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Phytochemical screening, *in vitro* evaluation of antibacterial and GC-MS analysis of bioactive compounds from *maeruaapetala*roth (Jacobs) (*Capparaceae)*

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Abstract: This study was carried out with an objective to investigate the antibacterial activity, phytochemical screening, and GC-MS analysis of *maeruaapetala*roth (Jacobs) (*Capparaceae)*. The antibacterial activity was evaluated against gram positive organism methicillin-sensitive s*taphylococcus aureus* (MRSA) and gram negative organism *escherichia coli, klebsiella pneumonia, pseudomonas aerunginosa*by broth dilution and agar diffusion method. The butanol fraction showed maximum zone of inhibition 21mm against*E. coli* and 12mm againstMRSA. The phytochemical analysis was performed with plant extracts petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract.The results obtained from phytochemical screening revealed that the presence of steroids, tannin, phenol, saponins and flavonoids in the plant extracts. In addition to that qualitative analysis of methanolic crude extract of *m. apetala*was analyzed by using GC-MS showed that the presence of eighteen different phytochemical compounds. The components were identified by comparing their retention time and fragmentation patterns with those data stored in the National Institute of Standard and Technology (NIST) library. The reported chief constituents are Z-10-Pentadecen-1-ol, Phytol, Vitamin E and 1-Naphthalenepropanol, à-ethyldecahydro-5- (hydroxymethyl)-à,5,8a-trimethyl-2-methylene-, [1S-].
Keywords: Antibacterial activity, phytochemical screening, *maeruaapetala,Capparaceae,* MRSA, GC-MS analysis.

Introduction

Traditional medicine has provided a number of therapeutic applications for the control of infectious microbes, diabetics and other diseases. *Maerua* is an important ayurvedic drug used as one of the ingredients in many Ayurvedic preparations [1]. Ethnomedical survey reveals that *maerua*is used to cure various diseases such as fever, stomach ache, skin infections, urinary calculi, diabetes mellitus, epilepsy, pruritis, rigidity in lower limbs, and abdominal colic [2, 3]. Antibiotics are usually prescribed to restore the health of patients however the development of microbial resistance lead to reduce the persons individual immunity for that need to alter the treatment method by administering natural products. Based on the literatures newer antibacterial source is needed to reduce the microbial resistance. In the current study an attempt was made to evaluate the potency of the antibacterial activity of newly identified plant. So far, no attempts have been made to evaluate the phytochemical analysis, antibacterial activity and GC-MS analysis of *m. apetala*.

Materials and methods

Collection and Preparation of Plant material

The aerial parts of plant *maeruaapetala* were collected from the natural habitat of Kanyakumari District

in Tamilnadu, India. The plant was authenticated by Botanist Dr. V. Chelladurai, Research officer-Botany (Retd.), Central council for research in Ayurveda and Siddha, Government of India and the herbarium has been deposited at the herbarium of Entomology research Institute, Loyola College, Chennai (India). The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The aerial parts of plant were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

Preparation of extract

Shade-dried powder 250gm was filled in the thimble and extracted successively with petroleum ether, chloroform, and ethyl acetate and ethanol solvents in Soxhlet extractor for 48h. The solvent extracts were concentrated under reduced pressure and preserved at 5°C in airtight bottle until further use.

Phytochemical analysis:

For phenolic compounds: The extract (500 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution was added which showed the dark green color indicated the presence of phenolic compounds.

For alkaloids: One gram of extract was taken in a conical flask and ammonia solution added (3 mL). It was allowed to stand for few minutes to evaluate free alkaloids. Chloroform (10 mL) was added to the conical flask and shaken by hand then filtered. The chloroform was evaporated from the crude extract by water bath and added Mayer's reagent (3 mL) [4]. A cream color precipitation was obtained immediately that showed the presence of alkaloids.

For flavonoids: The extract (1 mL) was taken in a test tube and added few drop of dilute NaOH solution. .An intense yellow color was appeared in the test tube. It became colorless when on addition of a few drop of dilute acid that indicated the presence of flavonoids.

For saponins: The extract (1 mL) was taken in a test tube and diluted with 20 mL of distilled water. It was shaken by hand for 15 min. A foam layer was obtained on the top of the test tube. This foam layer indicated the presence of saponins.

