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TLC Bio-autography guided identification of antioxidant and antibacterial activity of *Acacia senegal*

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ABSTRACT

Using plants for treating diseases is followed since ages. Phytochemicals present in the plant have been proven to show various bioactivities like antibacterial, antifungal, antimicrobial, anticancer, antioxidant activities etc. In this study, Acacia Senegal was collected from Chennai, Tamil Nadu, India. The crude extracts were obtained using various solvents like ethanol, chloroform and petroleum ether. The obtained extracts were exploited for phytochemical analysis, and TLC bio autography analysis for antioxidants and antibacterial activity. The extracts which showed activity were fractionized using column chromatography and subjected for spot assay for antioxidant activity and antibacterial activity.

Key words: Plant extract, Phytochemical Screening, antioxidant, Bio autography, Spot assay

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INTRODUCTION

Ayurveda treatments involve the use of plants as a sole source of medicine [1]. There are a few or more components present in plants help in achieving this, commonly known as bioactive compounds or phytochemicals [2]. It is true that anything derived out of natural are compatible, less harmful, economically viable, safe and dependable, these characteristics of plants kindle the interest of many of researchers and scientists into the discovery of drugs from these sources[3]. Plants are known to show antibacterial, anticancer, protective activity[4-8]. There are more assays are available to screen potential antioxidants, Thin layer chromatography (TLC) bioautography assay is the best quick, convenience, simple and efficient method among them, where the TLC plates are sprayed 2,2-diphenyl-1-picrylhydrazyl (DPPH) as derivatizing agent and looked for yellow or pink colored spots[9-15].

Acacia sp has been proven to have been used to treat various diseases like malaria, sore throat (aerial part), viral diseases, toothache etc[16-20]. *Acacia senegal* also known as the Khor or Hashah, a small thorny tree found in the suburbs. This tree was most profoundly used for its gums, which got demulcent and enmollient properties. The plant was used externally to cover inflamed surfaces and used for diabetes treatment[21]. This study was aimed to find out the antibacterial and antioxidant activity of *Acacia senegal* by TLC guided bioautography.

MATERIALS AND METHODS

The leaves of *Acacia Senegal* were collected from Chennai, Tamil Nadu, India. Shade dried leaves were ground mechanically using a mixer grinder, obtained powder was added with different polar solvents like Acetone, Chloroform, Ethanol, Petroleum ether in the ratio of 1:10 (ie.10g of leaf powder in 100ml of solvent) in a 250ml

conical flask and kept in the rotatory shaker at 60 rpm for 24h. Samples were filtered dried, weighed and stored in refrigerator at 4^0C till use. The dried samples were with appropriate solvent (1mg/ml) before study [22,23].

Qualitative phytochemical analysis
Qualitative phytochemical analysis for flavonoids, phenols, saponins, Cholesterol, Cardiac glycosides etc were done [24-26].

Thin Layer Chromatography
TLC was carried out on a TLC silica plate (Merck, F245) having Chloroform as mobile phase. Bands were visualized by exposing to iodine saturated chamber.

TLC DPPH bio-autography for antioxidant activity
The extracts were chromatographed using TLC silica plate (Merck, F245) having chloroform as mobile phase. Developed plates were dried, sprayed with DPPH (0.004% w/v in 95% methanol) and observed for development of bright yellow to pink colour for confirmation as antioxidant molecule [27]. The Rf value of the samples were calculated.

TLC bio-Autography for antibacterial activity
The extracts were run on TLC plate and were air dried. 24h culture of *Pseudomonas aeruginosa* was sprayed over the TLC plate, which was followed with incubation for 37˚C for 24h.The plates were then sprayed with MTT reagent (0.001% w/v in Distilled water) and the plates were observed for zone of inhibition around the separated molecules. The Rf value of the samples were calculated and recorded [28].

Column Fractionation and spot assay
Silica gel was mixed with 10ml of Ethylacetate and made into slurry. The gel was packed in a glass column. 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 earlier[27-32].

RESULTS AND DISCUSSION

Different polar solvents tend to extract different components hence various solvents like Acetone, Ethanol, Chloroform, Petroleum ether were used to prepare the extracts of *Acacia senegal*. The obtained extracts were screened qualitatively for secondary metabolites. Most predominantly found phytochemicals were glycosides, alkaloids and flavonoids. Amongst the various solvents, ethanol extract showed the presence of most of the secondary metabolites (Table 1). Several polyphenolic compounds have been reported in the bark of *Acacia nilotica* viz. catechin, epicatechin, quercetin, gallic acid and leucocynidin gallate [33,34]. Presence of tannins, steroids, cardiac glycosides, flavonosides, saponins and alkaloids in *A.senegal* were reported by Mudi and Salisu [35], whereas they did not find flavonoids, anthraquinone, resins and phlobatannins in all the fractions. In our study, *A.senegal* was found with flavonoids and anthraquinone. Lawrence et al [36] showed the presence of tannins, carbohydrates, terpenoids, phenols, anthraquinone, cardiac glycosides, flavonoids and alkaloids in *Acacia nilotica*.

