

Isolation, Identification of Secondary Metabolites and Anti Bacterial Property of *Memecylon edule* Leaves Extract

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Abstract

The present study aims to determine the preliminary phytochemical screening, revealed the presence of alkaloids, flavanoids, saponins, glycosides and oil compounds using FT-IR and GC-MS analyses. The methanolic extract of *Memecylon edule* to check the antibacterial activity for the maximum inhibitory concentration against E.coli, (15mm) and Staphylococcus aureus (14mm) with minimum inhibitory concentration 6.25µg/ml. The minimum inhibitory growth against *Pseudomonas auroginosa* and *Klebsella pneumoniae* (12mm, 11mm) respectively. The result of GC-MS study was confirmed the squalene, palmitic acid, fatty acid and also related functional group were identified using FT-IR spectra. Hence in this research work suggests the traditional plant *Memecylon edule* contain various phytochemical compounds. It represents effect of pathogenic bacteria to inhibit the growth response.

Keywords: *Memecylon edule*, antibacterial, phytochemicals, GC-MS, FT-IR, squalene, palmitic acid.

1. Introduction

Plant secondary metabolites present chemicals and pharmaceutical properties interesting for human health compounds belonging to the terpenoids, alkaloids and flavanoids are currently used as drug or as dietary supplements to cure or prevent various chronic and acute diseases (Raskin et al., 2002). Many thousands of wild plants have great economic and cultural importance, providing food, medicine, fuel, and clothing around the world. Plants also play a key role in maintaining the earth's environmental balance and ecosystem stability. They also provide habitats for the world's animal and insect life (Ahmed et al., 2001).

Memecylon edule (Melastomataceae) are growing as small shrubs or trees in tropical and sub tropical regions in the various part of India, Malaysia (clausing G. et al., 2001). M.edule from india is mostly found in Karnataka, Andhra Pradesh and Tamil Nadu (Ranjit Daniels RJ et al., 2007). The plant is well known for its use as a mordant in silk dyeing in the Northeast of Thailand. *Memecylon edule* trees are not only beautiful but also useful. It provides hard timber used for building house boat and walking sticks. Phytochemical screened for alkaloids saponins, steroid components, essential oils, glycosides and hydrogen cyanide in melastomataceae families (Ikram et al., 1998).

A decoction prepared from leaves of *Memecylon edule* is used internally for gonorrhoea, stomach pain and fever symptoms. Roots of this

plant are used for the excessive menstrual discharge (Khare CP 2007). Roots extract and heart wood has been also used to get relief from fever symptoms of several diseases such as common cold, measles, chicken box (Suriyajunrathong et al., 1999).

The traditional uses of *Memecylon edule* leaves as an anti burning and wound healing medicine without scars. Leaves of *M.edule* have strong anti-inflammatory and analgesic activity (Naulkeaw et al., 2009). Secondary metabolites of *Memecylon edule* showed the presence of flavonoids and triterpenoids in crude ethyl acetate extract of leaves (Naulkeaw et al., 2009). Fruits of this plant are used cooling astringent and leaves are used for the treatment of anti leucorrhoeic, spasmolytic, hypoglycemic and lotion prepared from leaves was used for ophthalmia and conjunctivitis. The aim of the work was to carry out the phytochemical investigation in methanolic extract of leaves of *Memecylon edule* and also evaluated for its antibacterial activity.

2. Materials and methods

2.1 Plant and preparation of extract

The plant *Memecylon edule* leaves were collected in July 2010 from vandalur shrubby forest near in Chennai, Tamil Nadu, India. The extract was taken using hot extraction by Soxhlet apparatus. Fresh *Memecylon edule* leaves samples were collected (1kg) thoroughly washed with running tap water. Shade dried at room

temperature and ground in a mixer grinder. The powder (250g) was extracted with 750ml of methanol (1:3 w/v) for 48 h at 60°C. The extract was filtered through a Buchner funnel with Whatman no.1 filter paper. The filtrate was evaporated to dryness under reduced pressure using rotary vacuum evaporator at 60°C. Finally crude extract was obtained. The crude extract was stored at 40°C until further use.