For steroids: The crude plant extracts (1 mg) was taken in a test tube and dissolved with chloroform (10 mL), then added equal volume of concentrated sulphuric acid to the test tube by sides. [5] The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

For tannins: The plant extract (3 mL) was taken in a test tube and diluted with chloroform and added acetic anhydride (1 mL). Finally, sulphuric acid (1 mL) was added carefully by the side of test tube to the solution. A green color was formed which showed the presence of tannins.

For reducing sugars (Fehling's test): The aqueous ethanol extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution in a test tube. The solution was observed for a color reaction [6].

For triterpenoids: The plant extract (5 mg) was dissolved in chloroform (2 mL) and then acetic anhydride (1 mL) was added to it. Concentrated sulphuric acid (1 mL) was added to the solution. Formation of reddish violet color shows the presence of triterpenoids[6,7,8,9].

Preparation of media and inoculum

The liquid media used for this study was Muller Hinton Broth (MHB), Solid media was Mueller Hinton agar (MHA), obtained by solidifying the liquid media with 1.2% (w/v) agar.. The medium was completely dissolved by heating and sterilized by autoclaving at 121^{o}C for 15min. The bacterial isolates methicillin-sensitive s*taphylococcus aureus* (MRSA) and gram negative organism *escherichia coli, klebsiella pneumonia, pseudomonas aerunginosa obtained* from K.A.P. Visvanathan Medical College, Tiruchirappalli. The inoculum for each bacterial strain was prepared by taking four or five pure colonies from a stock culture using a sterile inoculation loop. The culture flask was then incubated for 24 hrs in an incubator at 37^{o}C. After the incubation the bacterial growth was identified by taking OD in spectrophotometer. These colonies were mixed in sterile normal saline. Gentle dilution was performed, till the turbidity was comparable visually to 0.5 to 1.0 McFarl

and turbidity standard (10).

Determination of Minimum Inhibitory Concentration (MIC) - Serial tube dilution method

The MIC of Ciprofloxacin and methanolic extract with respect to different test bacteria were determined by broth dilution methods. For broth dilution 0.1mL of standardized suspension of a strain (1Mcfarland standard) was added to each tube containing methanolic extracts at concentrations of 1000 µg/ml serially diluted up to 1.95µg/ml in MHB. The tubes were incubated at 37°C for 24 hours, and looked for visible growth after vortexing the tubes gently. The optical densities were measured by determining the absorbance at 530 nm in spectrophotometer (UV-Thermoscientific BIOMATE 35). The lowest concentration of methanolic extract in a tube or plate that failed to show any visible macroscopic growth was considered as its MIC. The MIC determination was performed in duplicate for each organism, and the experiment was repeated where necessary (11,12,13,14).

Plate method by well diffusion process

The sterile agar plate was prepared by pouring MHA to the sterile petriplate at 45^{o}C. After solidification, the agar plate was inoculated by spreading technique with sterile swab in different directions to get a uniform growth of MRSA and *Escherichia coli*. The required concentration of the extracts petroleum ether, chloroform, ethyl acetate, and ethanol were taken in prepared wells in agar plates by using micro pipette. The extract loaded inoculated plates were allowed to dry for 30 minutes and then kept in incubator for 18 hours at 37°C. The plates were viewed against a black background and zone of inhibition were measured by zone reader (11,12,13,14).

GC-MS analysis

The Clarus 500 GC (Perkin Elmer) used in this analysis (15). It employed a fused silica column packed with Elite -5MS (5%Diphenyl / 95% Dimethyl poly siloxane, 30mm x 0.25mm x0.25µm df) and the components were separated using helium as carrier gas at a constant flow of 1 mL/ min. The 2 µL sample extract injected into the instrument. It was detected by the turbo gold mass detector (Perkin Elmer) with the aid of Turbo mass 5.2 software. During the GC process the oven was maintained at a temperature of 110^{0}c with 2 min holding. The injector temperature was set at 250^{0}c. The different parameters involved in the operation of the Clarus 500 MS were also standardized. The inlet line temperature was 200^{0}C and source temperature was 200^{0}C. Mass spectra were taken at 70 eV; a scan period of 0.5 s and fragments from 45-450 Da. The MS detection was completed in 36 min. The NIST ver. 2.0 year 2005 library was employed for detection(15).

