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Research Article

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TLC bioautography guided identification of antioxidant and antibacterial activity of various extracts of *Punica granatum*

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ABSTRACT

Plants have been one the most important sources for producing medicines since ancient times. In this study, antioxidant and antibacterial activity of Punica granatum was analysed using TLC-bioautography analysis. The plant leaves were collected and subjected for crude extraction using several solvents. The crude extracts were analysed for phytochemicals followed with antibacterial and antioxidant activity by TLC bioautography assay. The extract which showed a promising result were subjected for column fractionation and the collected fractions were subjected for spot assay for antibacterial and antioxidant activity. The plant was found to possess antioxidant and antibacterial activity.

Keywords: *Punica granatum*, Phytochemical, Antioxidant, Spot Assay.

INTRODUCTION

Plant derived drugs have found extensively used in most countries because they are easily accessible, safer and cheaper. At present, there are more number of life saving drugs derived from plants. More than 2000 medicinal plant species are used in different forms to treat diseases. One such is *Punica granatum*, the tree of Iran and Himalayas, now been cultivated in Asia, Africa and Europe. *Punica granatum* is a shrub with multiple stems [1]. All the parts of the plant have displayed hypotensive, antibacterial, antivirtal, antioxidant, antisplasmodic and antihelminthic activities[2-9].

Bioautography offers faster and directed isolation identification of active molecules on the chromatogram itself [10]. After the chromatogram was run with the sample, stable radical scavenger 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) is sprayed and observed for the cream or yellow spot against purple back ground, thus it be a TLC bioassay [11-15]. Diana and Samrot [16] found Phenol.3,5-Bis(1,1-Dimethylethyl) using GC-MS in *Aeglemarmelos* after TLC bioautography followed with preparative TLC. TLC bioautography analysis for antibacterial activity is normally done by spraying the microorganism onto the TLC plate ran with sample and microbial growth is determined by tetrazolium salts, where these tetrazolium are converted by living microorganisms to colored formazan, white zone with purple background confirms the antimicrobial activity [17-20]. This study is aimed to identify the antioxidant and antibacterial activity of *Punica granatum* through TLC guided bioautography.

EXPERIMENTAL SECTION

Collection and extraction of plant samples

The leaves of *Punica granatum* was collected from Sathyabama University, Chennai, Tamil Nadu, India. Shade dried leaves were ground mechanically. Ground dried sample was added into a conical flask in the ratio of 1:10 to various solvents like ethanol, acetone, petroleum extract and chloroform. The conical flask was kept in an orbital

shaker for 24 hours and later filtered with gauze cloth. The filtrate was dried. Thus obtained extract were stored for further use [21].

Qualitative analysis for phytochemicals
All the extracts were subjected for qualitative phytochemical analysis for secondary metabolites were performed [22-24].

Thin Layer Chromatography
TLC was performed on a TLC silica plate (Merck, F245) having chloroform as mobile phase. After running the sample the plates were subjected to iodine exposure, bands were calculated for Rf value.

TLC DPPH bio-autography for antioxidant activity
The extracts were run on TLC silica plate (Merck, F245) having chloroform as mobile phase. Plates were air dried and then sprayed with DPPH (0.001g in 95% methanol. Bright yellow or cream colour was used for the confirmation of antioxidant molecule [11-15]. The Rf value of the samples were recorded.

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 [17-20].

Column Fractionation
Silica gel was mixed with ethyl acetate to make slurry and packed in a glass column. 10ml of sample was added to the column. Ten fractions of each 1 ml were collected. Collected samples were subjected for dot blot assay for antioxidant and antibacterial activity [25-27].

RESULTS AND DISCUSSION

All the solvents used in this study were tended to isolate most of the phytochemicals possessed by the plant chosen in this study. The plant extracts were lacking steroids, anthocyanins and leucoanthocyanins (Table 1). Sangeetha and Jayaprakash [28] also did not find anthocyanin in the peel extract whereas Bhandary et al [29]reported triterpenoids, steroids, glycosides, flavonoids, tannins, carbohydrate and vitamin C in the peel extract, whole fruit extract and seeds extract. Saponins and Glycosides are naturally cardioactive drugs [30].

