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ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF *Aegle marmelos*

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ABSTRACT

Either the whole plant or plant products having medicinal properties are commonly known as medicinal plants. These medicinal plants are known to possess various phytochemicals, which exhibit more bioactivities such as antibacterial, antifungal, anticancer activity, etc. In this study, *Aegle marmelos* was collected from Chennai, crude extract of fruit and pulp of the chosen plant was subjected for antimicrobial activity. Qualitative analysis for the phytochemicals of the plants was explored. Minimal inhibitory concentration of the crude extracts was identified.Crude extract was subjected to TLC bioautography for antibacterial activity. The fraction which showed antibacterial activity was subjected to GC-MS analysis.

KEYWORDS: Antimicrobial ,GC-MS, Aegle marmelos, Bioautography.

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INTRODUCTION

Plant derived compounds have got an increasing interest throughout the world as they possess potent, less or no toxic pharmacological compound, economic viable, safer and more dependable [1]. About 70 – 95% of the world population are relying on traditional medicines or traditional therapies where the whole or parts of plants is used as medicine [2]. Drug resistances in microorganism have become an unsolvable problem and treating an infectious disease with the existing drugs is becoming less use. This situation, truly made researchers to discover drug from various sources, one such source is plant based drugs. *Aegle marmelos* belong to the family *Rutaceae* is one such plants where all the parts of the plant are known to possess various pharmacologically active compounds.It has found that methanolic, toluene, water and chloroform extract *of Aegle marmelos* to possess antimicrobial activity against the plant pathogens of *Ducus carota*, *Capsicum* and *Pomigranata* sps [3]. Methanolic extract of *Aegle marmelos* was reported to have antibacterial activity against *Bacillus* sps, *E.coli* and *Klebsiella* sps [4]. Various separation and detection techniques like TLC, HPLC, GC-MS etc. to analyse compound present in *Aegle marmelos* have been standardized [5]. Compounds such as skimimianine, ageline, lupeole, citral and marmesinin from leaves, marmelosin luvangetin, aurapten, psoralen from fruit and faganine, marmemin from bark were identified from *A.marmelos* [6] . Ethanolic extract to have antioxidant activity and they also estimated the phytochemicals like total phenolics and flavonoids of *Aegle marmelos* . Having understood the bioactive potency of *Aegle marmelos*, this study was done with the intention of analyse the antibacterial activity of *Aegle marmelos* by conventional methods as well as by performing TLC –bioautography and also to identify the possible active compounds which showed antibacterial activity by Gas Chromatography-Mass Spectroscopy.

MATERIALS AND METHODS

Collection And Extraction

Leaves and fruits of *Aegle marmelos* were collected from Kodambakkam, Chennai, Tamil Nadu-600 024, India. Extractions using various solvents were done.

Qualitative Phytochemical Analysis

Qualitative phytochemical analysis of flavonoids, phlabotannins, tannins, phenols and saponins were done [7],[8].

Antibacterial Activity

Bacterial cultures used for this study were *Bacillus subtilis, E.coli, Klebsiella pneumoniae*. Organism was inoculated into 50ml nutrient broth and incubated at 37ºC for 24 hours. The organism was swabbed all over the surface of the sterile nutrient agar plate using a sterile cotton swab. Six wells of 3mm diameter were bored with the medium with the help of sterile cork-borer and 20 µl of the working suspension solution of different concentration of was poured into the well. Positive control (erythomycin) and negative control (solvent used for dissolving the extract) were also kept. Plates were left with the lid closed for some time till the extract diffuses into the medium and then incubated at 37°C for 24 hours. After incubation, the zone of inhibition was measured using a scale[9].

Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) was determined by the microdilution method (96 well micro titre plate) against serially diluted plant extracts according to the NCCLS protocol[10]. The ethanolic extracts were diluted to get series of concentrations from 1.56mg/ml to 50mg/ml in sterile nutrient broth. Volumes of all the wells were made upto 150 µl by adding sterile nutrient broth. 50µl of culture was added to the broth dilutions and were incubated for 18hour at 37ºC. MIC of each extract was developed by adding MTT[11].

Thin Layer Chromatography

The ethanol extract of Fruit pulp and the coat was chromatographed on an Aluminum foil-backed normal particle silica gel 60F254 plates (TLC, #5554 from Merck, Darmstadt, Germany) TLC layer; and was developed with chloroform. Developed plates were dried, visualized and detected with iodine vapour exposure. Rf value for the developed band were calculated.