Results:

Results obtained from the phytochemical test results were tabulated in table.1

Table:1 The preliminary phytochemical screening of the methanolic extracts of *m. apetala*

Phytochemical test	Pet. ether extract	Chloroform extract	Ethyl acetate extract	methanol extract	Aqueous extract
Alkaloids	_	_	+	+	+
Tannins	_	_	+	+	+
Flavonoids	_	_	_	+	+
Saponins	+	+	+	+	_
Terpenoids	_	+	+	+	_
Reducing sugars	_	_	_	+	+
Steroids	+	+	+	+	_

\+ **- presence**
_ **- absence**

***In vitro* determination of antibacterial action of *m. apetala*extract:**

Minimum inhibitory concentration of methanolic extract was determined from the stock solution 1000 µg/ml serially diluted up to 3.95 µg/ml showed the antibacterial effect against MRSA and *E. coli*. The MIC of ciprofloxacin 3.95 to 15.625µg/ml against tested bacterias and MIC of the methanolic extract was 500µg/ml

against *E. coli*, 1000µg/ml for MRSA. The zone of inhibition of the extracts against tested organisms were determined and shown in Table 2 and fig 1, &2.

Table: 2 Antibacterial activity of various extracts of aerial parts of plant *m.apetala*(100µg /ml) against bacterial species tested by well diffusion assay

Micro organism	Zone of inhibition in mm(100µg/ml)					
	Pet.ether Extract	**Chloroform Extract**	**Ethyl acetate Extract**	**Methanol Extract**	**Aqueous extract**	**Butanol fraction**
Klebsiella pneumonia	NA	10±0.12	18±0.23	9±0.15	ND	ND
Pseudomonas aerunginosa	NA	17±0.5	18.5±0.14	9±0.24	ND	ND
Escherichia coli	NA	16±0.30	12±0.65	12±0.31	11±0.12	21±0.19
MRSA	NA	9±0.25	9±0.45	8±0.26	10±0.32	12±0.34

Fig.1&2: The zone of inhibition of the extracts against tested organisms

Table :3Componentsidentifiedinthe methanolic extract of aerial parts of *M.apetala* usingGCMS

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area
1.	11.01	2-Tridecen-1-ol, (E)-	$C_{13}H_{26}O$	1	2.22
2.	11.26	Z-10-Pentadecen-1-ol	$C_{15}H_{30}O$	2	1.13
3.	11.46	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	2	1.42
4.	12.72	Octadecanoicacid, ethylester	$C_{20}H_{40}O_2$	3	30.31
5.	13.89	n-Hexadecanoicacid	$C_{16}H_{32}O_2$	2	0.79
6.	14.16	Phytol	$C_{20}H_{40}O$	2	4.09
7.	14.74	9,12-Octadecadienoicacid,methylester, (E,E)-	$C_{19}H_{34}O_2$	2	6.80
8.	14.81	E-11-Hexadecenoicacid, ethylester	$C_{18}H_{34}O_2$	2	15.78
9.	15.15	Docosanoicacid, ethylester	$C_{24}H_{48}O_2$	3	3.25
10	17.41	cisZ-11,12-Epoxytetradecan-1-ol	$C_{14}H_{28}O_2$	2	1.33
11	17.83	Z-8-Methyl-9-tetradecenoicacid	$C_{15}H_{28}O_2$	2	1.05
12.	20.60	Pentadecanoic acid, 2,6,10,14-tetramethyl-,methyl ester	$C_{20}H_{40}O_2$	3	1.31
13.	24.16	9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethylester,(Z,Z,Z)-	$C_{25}H_{40}O_6$	436	1.43
14.	26.77	2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1- pentenyl]cyclohexan-1-	$C_{14}H_{24}O_4$	2	1.52
15.	27.65	Vitamin E	$C_{29}H_{50}O_2$	43	7.76

16.	30.51	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3- hydroxy-, (3á,17á)-	$C_{22}H_{32}O_2$	32	3.27
17.	31.44	1-Naphthalenepropanol, à-ethyldecahydro-5-(hydroxymethyl)-à,5,8a-trimethyl-2-methylene-, [1S- [1à(S*),4aá,5à,8aà]]-	$C_{20}H_{36}O_2$	308	9.59
18	32.02	4,8,12-Tetradecatrien-1-ol, 5,9,13-trimethyl-	$C_{17}H_{30}O$	2	6.96

The GC-MS investigation directed to recognition of lot of compounds from GC fractions of the methanolic extract of *M. apetala*. These compounds were acknowledged through mass spectrum attached with GC in fig 3. The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (%) are accessible in Table 3. The present study characterized the chemical profile of *M. apetala* using GC-MS. The GC chromatogram shows the relative concentration of various compounds getting eluted as a function of retention time. The heights of the peak point out the relative concentration of the presented components. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. These mass spectra are figure print of that compound which can be identified from the National Institute of Standards and Technology (NIST) data library fig 4-8. Table 4 shows the biological activity of the predicted compounds.