Table 1: Phytochemical screening of *Punica grantum*

No	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	-	-	+	-

+ - presence, - - negative, AE-Acetone Extract, CE-Chloroform Extract, EE-Ethanol Extract, PE-Petroleum Ether Extract

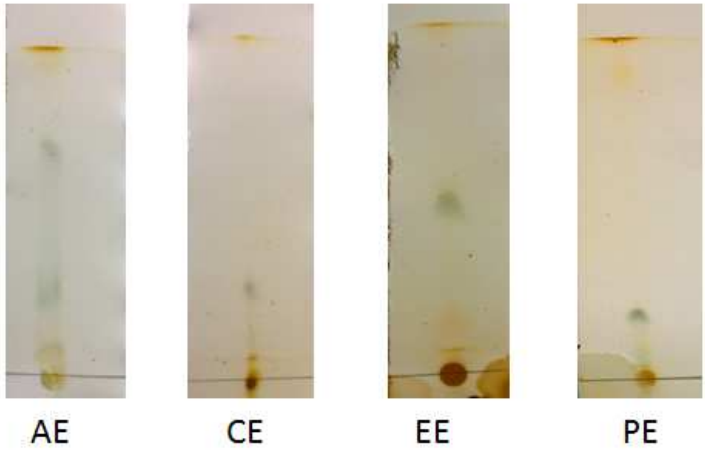

Fig.1 TLC of *Punica granatum* AE- Acetone Extract, CE- Chloroform Extract, EE- Ethanol Extract, PE- Petroleum ether extract

Table 2: Rf value of compounds separated

Acetone Extract	Chloroform Extract	Ethanol Extract	Petroleum ether extract
0.07 0.20 0.64 0.98	0.05 0.58 0.78 0.98	0.09 0.16 0.20 0.25 0.99	0.04 0.08 0.14 0.22 0.97

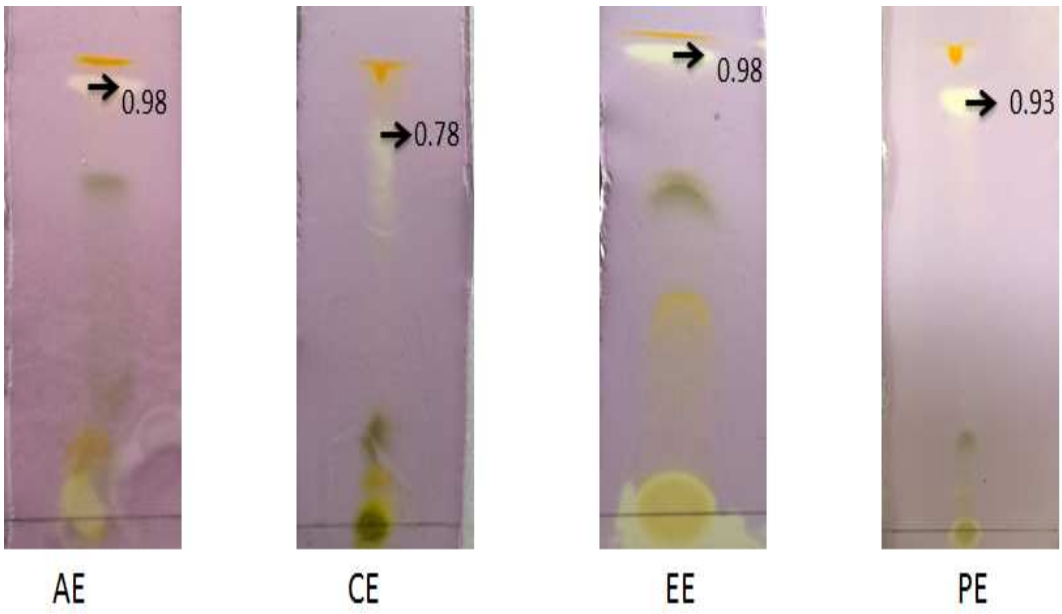

Fig.2 TLC bioautography for antioxidant activity in *Punica granatum*
AE- Acetone Extract, CE- Chloroform Extract, EE- Ethanol Extract, PE- Petroleum ether Extract

TLC confirmed the presence of more components extracted ethanol and petroleum ether, the polarity of these solvents might have favoured this isolation (Fig.1).

Antioxidants bands were observed in the TLC plate exposed to DPPH. Flavonoids and tannins are phenolic compounds primary antioxidants seen in plants which acts as free radical scavengers [29]. Flavanoids were found in the prelimnary screening too. Hence the flavanoids might be the reason for the antioxidant property which has been evidenced by the TLC assay (Fig.2) .When the samples are subjected for column fractionation followed that the fractions were subjected for spot assay, ethanol extract fractions were showing the most activity (Fig.3). Rajan et al[31] found dose dependent DPPH scavenging activity by the extracts by dot blot assay.