Direct Bio – Autography

The antibacterial effect of Fruit pulp and coat was evaluated invitro with direct bioautography [12],[13],[14] against a Gram-positive *Bacillus subtilis* and Gram-negative *Klebsiella sp* and *Proteus sp*, which were grown in nutrient broth at 37^0C. All the bacterial cultures were grown to attain late exponential phase,at which give an optical density of 1.2 at 600 nm. The dried, developed chromatoplates with separated spots were sprayed with these bacterial cell suspensions and incubated for 2h in a humidity chamber at 37^oC and then sprayed with an aqueous solution of MTT (100 mg Triton X-100 and 80 mg MTT in 100 ml distilled water). The treated chromatoplates was incubated for 1h until inhibition zones appeared as clear regions against a darker background, where reduction of the bluish MTT-formazan to the yellow MTT was carried out by the viable, metabolically active cells .

GC MS

The band showed antibacterial activity in TLC bioautography was scrapped and subjected to GC-MS (SHIMADZU QP2010) at Sargam laboratory, Chennai, Tamil Nadu as explained earlier[15]. The spectrum obtained was compared with NIST library.

RESULTS & DISCUSSION

Qualitative analysis of phytochemicals of various extracts of Fruit pulp, Fruit rind and Leaves of *Aegle marmelos* are listed in Table 1. The results revealed the presence of pharmacologically active compound such as alkaloids, flavonoids, terpenoids, saponins and phlobatannins in leaves, fruit pulp and rind. [16],[17],[18]. Presence of alkaloids, Flavonoids, Phenolic Compounds, Steroids, Saponins and Xanthoproteins in invitro generated *Aegle marmelos* was detected [16, 17,18, 19]. The antimicrobial activity of Leaves, pulp and rind extracts of ethanol was assessed by agar plate diffusion technique. Ethanolic extract of fruit rind and fruit pulp had good antimicrobial activity. The antibiogram analysis of pulp extract showed a zone of inhibition of 1.0 cm against *Bacillus.* From the above analysis, it has been confirmed that ethanolic extract of fruit rind and pulp are having potent antimicrobial activity (Table 2), this was previously reported[19]. The MIC values of pulp and coat extracts against *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus* are tabulated in Table 3. MIC of ethanolic extract of pulp was 0.78mg/ml, 1.56mg/ml and 1.56mg/ml against *Bacillus subtilis*, *Klebsiella pnemoniae* and *Proteus* respectively. The MIC of rind against *Proteus* sp was found to be better i.e.0.78mg/ml than the MIC against *Bacillus subtilis* (1.58mg/ml) and *Klebsiella* (3.12mg/ml). Pandey and Mishra (2011) have obtained the MIC of 1.98 mg/ml in ethanolic and ethyl acetate extract of fruits against *S. aureus* and by methanolic extract (11.90mg/ml) in against *P. aeruginosa*. The variation in Rf values of these extracts helps in identifying the polarity and solvent system for separation of pure compounds by other chromatographic techniques. Compounds of high Rf values are low polarity compounds and with less Rf values have high polarity. In this study, separation of compounds by thin layer chromatography using Chloroform as the mobile phase revealed the presence of 13, 8, 6 Iodine bands with Rf value ranged from 0.8 - 6.2, 1.1 - 5.8 and 3.3 - 6.8 for Ethanolic extracts of leaves, pulp and rind respectively (Table 4, Figure 1). TLC profiling of ethanol extract of *Aegle marmelos* and found three major bands in long UV and iodine sprayed plates with Rf value of 0.72, 0.70 and 0.58. After performing direct TLC Bio autography of ethanolic extract of fruit rind the band with 0.96 Rf value was found to active against *Klebsiella* sps, the band was scraped and subjected for GC-MS analysis (Figure 3). The compounds present in the fractions is listed in Table 5. Pharmacologically active compound such as of Di-n-octyl pthalate (12.8%), 1,2-benzenedicarboxilic acid (4.84%) which were proven found to be with antimicrobial activity. Alpha-pinene (mono terpene) and Squalene (triterpene) were also found.Terpenes are phenolic compounds which exhibits antibacterial and antifungal activity [20]. Squalene which is a triterpene which is found to be possesing chemopreventive activity against colon cancers [21]. Trace amounts of other antimicrobial compounds such as tetradecanoic acid, octa decanoic acid, D-limonene were also found which is on poor with the earlier reported result [22].

