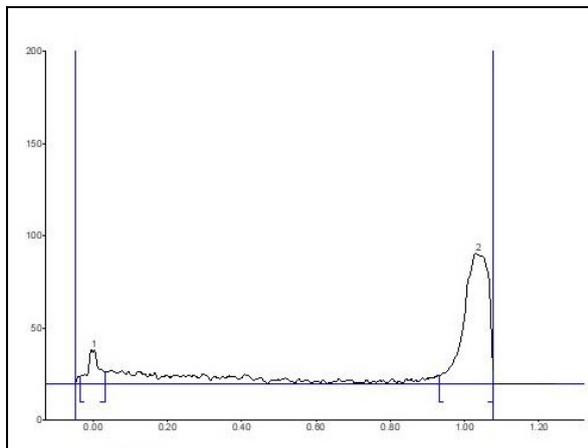
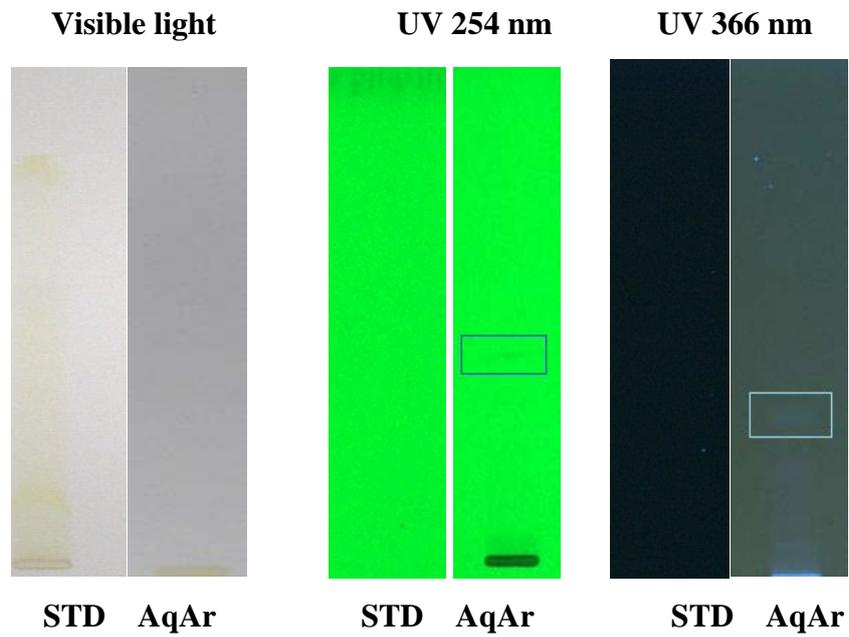
The finding of Goswami *et al.*, (2019) also affirmed the presence of quercetin along with other phenolic compounds from ethanolic extract of *Schleichera oleosa (Lour) Oken*.

2. HPTLC FINGERPRINTING ANALYSIS OF TERPENOIDS OF *Annona reticulata* LEAVES

Terpenoids are secondary metabolites with molecular structures containing carbon which contains isoprene units and they constitute one of the largest families of natural products. They also possess antimicrobial, anti-inflammatory, gastroprotective and hepatoprotective properties. Besides the wide range of biological properties, these natural compounds have significant commercial value as pharmaceuticals, flavors and fragrances, commodity chemicals and more recently as potential biofuels (Senguttavan *et al.*, 2016).

The results of HPTLC analysis of terpenoids of *Annona reticulata* leaves were depicted in Plate 2, Figure 2 and Table 2. The lupeol was used as a standard which developed with the Rf value 1.08.

Plate 2. HPTLC chromatogram of terpenoids of aqueous extract of *Annona reticulata* leaves



Standard - Lupeol

Plant extract - *Annona reticulata*

Figure 2. Peak densitogram display of terpenoids of aqueous extract of *Annona reticulata* leaves (Scanned at 254 nm)

Table 2. Effect of *Annona reticulata* leaves on HPTLC analysis for Terpenoids

Track	Peak	Rf value	Height	Area	Assigned substance
Standard	1	1.08	6.6	3368.1	Lupeol
Sample	1	0.01	459.4	11034.9	Terpenoids 1
Sample	2	0.09	54.2	1159.9	Unknown
Sample	3	0.21	14.5	360.1	Unknown
Sample	4	0.27	10.5	152.2	Unknown
Sample	5	0.47	11.5	153.9	Unknown
Sample	6	0.62	29.6	829.8	Unknown
Sample	7	0.74	39.4	1113.9	Unknown
Sample	8	1.01	33.8	316.4	Terpenoids 2

The Rf value of the different compounds present in the plant extract was found to be 0.01, 0.09, 0.21, 0.27, 0.47, 0.62, 0.74 and 1.01 of peak 1,2,3,4, 5,6,7 and 8 whereas the Rf value of standard Lupeol was found to be 1.08 at peak 1. Comparing Rf values and peak regions of both leaf extract and standard showed the presence of two terpenoid compounds at the peak region 1 and 8 in the aqueous extract of *Annona reticulata* leaves.

Terpenoids have been identified to relax cardiovascular smooth muscle by inhibiting calcium influx in vascular smooth muscle or by stimulation of nitric oxide synthesis and quenching of reactive oxygen species (Olivia *et al.*, 2021). The extracts also contained unknown compounds as evident from the HPTLC data whose characterization would serve to further evaluate the beneficial properties of this plant.

Ruddaraju *et al.*, (2019) reported that the HPTLC analysis reveals the diverse

phytochemicals present in *Annona squamosa* leaf extract. The result is also in accordance with earlier report of Rajamani *et al.*, (2018) who confirmed that the ethanolic extract of South Indian orthodox black tea contained several potential active components such as terpenoids.

CONCLUSION

The results obtained from HPTLC analysis showed the presence of phenols and terpenoids in aqueous extract of *Annona reticulata* leaves and it may be helpful to identify the secondary metabolites present in the plant extract and ensure its therapeutic potential.

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