Possible components present in all the extracts were seen by TLC followed with exposure to iodine (Fig.1). Rf value of the separated components are listed in Table 1. All the extracts were found to possess antioxidant molecules which has been evidenced through the TLC bio-autography analysis for antioxidants (Fig.2), the solvent used and the Rf value of antioxidants molecules are as follows:- acetone extract - 0.03,0.14,0.46 , chloroform extract - 0.20,0.98, ethanol extract - 0.02,0.14,0.22,0.24,0.57 and petroleum ether extract -0.04,0.12,0.27,0.29. Abdelhady et al [37] found leaf extract of *Acacia senegal* to have antioxidant activity by DPPH activity. Contradictory to this Hilmi et al [38] found the exudates of *Acacia senegal* to not to have antioxidant activity in DPPH free radical scavenging assay. Since the plant chosen in this study has proven to show antioxidant activity, all the extracts were subjected for fractionation using column chromatography. The obtained extracts were immediately involved in spot assay for antioxidant and antibacterial activity. 8^{th}, 9^{th}, 10^{th}fractions of acetone extract, 9^{th},10^{th} fractions of Choloroform extract, 6^{th},7^{th},8^{th},9^{th},10^{th} fractions of Ethanol extract and 8^{th},9^{th},10^{th} fractions of petroleum ether extract showed antioxidant activity (Fig.3).

The plant was exhibiting antibacterial activity against *Pseudomanas aruginousa* which was evidenced through TLC bio-autography analysis for antibacterial activity (Fig.4). the solvent used and the Rf value of antibacterial molecules

are as follows:-acetone extract- 0.74,0.96, chloroform extract -0.95,0.83,0.73,0.62, ethanol extract - 0.99,0.76,0.63 and petroleum ether extract - 0.4. Saini et al [39] reported *A.senegal* to have antibacterial activity against *S. aureus* alone. Renuka et al [40] reported dichloromethane extract of *Acacia senegal* root heartwood to exhibit antibacterial activity against *E. coli* and *S. aureus*. Acetone extract of *Acacia nilotica* has been found to be effective against *E. coli, S. pyogenes, V. cholera, S. aureus, P. aeruginosa* etc [36]. All the extracts were fractionized by column chromatography and the fractions were subjugated for spot aasay, the results are as follows - 2^{nd},3^{rd},4^{th},5^{th} fractions of acetone extract,7^{th},8^{th},9^{th},10^{th} fractions of Choloroform extract, 7^{th},8^{th} fractions of ethanol extract and 3^{rd},4^{th},5^{th},6^{th}fractions of petroleum ether extract showed antibacterial activity (Fig.5). Mudi and Salisu [35] reported n- hexane soluble fraction of *A.senegal* to be more active against the respiratory tract pathogenic bacteria such as *Klebsiellapneumonia* and *Streptococcus pneumonia*. Lawrence [36] found the fractions of acetone extract obtained from column chromatography of *Acacia nilotica* to exhibit antibacterial activity against *S. aureus* and *S. dysenteriae* by spot assay.

Table 1: Phytochemical Screening

Si	Phytochemical	AE	CE	EE	PE
1	Carbohydrates	+	-	+	-
2	Amino acid Proteins	-	-	+	-
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 Extract

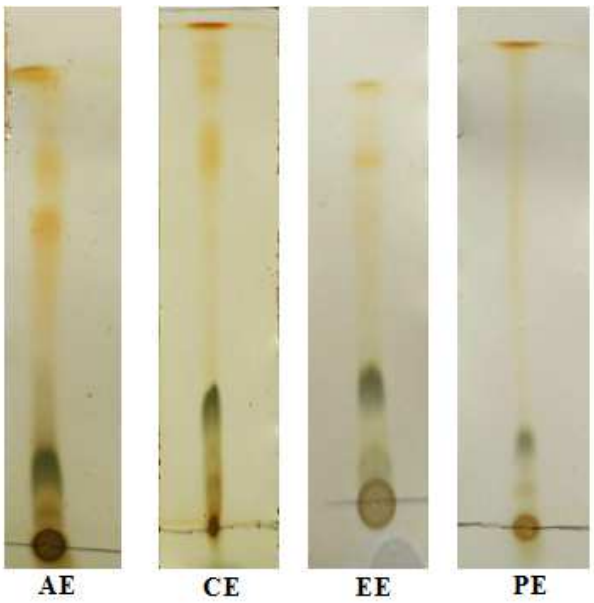

Fig.1 TLC of *Acacia sp.*
AE- Acetone Extract, CE- Chloroform Extract, EE- Ethanol Extract, PE- Petroleum Extract