Table 1
QUALITATIVE PHYTOCHEMICAL SCREENING

Metabolites	Solvents	Pulp	Coat	Leaves
Terpenoids	Ethanol	++	++	--
	Chloroform	--	--	--
	Acetone	++	--	++
Phlabotannins	Ethanol	--	++	--
	Chloroform	--	--	--
	Acetone	--	--	--
Saponins	Ethanol	++	++	--
	Chloroform	--	--	--
	Acetone	--	--	--
Flavonoids	Ethanol	++	++	--
	Chloroform	--	--	--
	Acetone	--	--	--
Tannins	Ethanol	++	++	--
	Chloroform	--	--	--
	Acetone	++	++	--

++ = presence, -- = absence

Table 2
ANTIMICROBIAL ACTIVITY OF THE CRUDE EXTRACTS

Cultures	Solvents	Coat	Pulp	Leaves
E.coli	Acetone	-	-	-
	Chloroform	0.6	-	-
	Ethanol	0.8	-	-
Bacillus subtilis	Acetone	0.8	0.7	0.5
	Chloroform	0.5	0.5	-
	Ethanol	0.9	1.0	0.8
Klebsiella pneumoniae	Acetone	0.7	-	0.6
	Chloroform	-	-	-
	Ethanol	0.8	0.5	-

Table 3
MIC CONCENTRATIONS OF PULP AND COAT

Sample	Pulp			Coat		
Organism	*Bacillus subtillus*	*Klebsielle*	*Proteus*	*Bacillus subtillus*	*Klebsielle*	*Proteus*
Concentration	0.78mg	1.56mg	1.56mg	1.56mg	3.12mg	0.78mg

Table 4
R_f VALUES OF LEAVES, PULP AND RIND

S. no	R_F values		
Fractions no	Leaves	Pulp	Coat
1	0.97	0.89	0.95
2	0.76	0.75	0.76
3	0.64	0.64	0.67
4	0.56	0.41	0.60
5	0.5	0.33	0.53
6	0.43	0.26	0.46
7	0.37	0.21	
8	0.31	0.16	
9	0.28		
10	0.23		
11	0.17		
12	0.12		
13	0.09		

Figure 1
TLC of ethanol extract of Aegle marmelos

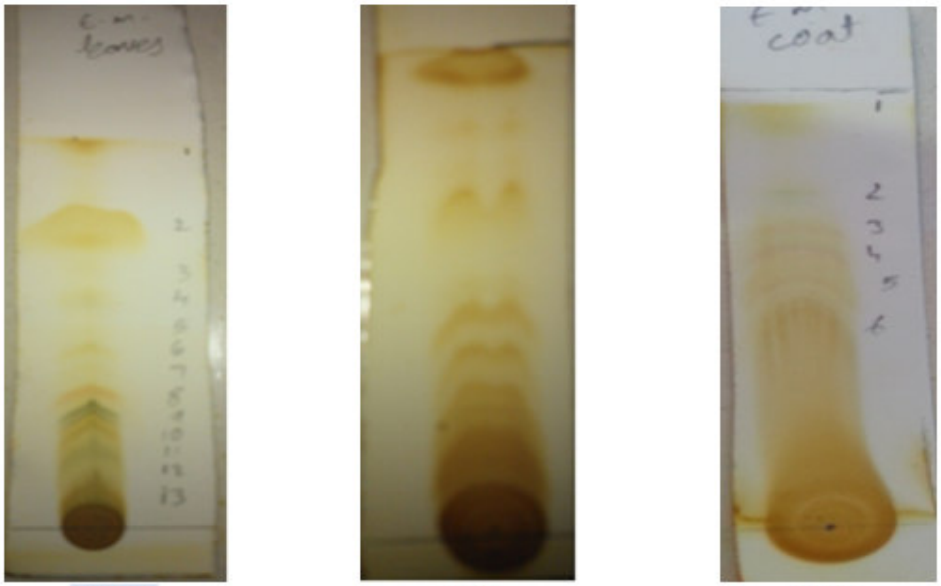


Table 5
COMPOUNDS

NAME	R_f /mins	Area%	Quality
Alpha-pinene	3.958	7.06	86
D-Limonene	5.614	2.08	99
Tetradeconoic acid	15.635	0.38	95
1 2 benzene di carboxilic acid	17.726	4.84	97
Octadecanoic acid	19.541	1.44	99
Di-n-octyl phtalate	22.766	12.80	96
Squalene	24.784	3.47	99