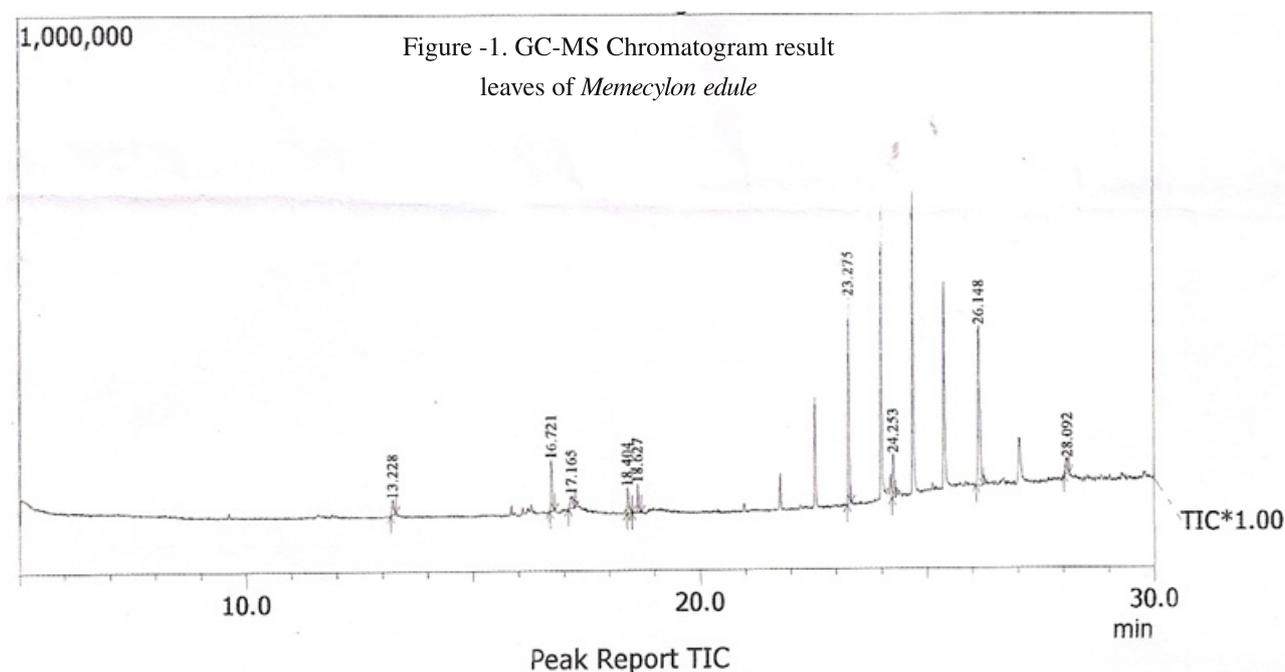
2.2 Phytochemical Screening

Chemical tests were carried out on the methanolic extract leaves of *Memecylon edule* using standard procedures to identify the phytochemical constituents such as alkaloids, flavanoids, saponins, tannins, glycosides, triterpenoids and essential oil, described by (Harborne 1973, Evans 1989).

2.3 Instrumentation Analysis

2.3.1 GC-MS Analysis

GC-MS Analysis was performed on a 188 LSD ES GC-3 with a capillary column ES 23m x 0.2mm 0.5µl thickness. Helium was used as a carrier gas with a temperature 100-315°C at 5°C min⁻¹ sample glow rate at 1µl/min. GC-MS result was based on the interpretation of the mass spectra fragmentation followed by comparisons of the obtained spectra with those of authentic sample. The sample was analyzed by Sargam Laboratory Chennai, Tamilnadu, India.



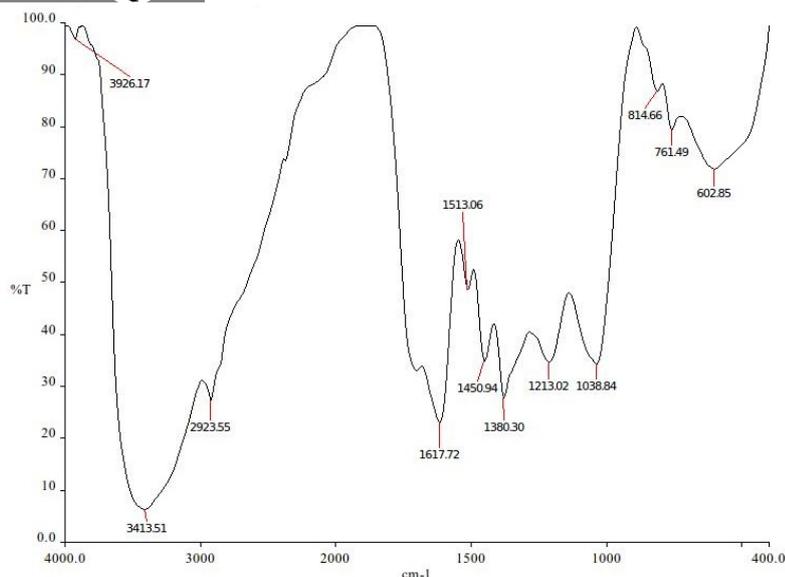


Figure - 2. Depicts FT-IR spectroscopy results Memecylon edule crude extract

S. No.	Phytochemical Compounds	Results Obtained
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Terpenoids	-
5	Saponins	+
6	Glycosides	+
7	Phlobatannins	-
8	Oils	+

Table -1. Phytochemical screening of M.edule leaf extract

2.3.2 FT-IR Spectra

FT-IR Spectrum of the *Memecylon edule* crude extract were taken FT-IR Gms 8000 Spectrometer using 800 reflectance and integrated using Kubelck Munk conversion. FT-IR Spectra we get a plot of transmittance version wave number, and each functional group has its own absorption value in IR region.

