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DETECTION OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF *MANGIFERA INDICA* USING TLC BIO-AUTOGRAPHY

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ABSTRACT: *Mangifera indica* is commonly found in India, which contains exalted medicinal values. TLC bioautography help us to identify the bioactivity of separated molecules in less time. In this study, leaves of *Mangifera indica* was subjected for various solvent extraction, followed with phytochemical screening and TLC bioautography for antioxidant and antibacterial activity. Column fractionation was done for all the extracts and subjected for spot assay. The plant chosen for this study was found to have antioxidant and antibacterial activity.


INTRODUCTION: Although modern medicine is being predominantly used in recent times, herbal based natural medicine always maintains its eminence in curing diseases^{1, 2}. Plants are known to possess phytochemicals which exhibit bioactivity and commonly mentioned as bioactive compounds³. Natural derived foods or products are generally free from radiations, chemical exposure, toxic and harmful synthetics etc⁴ and are thus safe, economic and highly reliable. These above characteristics pull the researchers towards it for drug discovery and development⁵⁻⁹. Screening for antioxidants, antibacterials and phytochemicals can be performed using Thin layer chromatography (TLC), the assay is finest, rapid, expedience, uncomplicated and efficient method for screening¹⁰⁻¹⁵.

Mangifera indica grows upto 10-45m with dense foliage, typically heavy branched with stout trunk. The fruit is well known large drupe, but shows a great variation in shape and size. *Mangifera indica* has been proven to have the following properties - analgesic, anti-inflammatory, antioxidant, immunomodulatory, hepatoprotective, laxative, diuretic, diaphoretic, astringent, refrigerant¹⁶⁻²¹. *Mangifera indica* is an evergreen tree in the anacardiaceae family. As this plant is commonly available plant and having high enriched property, the plant has been utilised in this study to screen for its antioxidant and antibacterial activity through TLC guided bioautography.

MATERIALS AND METHODS:

Collection and extraction of plant materials:

Fresh leaves free from insect infestation and infection were collected from Sathyabama University, Chennai, Tamil Nadu, India. The collected leaves were washed in tap water followed by distilled water and shade-dried. The dried samples were powdered using a blender. A known weight of the powder was kept immersed in

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Acetone, Chloroform, Ethanol, Petroleum ether in the ratio of 1:10 (ie.10g of leaf powder in 100ml of solvent) for 24h. The supernatant was filtered using Whatmann filter paper. The collected filtrate is kept for evaporation of solvent. Then extract is dried. Thus, obtained extract was stored in air tight tubes at 4°C till use^{22, 23}.

Qualitative phytochemical analysis: Qualitative phytochemical analysis for Carbohydrates, Amino acid, Proteins, Phenols, Sterols and Steroids Glycosides, Saponins/Saponin, Glycoside, Quinones/Anthra Quinones, Alkaloids, Flavanoids, Leucoanthocyanins, Anthocyanins, Volatile oils, Lignin, Terpenoids were performed²⁴⁻²⁶.

Thin Layer Chromatography: TLC silica (Merck, F245) plates were used to perform Thin Layer Chromatography. Chloroform: Methanol in the ratio of 9:1 was used as mobile phase²⁷. After running the samples, TLC plate was exposed to iodine to identify R_f value.

TLC bio-autography for antioxidant activity: Chromatographed extracts were sprayed with 0.004% DPPH in 95% methanol to identify the compounds with radical scavenging activity and observed under visible light, yellow to pink colour in purple background confirmed the antioxidant molecule²⁸⁻³³. The R_f value of the samples were calculated.

TLC bio-autography for antibacterial activity: The prepared chromatograms were air dried and sprayed with 24h culture of *Pseudomonas aeruginosa*, this was followed with incubation for 37 °C for 24h with 100% relative humidity in the dark. After incubation, chromatograms were sprayed with 0.001% MTT in distilled water. Plates were observed for zone of inhibition due to the presence of compounds that inhibited the growth of tested organisms. The R_f value of the samples were calculated and recorded³⁴⁻³⁸.

Column Fractionation and dot blot assay: The crude extract of plant was run in a column chromatography. The column was packed with silica gel in a glass, the mobile phase used here was ethylacetate. The sample was added to the column and ten fractions were collected. All ten fractions were subjected for spot assay for antioxidant as

well as antibacterial activity following the method as prescribed earlier³¹⁻³⁸.

