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Identification of antioxidant activity of bark of *Aegle Marmelos*

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ABSTRACT

To identify potent antioxidant molecule in chloroform extract of bark of Aegle marmelos. Bark of Aegle marmelos was collected and subjected for chloroform extract. TLC was run having solvent system of Chloroform/Methanol (5:1), and subjected for on exposure to iodine and to DPPH. Further the extract was subjected for column chromatography. The fractions were subjected for identification of antioxidant activity by dot blot assay. The fractions showed activity was pooled together and subjected for GC-MS analysis. A band with Rf value of 0.87 was found to show antioxidant activity. Fraction 2 to 5 was found to show antioxidant activity and these fractions were pooled together and subjected for GC-MS analysis. GC-MS revealed the presence of seven major compounds responsible for antioxidant activity. Thus potent antioxidant molecule present in the extract was identified through TLC bioautography followed with GC-MS analysis.

Keywords: TLC bioautography, antioxidant activity, GC-MS analysis

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INTRODUCTION

Plants are being used to treat various diseases since the time unknown.. Medicinally potent plants are traditionally recommended for primary health care system due to its effectiveness and cultural preference [1]. 80% of the world's population rely on either partially or wholly depends on plant derived medicine [2]. With proper investigations, various secondary metabolites can be extracted from the plants and analysed for their impressive medicinal properties such as antibacterial, anticancer, anti inflammatory, diuretic etc [3,4]. Extracting these medicinal compounds is done by using a proper solvent, whereas the identification and separation of a particular bioactive compound is a quite tedious process [5]. Among the various approaches available for the identification of the bioactive molecules , the commonest method id fractionating the extracts by column chromatography and screening the fractions by thin-layer chromatography (TLC)-bioautography analysis, which is time saving, simple and cost effective[6].

A. marmelos has been proven to scavenge reactive oxygen species and reactive nitrogen species, commonly this property is called as antioxidant activity. This property mostly conferred by total phenolic compounds, where it is richly found in *A.marmelos* [7]. Various invitro studies were also have proven the antioxidant potential of *A. marmelos* [8-13]. TLC bioautography guided identification of antioxidant is simple and time consuming one, various studies have proven it as sensitive [9,11]. In this study, chloroform extract of bark was taken and the extract was subjected TLC guided identification for antioxidant molecule. The band which showed antioxidant activity was subjected for GC-MS analysis to identify the molecule.

MATERIALS AND METHODS

Collection and extraction of samples

The bark of medicinal plant *Aegle marmelos* was collected from Chennai, Tamil Nadu was washed thoroughly, chopped into small pieces, shade dried and ground to powder. Ground bark sample was taken in a conical flask and added with Chloroform in the ratio of 1:10(w/v). The conical flask was kept in an orbital shaker for 48h and later filtered using a gauze cloth. The filtrate was dried. Thus obtained extract were stored for further use [14].

Thin Layer Chromatography

TLC silica plate (Merck, F245) plate loaded with the sample was placed vertically and made to run in various solvent systems as follows - Chloroform/Ethyl Acetate/Formic Acid (10:8:2), Ethyl Acetate/Methanol/ Water (10:1.35:1) and Chloroform/Methanol (5:1). Bands were visualized by keeping them exposed to iodine. Rf value was calculated and recorded.

TLC-bioautography for antioxidant activity

TLC plates were run having the above mentioned solvent systems. The plates were dried in the fume-hood and then sprayed with 0.2% 2,2-diphenyl-2-picrylhydrazyl (DPPH) in methanol. Yellow spots against a purple background confirm the antioxidant activity [15,16].

Column chromatography

The column was packed 10g silica gel added with 50ml of Chloroform. 1mg chloroform extract of bark was dissolved with 1ml of chloroform and further on top of it 10ml of chloroform:methanol (5:1) was added and made to elute slowly. 1ml/fraction was collected in eppendorf vials, thus 10 fractions were collected.