Petroleum ether extract was found to have potent antibacterial compounds, which was confirmed by TLC bioautography analysis as well as spot assay for antibacterial activity. Growther et al [32] showed the presence of ellagic acid in TLC analysis. They also showed the methanol extract of *P. granatum* peel to have activity against shiga toxin producing *E.coli.* Benzene fraction of *Punica granatum* had good activity against methicillin resistant*S. aureus*[33]

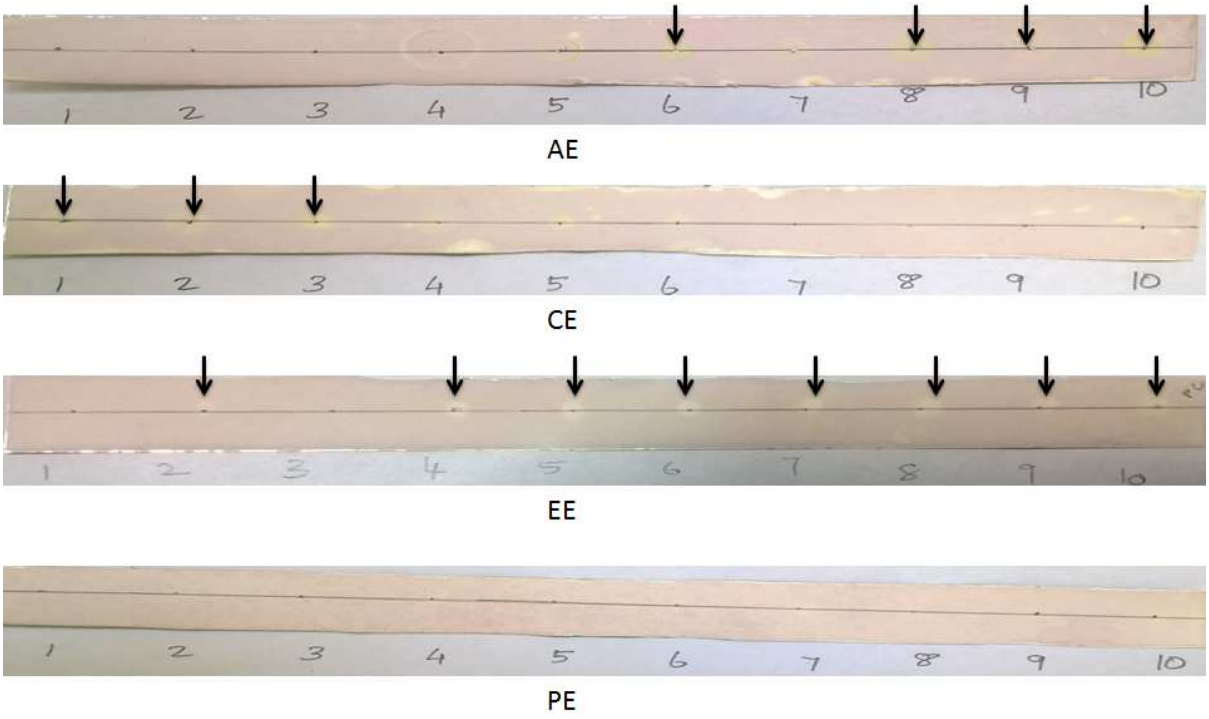

Fig.3 TLC Spot assay of fractionated columns for antioxidant activity in *Punica granatum*
AE- Acetone Extract, CE- Chloroform Extract, EE- Ethanol Extract, PE- Petroleum ether extract