2.4 Antibacterial Assay

Antibacterial activity of methanolic extract of plant leaves was determined by agar well diffusion method (Anon 1996). Wells were made in nutrient agar plate using sterile cork borer (5mm), and inoculum containing 106 CFU/ μ l of bacteria were spread on the solid plates with a sterile cotton swab. The extract used various concentrations like 50 μ l, 75 μ l and 100 μ l respectively (1% solution prepared in extract). Different concentration added in the different well. The treatment also include 50 μ l of methanol added well which served as control. The plates were incubated for 24 hrs at 37oC and zone of inhibition around the wells were measured in mm scale (millimeter). For each treatment three replicates were maintained. The data was subjected to statistical analysis using SPSS for windows software.

Minimum Inhibitory Concentration

Minimum Inhibitory Concentration (MIC) was performed according to the Standard Reference

methods. The required concentration of the extracts was dissolved in 2% DMSO and dilutes to give serial two fold dilutions that were added to each medium well. Each well was inoculated with 5 μ l of suspension containing 107 CFU/ μ l of bacteria. The antibacterial agent streptomycin was included in the assays as positive control. For bacteria the plates were incubated for 24hrs at 37o C. The MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate.

3. Results

The phytochemical analysis data was indicate that the methanolic extract of *Memecylon edule* leaves containing various secondary metabolites such as alkaloids, flavanoids, saponins, glycosides, tanrins, triterpenoids shows Table – 1.

Methanolic crude extract of *Memecylon edule* contained various metabolites and trace elements (R.S David Paulraj et al., 2010). Based on GC-MS total in Chromato gram (TIC) (Fig-1) of plant clude extract of *Memecylon edule* leaves showed 16 major peaks indicating its major secondary metabolites such falty acids, Squalue, Carboxylic compound, steroids and glycosides. Some of the trace components where also containing 5 peaks, pathalic acid rt 13.228, palmitic acid rt 16.721, Hexa deconoic acid rt 17.165, 9-

octadecanoic acid, rt 18.404, Tetra tetra contane rt 23.275, squalene rt 24.253, Hexa tri contane rt 26.148, Hexa decane rt 28.092 respectively. (Table 2).

Peak #	Retention time Rt	Area	Area%	Name of the Compound
1	13.228	95162	3.64	Pathalic acid
2	16.721	220993	8.45	Palmitic acid, methyl ester
3	17.165	134019	5.13	Hexa decanoic acid
4	18.404	112297	5.11	9-Octadecanoic acid
5	18.627	698062	4.30	9-Octadecanoic acid
6	23.275	185806	26.70	Tetra tetra contane
7	24.253	921514	7.11	Squalene
8	26.148	112728	35.25	Hex atria contane
9	28.092	2614289	4.31	Hexa decane

Table - 2. GC-MS results obtained from Memecylon edule methanolic crude extract

On the basis of the FT-IR Spectra analysis of *Memecylon edule* crude extract the maximum peak value 3413.51 and minimum peak value is 761.49 cm⁻¹. (Fig.2) the

S. No	Microorganisms	Zone of Inhibition(mm)					MIC (µg/ml)
		50 µl	75 µl	100 µl	Control		
1	<i>Escherichia coli</i>	12	13	16	1	6.125	
2	<i>P. auroginosa</i>	11	11.5	12	2	-	
3	<i>Bacillus subtilis</i>	11	13	14	2	6.125	
4	<i>Staphylococua aureus</i>	13	14	15	-	3.245	
5	<i>Klesella pneumoniae</i>	11	13	14	3	-	

Table - 3. Functional group Identification of leaves of *Memecylon edule* crude extract using FT-IR

FT-IR studies confirmed presence of amines, aromatic, alcohol compound, phenolic compound and amides as shown in Table 3.

The result of antimicrobial activity indicated that methanolic extract of *Memecylon edule* showed promising activity methanolic crude extract inhibit the growth of tested bacteria such as E-coli (14mm) and Staphylococcus aureus (12mm) high inhibition rate against in bacteria and followed by Bacillus subtilis (10mm), Pseudomonas auroginosa (9mm) and klebsella pneumoniae (8mm) moderate growth of bacteria. The lowest MIC value (0.312 µg/ml) was in plant extract against E-coli strain.

4. Discussion

In earlier phytochemicals explore of whole *Memecylon edule* genus evidenced the presence of 13 fattyacids, 12 methyl tetradecanoate, glucose, aminoacids, carotenoids, glycosides and saponins (stephan L., 1981). The strongest antibacterial effect was found in chloroform extract of seed extract of *Memecylon edule* against Bacillus subtilis and the weekest activity against Vibrio chlorae. In case the anti bacterial studies have shown that the chloroform and ethyl acetate extract against werite less than ampicillin (Tamizhamudu elavazhagan et al.,2010).Memecylon malabaricum (Malestomataceae) shown antibacterial agent (Kiran kumar Hullatti et al., 2004).

5. Conclusion

The present work was explored the bioactive compounds in methanolic extract of *Memecylon edule* and antibacterial activity against harmful human pathogens. The crude extract os *Memecylon edule* GC-MS result was showed the presence of long chain fatty acids, steroids and saponins. In that compounds are responsible for inhibit growth of disease causative agents. From the study conclude methanolic extract of *Memecylon edule* act as good antimicrobial agents and novel pharmacological product development plant resources.

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