GC MS Analysis

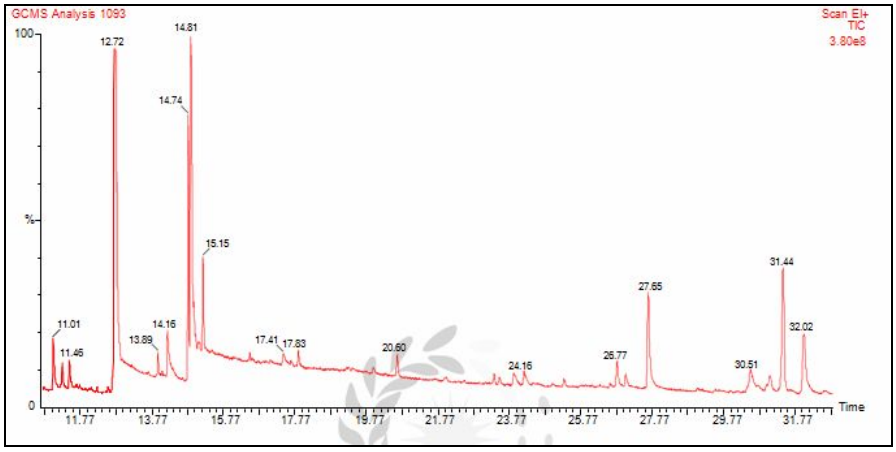
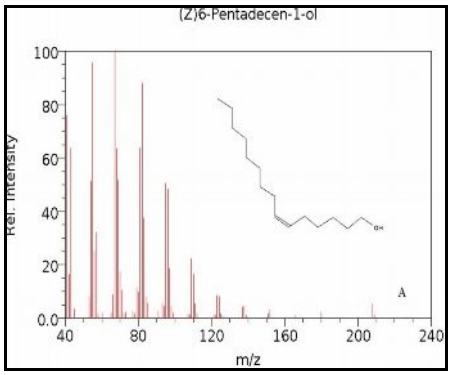
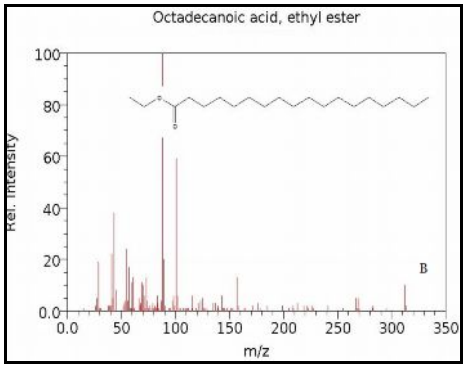