Dot Blot Assay

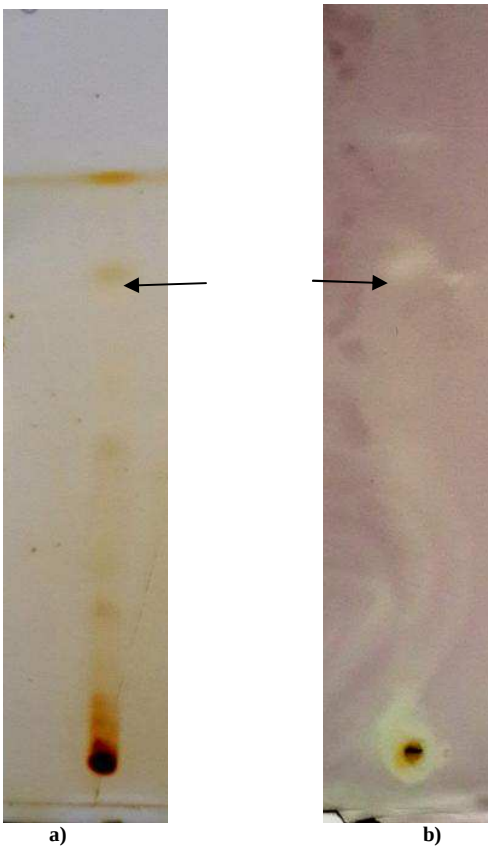
A drop from the collected fractions was carefully placed on TLC silica plate (Merck, F245) and allowed to dry. The spots were sprayed with 0.2% DPPH dissolved in methanol [17]. Fraction with positive response was subjected for further analysis.

GC-MS

The purified sample of the fraction was further analysed under Perkin Elmer, Clarus 680-Clarus 600(EI) to perform Gas Chromatography – Mass Spectrometry. The acquisition parameters were maintained with the initial temperature at 60°C for 2 min, ramp 10°C/min to 300°C held for 6 minutes and running it for a total time of 32 minutes. The gas carrier was helium and the flow rate of the sample was 1mL/min. The sample was further checked against various libraries.

RESULTS

The extract run in the solvent system of Chloroform/Methanol (5:1) showed a distinct band at the same region on exposure to iodine and to DPPH (Fig 1a and 1b). Rf was calculated and tabulated in Table 1.



a) **b)**

Figure 1. TLC analysis of chlroform extract of bark. a)TLC ran with solvent system of chloroform:methanol (5:1) and exposed to iodine, b) TLC bioautography for antioxidant activity of chloroform extract

Table 1. Rf value of the components separated through TLC having chloroform:methanol (5:1) solvent system

s.no	Rf value of compounds deteced after exposure to iodine	Rf value of compound deteced after spraying with DPPH
1.	0.87	0.87
2.	0.57	
3.	0.31	
4.	0.17	

Using the same solvent system used for running the TLC was used for separation of the antioxidant potent fraction through column chromatography. About 10 fractions were collected from silica column. In order to screen free radical scavenging activity, each fraction eluted by column chromatography was applied as a dot on TLC plate that was later sprayed with DPPH solution, where the fractions between 2 and 5 were found to have antioxidant activity (Fig.2) evidenced by the formation of light yellow coloration around it. These fractions were mixed together used for GC-MS analysis and revealed the presence of compounds (Fig.3 and Table 2).