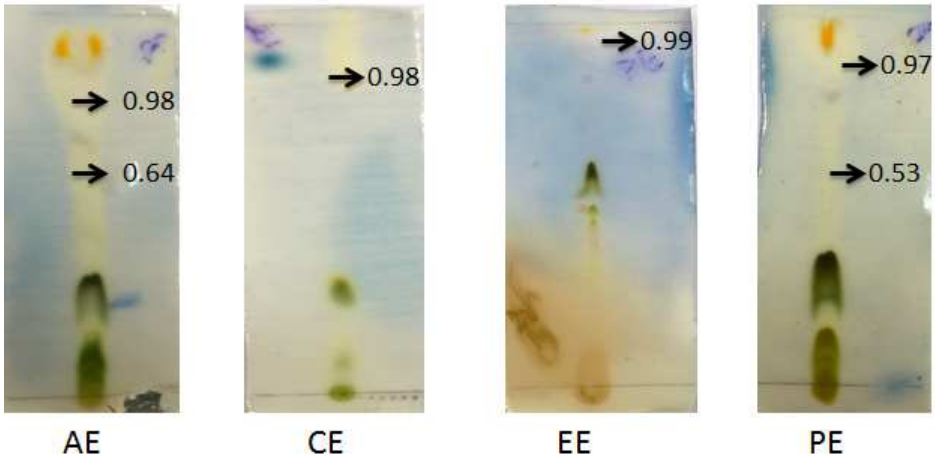

Fig.4 TLC bioautography for antibacterial activity in *Punica granatum*
AE- Acetone Extract, CE- Chloroform Extract, EE- Ethanol Extract, PE- Petroleum ether extract

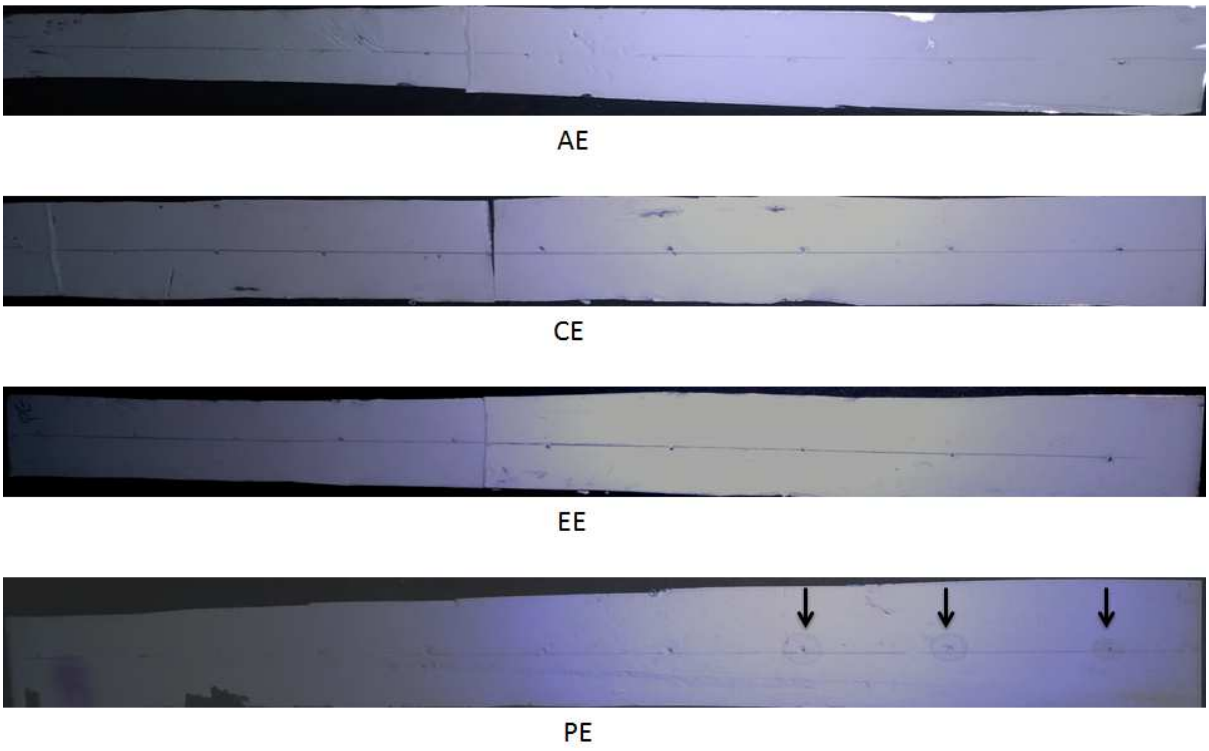

Fig.5 TLC Spot assay of fractionated columns for antibacterial activity in *Punica granatum*
AE- Acetone Extract, CE- Chloroform Extract, EE- Ethanol Extract, PE- Petroleum ether extract

CONCLUSION

This study revealed that Punica granatum to possess antioxidant and antibacterial activity through TLC guided bioautography and dot blot assay.

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