Fig 3- The GC-MS chromatogram of methanolic extract of *C. retusa*





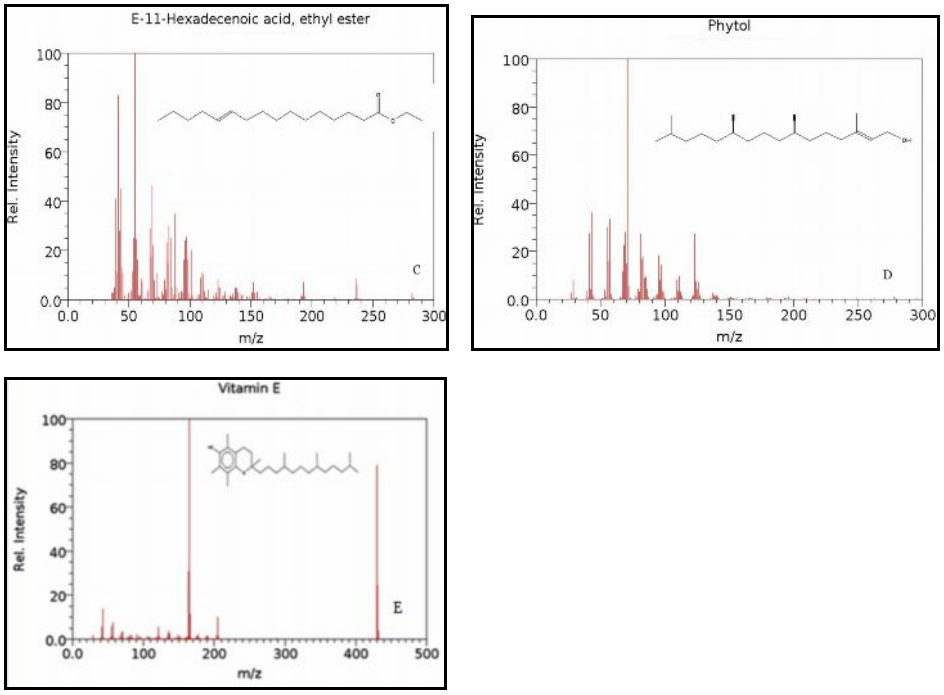


Fig 4-8: The individual fragmentation pattern of the important compounds

Table 4: Reported activity of phyto-components identified in ethanol extract of aerial parts of *M. apetala.* NA-No activity

S.No	Name of the compound	Biological activity reported
1.	2-Tridecen-1-ol, (E)-	NA
2.	Z-10-Pentadecen-1-ol	NA
3.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Anti microbial, Antinflammatory, Anti oxidant, Diuretic
4.	Octadecanoicacid, ethylester	Anti microbial, Anti oxidant Hypertriglyceridemia
5.	n-Hexadecanoicacid	Anti microbial, Antinflammatory, rheumatic
6.	Phytol	Anti microbial, Antinflammatory, Anti oxidant, Diuretic
7.	9,12-Octadecadienoicacid,methylester, (E,E)-	Anti microbial, Anti oxidant
8.	E-11-Hexadecenoicacid, ethylester	Anti oxidant
9.	Docosanoicacid, ethylester	Anti microbial, Anti oxidant
10.	cisZ-11,12-Epoxytetradecan-1-ol	NA
11.	Z-8-Methyl-9-tetradecenoicacid	Anti protozoal activity
12.	Pentadecanoic acid, 2,6,10,14-tetramethyl- methyl ester	Anti microbial
13.	9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethylester,(Z,Z,)-	Anti microbial
14.	2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1- pentenyl]cyclohexan-1-perhydrol	NA

15.	Vitamin E	Antiageing, analgesic, antidiabetic, anti inflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, antiulcerogenic, antispasmodic, antibronchitic and anticoronary, hypocholesterolemic, vasodilator and hepatoprotective
16.	Spiro[androst-5-ene-17,1'-cyclobutan]-	NA
17.	1-Naphthalenepropanol, à-ethyldecahydro-5- (hydroxymethyl)-à,5,8a-trimethyl-2-methylene-, [1S- [1à(S*),4aá,5à,8aà]]-	NA
18.	4,8,12-Tetradecatrien-1-ol, 5,9,13-trimethyl-	NA

Discussion:

Medicinal plants have the ability to cure bacterial resistance with many no of antibiotics. The antibacterial effect of plant extract mainly depends on the plant components. In the present study the efficacy of plant extracts was determined by MIC and quantitatively measured the diameter of the inhibition zones around the wells in Table.2. In that MRSA strains inhibited by the methanolic extract 8mm. But against the *Escherichia coli* the inhibition was 12mm, butanol fraction have the ablity to inhibit the sensitive strain at marked potency. The above results show that the activity of hydroalcohol extracts of *Cassia fistula* shows significant antibacterial and antifungal activities. This study also shows the presence of different phytochemicals with biological activity that can be of valuable therapeutic index. The result of phytochemicals in the present investigation showed that the plant contains more or less same components like saponin, terpenoids, steroids, glycosides, flavonoids, proteins, and amino acids. Results show that plant rich in tannin and phenolic compounds have been shown to possess antimicrobial activities against a number of microorganisms (16). Gas Chromatography- Mass Spectrometry (GC-MS) is a valuable tool for reliable detection of bioactive constituents. This study results were interpreted. By interpreting these compounds, it is found that *m. apetala*possesses various therapeutical applications.

Conclusions

The methanolic extract of *m. apetala*could be used as a potential antibacterial source for various infections. This analysis showed the existence of various compounds with different chemical structures. Further research is needed to elucidate the mechanism of anti microbial actions and isolation of individual phytochemical constituents which may act as templates for novel drug molecules.

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