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TLC Bio-autography Guided Identification of Antioxidant Fraction from *Aegle marmelos* Rind.

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ABSTRACT

The present study was devised to identify the antioxidant potential fraction from rind of *Aegle marmelos*. The rind extract of *Aegle marmelos* was analyzed by TLC bioautography. The fraction showing DPPH reduction property in TLC bioautography were further ran on preparative TLC and the positive fraction was extracted and analyzed using GC-MS. The rind of *Aegle marmelos* has found to have anti-oxidant compound such as Phenol 3,5-Bis (1,1-Dimethylethyl).

Keywords: *Aegle marmelos*, TLC, Bio-autography, antioxidant

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INTRODUCTION

Reactive oxygen species (ROS) such as superoxide radical (O2), hydroxyl radical (OH·), peroxide radical (ROO) and nitric acid radical produced during metabolism in living organism [1] and these ROS lead to oxidative damage mediated degenerative diseases like cancer and plethora [2]. Antioxidants act against oxidative damage caused by ROS and implies the importance of antioxidants and necessity for the discovery of new antioxidant compound from various sources. Several screening assays are available for the screening of potential antioxidants, Thin layer chromatography (TLC) bioautography assay is the best method among them [3]. Comparing with other available methods, TLC bioautography is quick, convenience, simple and efficient method as active components from a complicated plant extract is also possible. So as, for the screening of antioxidants, the TLC bioautography assay is the good choice [4,5,6]. Several TLC techniques are developed and used for qualitative and quantitative analysis of antioxidants [7,8], but these methods use stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as derivatizing agent [9].

Medicinal plants are known to contain various high potential bioactive compounds which can act as lead compounds in drug discovery and design [10, 11,12]. *Aegle marmelos* (L.) Corr., belongs to the family *Rutaceae* which has been used to digestive and stomach problem and as astringent [13,14, 15]. In this present study, antioxidant potential of the *A.marmelos* rind was done.

MATERIALS AND METHODS

Collection of Samples

The fruit of *Aegle marmelos* was collected from around Chennai, Tamil Nadu, which was washed thoroughly, rind was removed and chopped into small pieces, shade dried and ground into powder form in a sterile condition.

Extraction

Various solvent extractions like Petroleum Ether, Chloroform, Ethyl Acetate, Acetone and Ethanol were taken by adding the solvent into a conical flask containing rind (10g of dried rind powder to 100ml of solvent) and the conical flasks were kept in an orbital shaker at 250 rpm for 24 hours and later filtered using a gauze cloth. The filtrate was dried and thus obtained extracts were stored for further uses [16].

Qualitative Phytochemical Analysis

Qualitative phytochemical analyses were performed for flavonoids, phytosterols, tannins, phenols, flavanoids and saponins [17].

Thin Layer Chromatography

Thin layer chromatography was carried out on a TLC silica plate (Merck, F245) in solvent pre saturated glass chamber. The solvent system used was Chloroform/Methanol (5:1). Based on time and solvent system used, the compounds start to separate and form bands. These bands were visualized by exposing to iodine.

Bio-autography for Antioxidant Activity

A rapid TLC screening method for antioxidant was done using the 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH) as a spray reagent. TLC was performed for all extracts as described earlier [18]. The plates were dried and antioxidant activity was detected by spraying 0.2% 2,2-diphenyl-2-picrylhydrazyl (DPPH) in methanol onto TLC plates. The development of yellow spots against a purple background indicated the presence of andioxidant compound.

Preparative TLC

A streak of crude extract was applied manually on a TLC silica plate (Merck, F245). After air drying, the plate was developed, using the same mobile phase which were used in the analytical TLC (TLC bioautography

for antioxidants), in a presaturated glass chamber. The plates were sprayed with DPPH radical (for antioxidants) and the bands that showed antioxidant activity were scraped off carefully from the plate. The scratched sample was dissolved in chloroform and centrifuged at 10000 rpm for 15 min in order to separate silica. The supernatant was filtered using Whattman filter paper and dried which was used for further characterization using GC-MS [19].

GC-MS

The purified 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 gas and the flow rate of the sample was 1mL/min. The sample was further checked against NIST library.

RESULTS AND DISCUSSION

In Indian traditional system of medicine plants play major role as source of beneficial therapeutics. Direct extraction of the *A. marmelos* rind was carried out using various solvents (Petroleum Ether, Chloroform, Ethyl Acetate, Acetone and Ethanol).

The preliminary phytochemical screening of various extracts of *Aegle marmelos* fruit rind revealed the presence of multiple chemical constituents (Table 1). It has been proven that phytochemical extracts from plants posses various bioactivity which make them as potential sources for allopathic medicine [20,21]. To study the antioxidant property of *A.marmelos* rind, the extracts were fractionated by TLC using chloroform: methanol (5:1) solvent system. R_f value was determined for all the resultant bands and tabulated (Table 2). Chloroform extract was shown with better separation of active compounds than the other extraction by the solvent system used in this study, thus chloroform extract was further used for analysis (Fig.1).

Antioxidant potential compounds on TLC plates were identified in situ with the help of DPPH reagent (Fig. 2), the fractions producing yellowish bands on the purple background were considered as antioxidants. Chloroform extract of rind showed a distinct band being formed at the region at Rf 0.85 on exposure to both iodine and to DPPH. DPPH method measures electron-donating activity (free radical scavenging activity) of compounds and provides an evaluation of antioxidant activity [22]. The antioxidant potential of combined plants extracts (*Cissus quadrangularis* and *Aegle marmelos)* was reported by TLC autobiography [23].