RESULTS AND DISCUSSION: Crude *Mangifera indica* extracts was done using solvent like Acetone, Ethanol, Chloroform, Petroleum ether with variable polarity. These extracts were then subjected to various screening procedures like Phytochemical/secondary metabolite screening, antioxidant activity and antibacterial activity. Most predominantly found phytochemicals were Carbohydrates, phenols quinones/anthraquinones, alkaloids, flavanoids and saponins. Amongst the various solvent extracts, ethanol and petroleum ether extract showed the presence of most of the secondary metabolites (**Table 1**).

TABLE 1: PHYTOCHEMICAL SCREENING

S.no	Phytochemical	AE	CE	EE	PE
1	Carbohydrates	+	+	+	+
2	Amino acids	-	+	+	-
3	Phenols	+	-	+	+
4	Sterols and Steroids	+	-	-	-
5	Glycosides	+	-	-	-
6	Saponins/Saponin Glycoside	+	-	+	-
7	Quinones/AnthraQuinones	-	+	+	-
8	Alkaloids	+	+	+	-
9	Flavanoids	-	+	+	+
10	Leucoanthocyanins	-	+	-	+
11	Anthocyanins	-	+	-	+
12	Volatile oils	-	-	+	+
13	Lignin	-	-	-	+
14	Terpenoids	+	-	-	+

AE- Acetone Extract, CE- Chloroform Extract, EE- Ethanol Extract, PE- Petroleum ether Extract

Joonal et al³⁹ also found the plant to possess the phytoconstituents like flavonoids, terpenoids, saponins, tannins and glycosides. Ediriweera et al⁴⁰ found steroids, flavonoids, phenolic compounds, tannins and reducing sugars, but not saponins in *Mangifera zeylanica*. When all the extracts were subjected for TLC and exposed to iodine it revealed the presence of more components of *Mangifera indica* (**Fig. 1**). The R_f value of all the extracts are tabulated in **Table 2**. Ethanol extract was not having any antioxidant molecule with it. Component with R_f value near 0.82 was observed in all the other extracts (**Fig. 2**) which may be due to flavonoids and terpenoids present in extract which has been already evidenced in phytochemical analysis. Joonal et al³⁹ found methanolic leaf extract of *Mangifera indica* to exhibit significant antioxidant and anticancer activity because of flavonoids and terpenoids level.

Dot blot assay revealed acetone and chloroform extract to have antioxidant activity whereas the fractions of other extract did not show any antioxidant activity (Fig. 3). Bhusari et al⁴¹ isolated two pure compounds from leaves of *M. indica* by DPPH scavenging guided fractionation strategy. One among that was further confirmed as mangiferin by UV, IR, 1H NMR, ESI-MS and HPLC studies. The compound with R_f value around 0.98 was showing antibacterial activity in acetone, chloroform and petroleum ether extract

(Fig. 4), when it was subjected for fractionation and dot blot assay only the fraction of acetone and chloroform was showing activity at 8th and 10th fraction respectively (Fig. 5). Biswas et al⁴² reported antibacterial effect of mangiferin isolated from *Mangifera indica*. Awad El-Gied⁴³ found methanol and ethanol extracts of mango bulb to have antibacterial activity most bacteria especially against *Mycobacterium smegmatis*. Vaghasiya et al⁴⁴ also found methanol extract of *M. indica* seeds to have potent antibacterial activity.

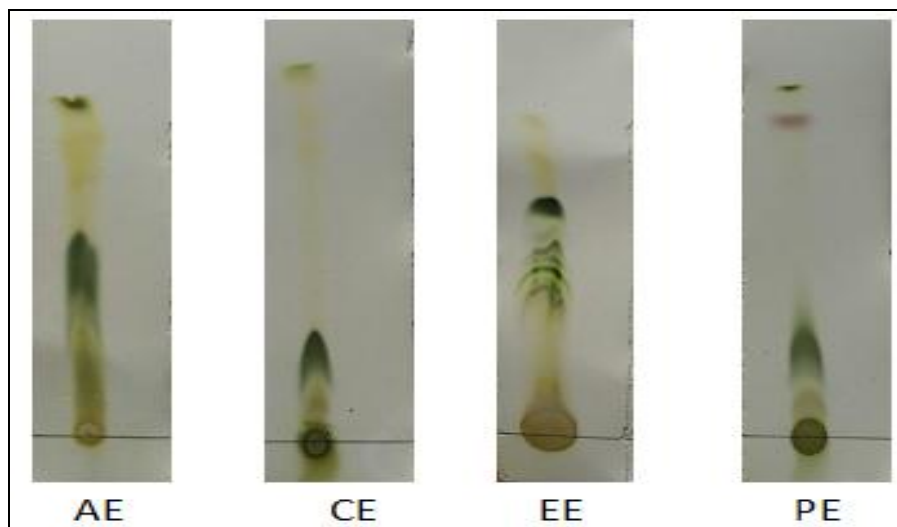


FIG.1 TLC OF MANGIFERA INDICA

AE- Acetone Extract, CE- Chloroform Extract, EE- Ethanol Extract, PE- Petroleum ether Extract