Table 2 Rf value of compounds separated

AE	CE	EE	PE
0.037	0.031	0.027	0.04
0.107	0.062	0.037	0.120
0.142	0.109	0.055	0.275
0.464	0.203	0.074	0.293
0.676	0.984	0.092	
		0.148	
		0.222	
		0.241	
		0.575	
		0.660	
		0.786	

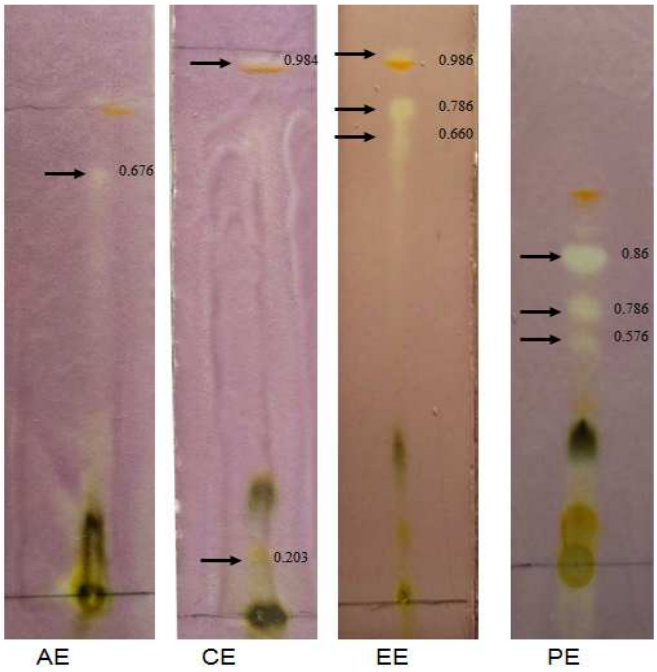

Fig.2 TLC bioautography for antioxidant activity in *Acacia sp.*
AE- Acetone Extract, CE- Chloroform Extract, EE- Ethanol Extract, PE- Petroleum Extract

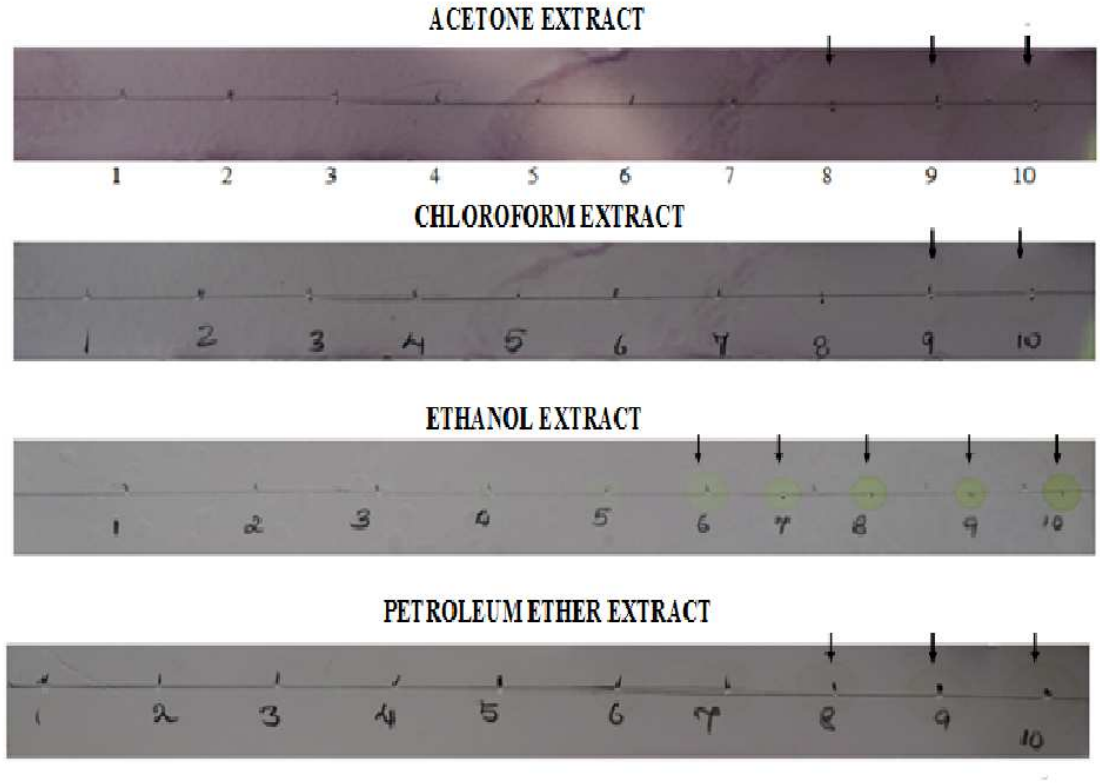

Fig.3 TLC Spot assay of fractionated columns for antioxidant activity in *Acacia sp.*