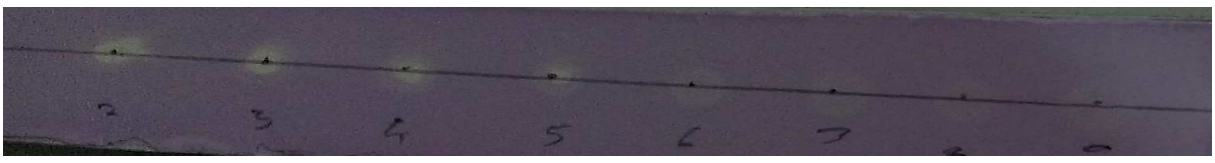


Figure 2. Dot blot assay for antioxidant activity of chlroform extract of bark

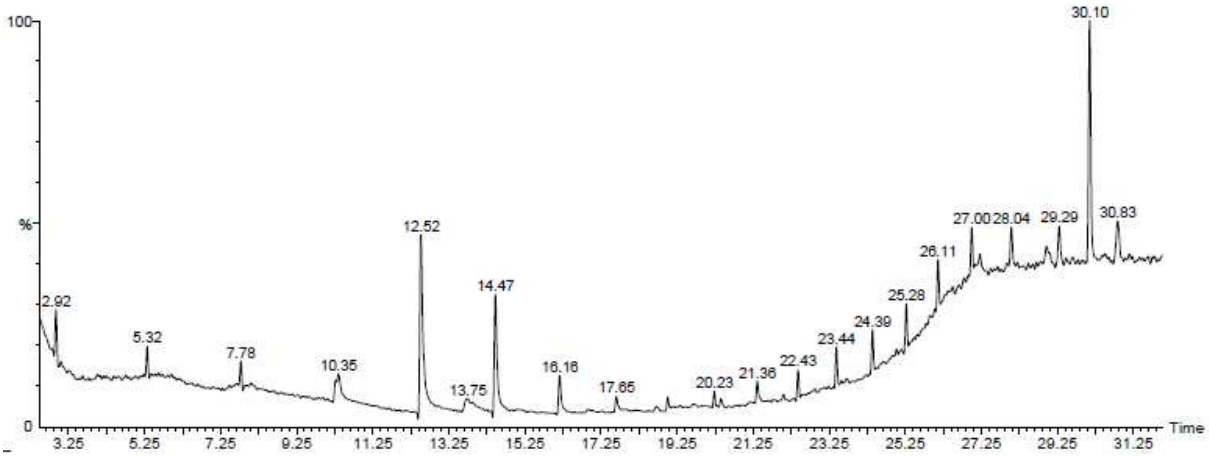


Fig. 3 GC spectrum of chloroform extract

Table 2 Compounds identified through GC-MS analysis

SL.NO	COMPOUND NAME	MOLECULAR FORMULA
1	1,2-BENZENEDIOL, 3,5-BIS(1,1-DIMETHYLETHYL)-	$C_{14}H_6O_2$
2	2,4-CYCLOHEXADIEN-1-ONE, 3,5-BIS(1,1-DIMETHYLETHYL)-4-HYDROXY-	$C_{14}H_{22}O_2$
3	4,6-DI-TERT-BUTYLRESORCINOL	$C_{14}H_{22}O_2$
4	PHENOL, 3,5-BIS(1,1-DIMETHYLETHYL)-	$C_{14}H_{22}O$
5	1,4-BENZENEDIOL, 2,5-BIS(1,1-DIMETHYLETHYL)-	$C_{14}H_{22}O_2$
6	2-(2-BUTOXYETHOXY)ETHYL 2,2,3,3,3-PENTAFLUOROPROPANOATE	$C_{11}H_{17}O_4F_5$
7	PHENOL, 3,5-BIS(1,1-DIMETHYLETHYL)-	C14H22O

DISCUSSION

TLC plates run with the sample was sprayed with the DPPH reagent and the antioxidant molecule was observed as yellow color band. The Rf value of the compound was found to be 0.87. Similarly, compounds which exhibited antioxidant activity by the extracts of rind of *Aegle marmelos* were identified using TLC bio-autography [9]. It was simpler and time consuming process [11]. After the samples were separated by column, the fractions were subjected for dot blot assay. Fractions between 2 and 5 was identified, likewise Samrot et al [11] also found potent antioxidant fractions in *Punica granatum* through dot blot assay. When these fractions were pooled and subjected for GC-MS analysis, it was found with major seven compounds. Diana and Samrot [9] identified five different molecules from TLC scrap.

CONCLUSION

In this study, antioxidant potent molecules were identified by performing TLC bioautography for antioxidant activity. Further, the extract was fractionated using column chromatography having Chloroform/Methanol (5:1) as eluent. The obtained fractions were performed with dot blot assay for antioxidant activity. The fractions showed antioxidant activity were pooled together and seven compounds were identified by GC-MS analysis.

Conflicts of interests

All authors have no conflict of interest.

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