Semi-purified antioxidant constituent was scrapped and collected after performing silica gel preparative TLC of chloroform extract. Thus obtained sample after preparative TLC was subjected for GC-MS analysis to identify the antioxidant compounds (Fig. 3). Six major compounds were identified using NIST library (Table 4). Among the identified compound, the possible pharmacological active compound was Phenol, 2, 4-Bis (1, 1-Dimethyl ethyl) (molecular weight 206 and molecular formula C_{14} H_{22} O $_{11}$) which been found in the Malaysian mango kernel and also reported to have antibacterial activity [24, 25].

Table 1: Qualitative Phytochemical Screening

Extract	Alkaloids	Tannins	Phenols	Flavanoids	Phytosterols
Petroleum Ether	-	++	++	+ +	++
Chloroform	+	+	+	+	-
Ethyl Acetate	++	++	+	+	+
Acetone	+	-	+	++	++
Ethanol	++	+	++	+	+

++ instant end point, + delayed end point, - no end point

Table 2: R_f value of TLC

Solvent system	Chloroform extract (R_f value)	Ethanol extract (R_f value)
Chloro: Methanol (5:1)	0.97	0.54
	0.92	
	0.85	

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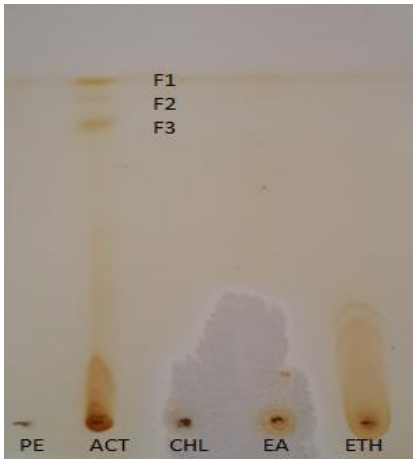
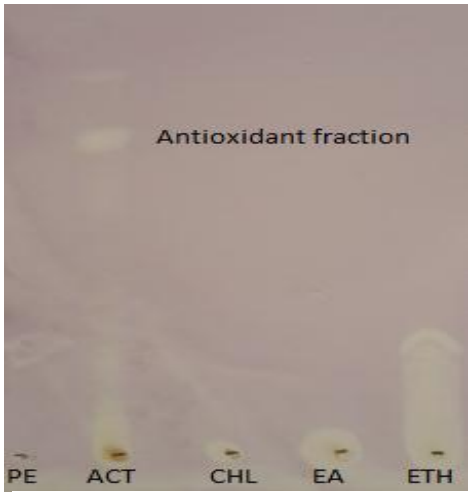


a b c d e

Figure 1: TLC chromatogram of various extracts

a) Petroleum Ether, b) Chloroform, c)Acetone, d)Ethyl Acetate, e)Ethanol



a b c d e

Figure 2: TLC- bioautography of various extracts

a) Petroleum Ether, b) Chloroform, c)Acetone, d)Ethyl Acetate, e)Ethanol

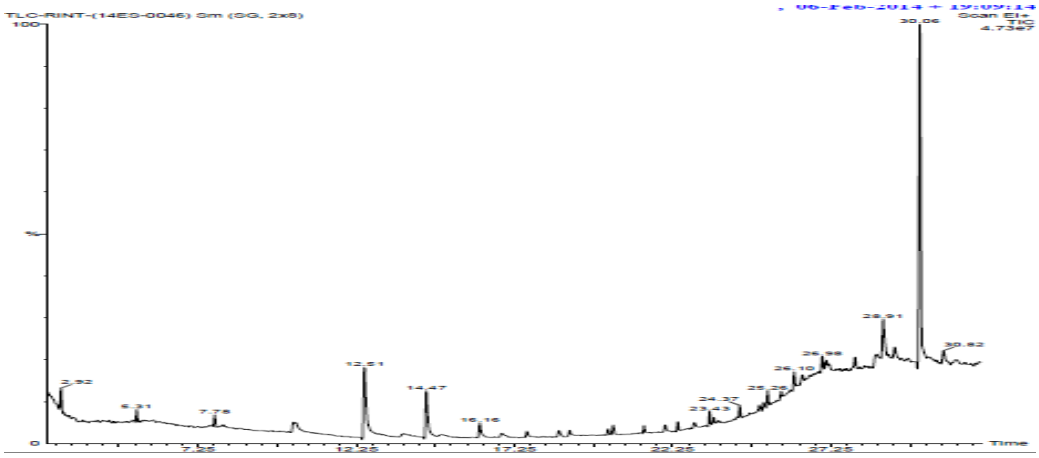


Figure 3: Gas Chromatography Spectrum

Table 4: Chemical list of GCMS

Sl.No.	COMPOUND	Mol. Wt.
1	2-T-Butyl-5-Chloromethyl-3-Methyl-4-Oxoimidazolidine-1-Carboxylic A	304
2	TriFluromethyl Butyl Disulfide	190
3	3-5-Decadien7-yne. 6-T-Butyl-2,2.9,9-Tetramethyl	246
4	Phenol 3,5-bis(1,1-dimethylethyl)	206
5	4-Pyrimidinecarboxylic acid. 2,6-Bis(Tert-butylomethylsilyl)oxy. te	498

SUMMARY AND CONCLUSION

The rind extract of *Aegle marmelos* was analyzed for its phytochemical and antioxidant properties. The compounds of the various extracts using different solvents were separated using TLC and were tested for their antioxidant properties by exposing it to DPPH. The fraction showing DPPH reduction property in TLC bioautography were further ran on preparative TLC and the positive fraction was extracted and analyzed using GC-MS. The chloroform extract of rind of *Aegle marmelos* has found to have Phenol.3,5-Bis(1,1-Dimethylethyl).

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