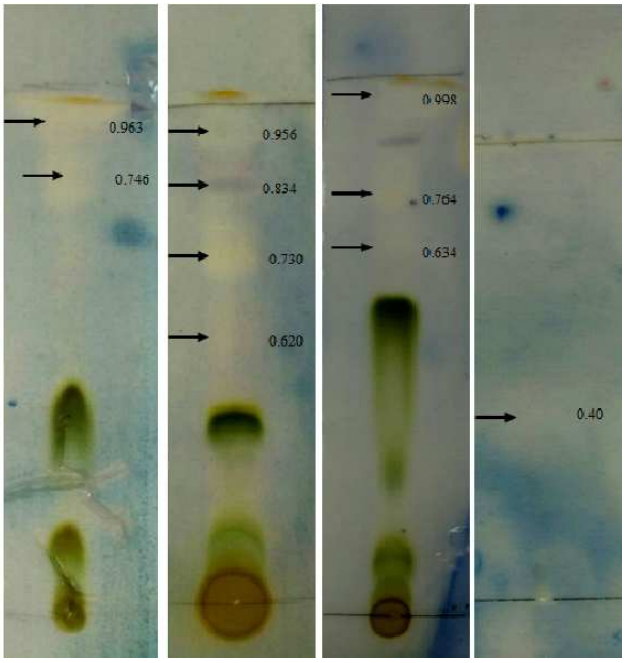

Fig.4 TLC bioautography for antibacterial activity in *Acacia sp.*
AE- Acetone Extract, CE- Chloroform Extract, EE- Ethanol Extract, PE- Petroleum Extract

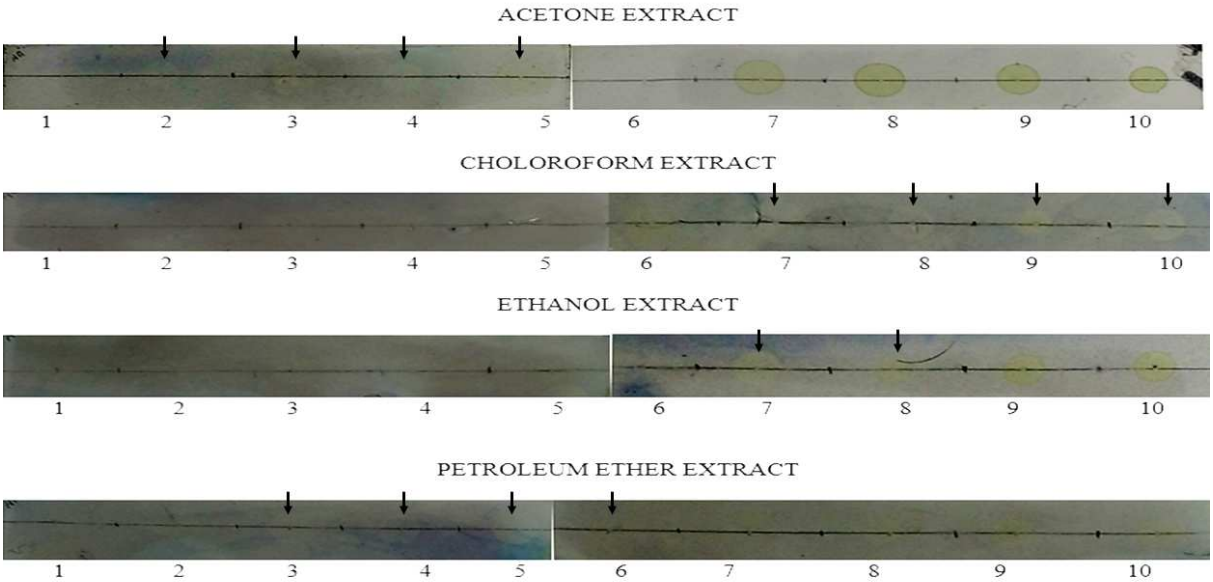

Fig.5 TLC Spot assay of fractionated columns for antibacterial activity in *Acacia senegal*

CONCLUSION

In this study, *A.senegal* was collected from Chennai. The plant was proven 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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