Figure 3
TLC BIO AUTOGRAPHY OF COAT

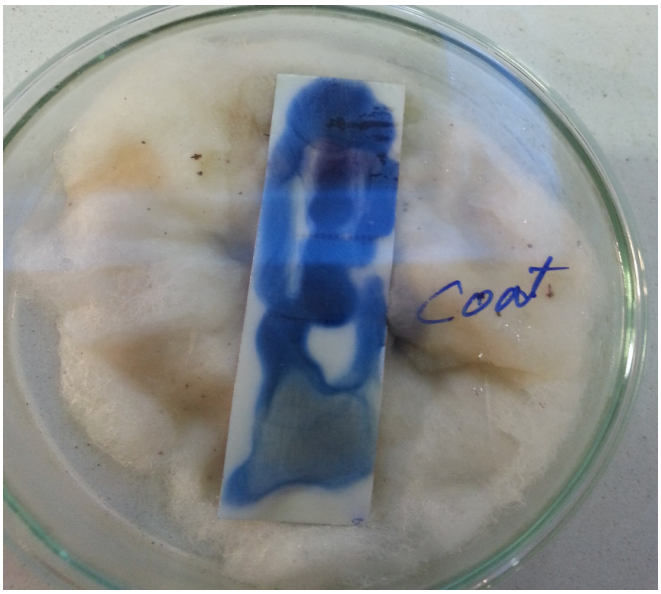
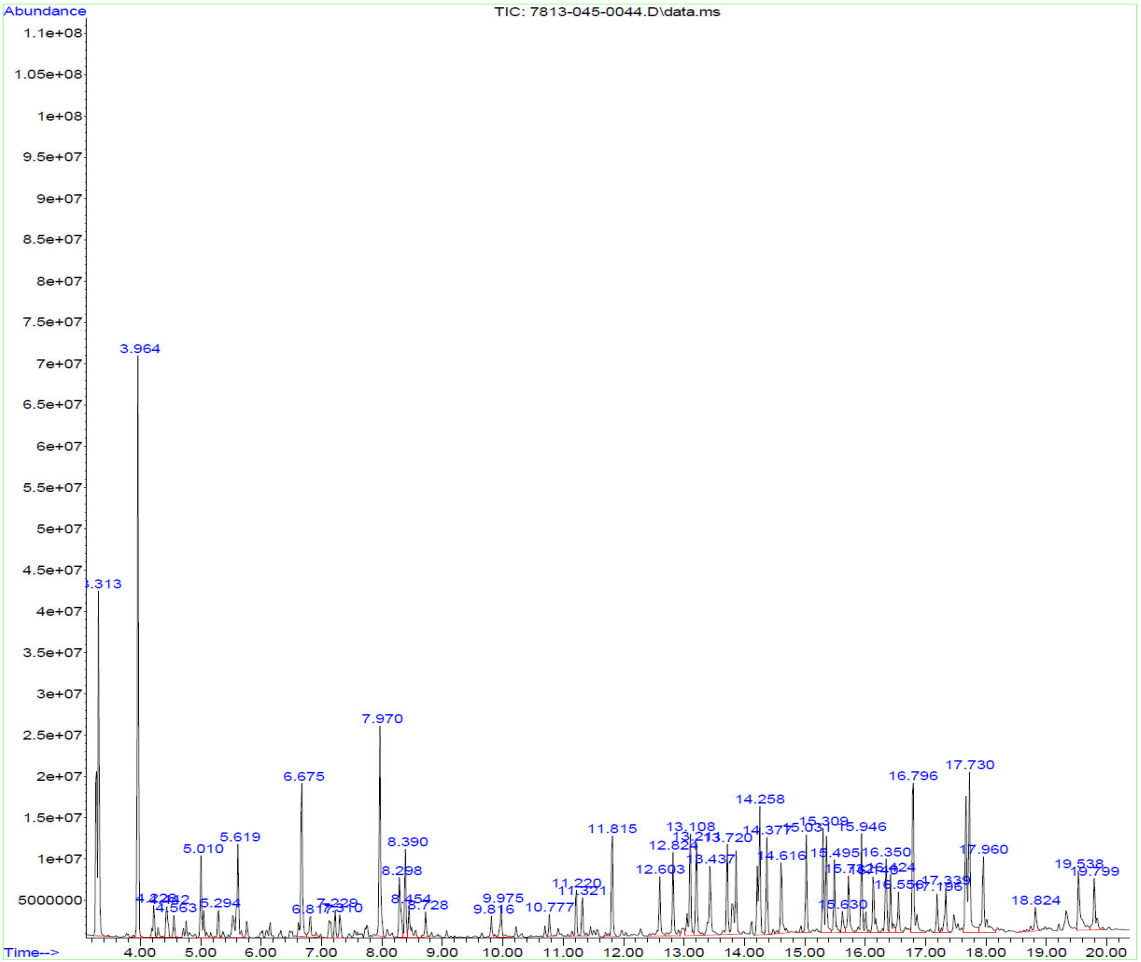


Figure 3
GC Mass spectra



CONCLUSION

In the present study qualitative phytochemical analysis and antimicrobial potentional of *A.marmeloes* plant part were carried out. Among various extract ethanol extract showed very good antimicrobial property whose MIC was found out. Further antimicrobial fraction was identified and extracted for GC-MS analysis which showed the presence of compound such as Di-n-octyl pthalate (12.8%), 1,2-benzenedicarboxilic acid (4.84%) with proved antimicrobial activity.

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