TABLE 2: R_f VALUE OF COMPOUNDS SEPARATED

AE	CE	EE	PE
0.07	0.05	0.09	0.04
0.20	0.58	0.16	0.08
0.27	0.78	0.20	0.14
0.98	0.99	0.25	0.22
		0.98	0.97

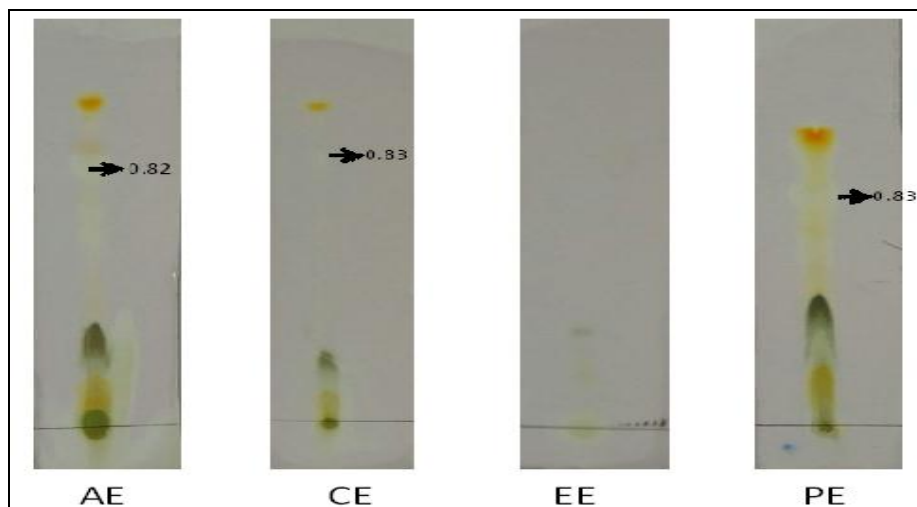


FIG. 2: TLC BIOAUTOGRAPHY FOR ANTIOXIDANT ACTIVITY IN MANGIFERA INDICA

AE- Acetone Extract, CE- Chloroform Extract, EE- Ethanol Extract, PE- Petroleum ether Extract

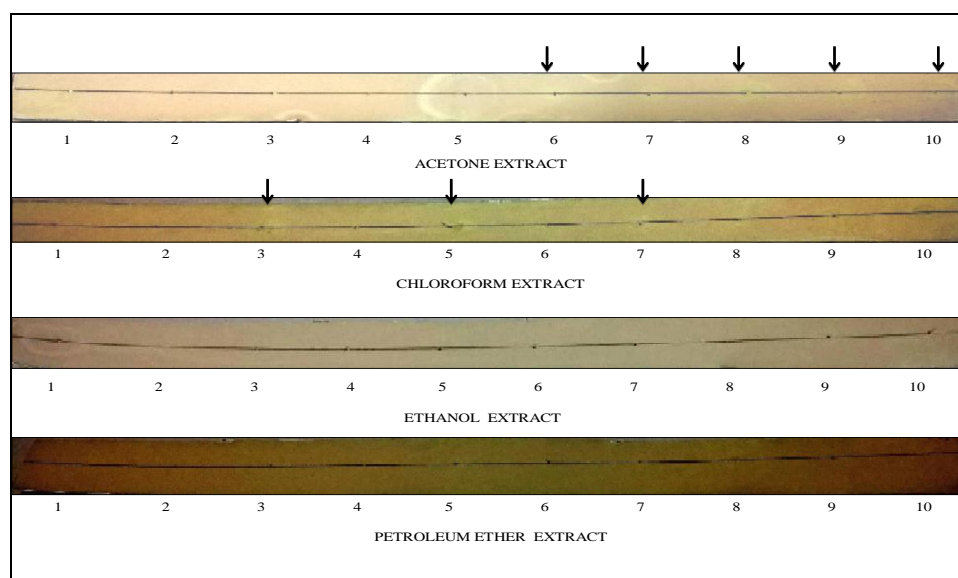


FIG. 3: TLC DOT BLOT ASSAY OF FRACTIONATED COLUMNS FOR ANTIOXIDANT ACTIVITY IN *MANGIFERA INDICA*

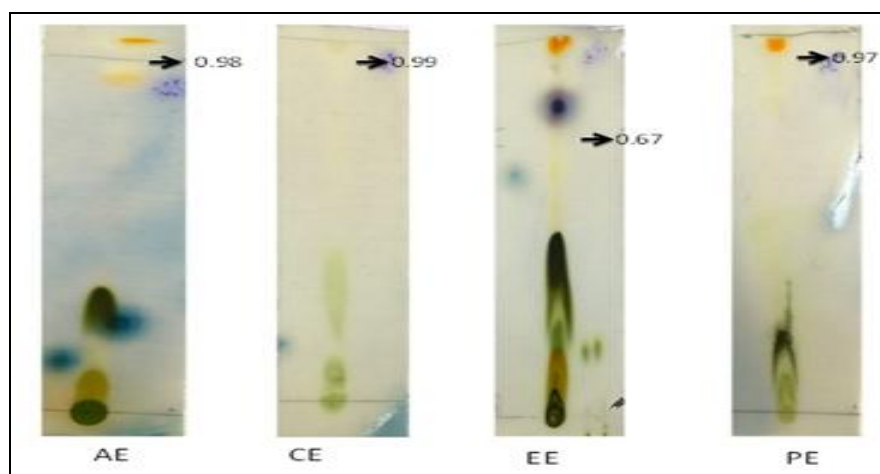


FIG. 4: TLC BIOAUTOGRAPHY FOR ANTIBACTERIAL ACTIVITY OF *MANGIFERA INDICA*
 AE- Acetone Extract, CE- Chloroform Extract, EE- Ethanol Extract, PE- Petroleum ether Extract

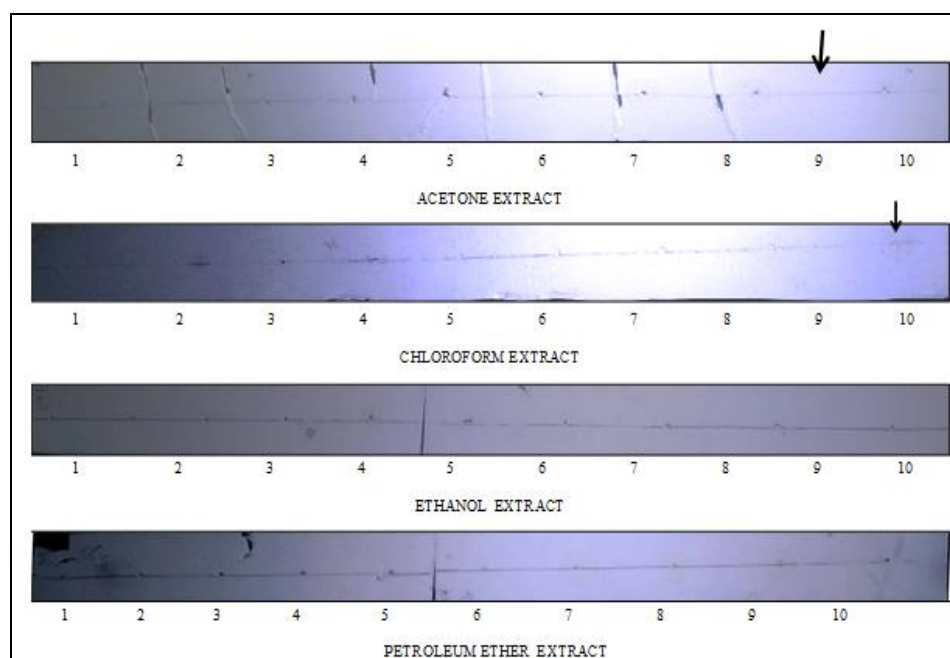


FIG.5: TLC DOT BLOT ASSAY OF FRACTIONATED COLUMNS FOR ANTIBACTERIAL ACTIVITY IN *MANGIFERA INDICA*

CONCLUSION: In this study, *Mangifera indica* was collected from Chennai. The plant is proved to have both antibacterial and antioxidant activity which has been confirmed through TLC guided identification. Even the fractions of all solvent extracts were confirmed with antioxidant and antibacterial activity. Further analysis required for the identification molecules responsible for the above bioactivities.

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