Original Article

Chemical Characterization, Antibacterial, Antibiofilm Activities of the Ethyl Acetate Flowers Extract of Memecylon Edule

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Abstract

Memecylon edule (Melastamataceae family) is a flower extract with botanical and phytochemical properties. The current work used a bioassay-guided isolation technique to examine the spectrum characterization, antibacterial activity antibiofilm from the memecylon module flowers. Plants used in traditional medicine are a great source for the discovery of novel therapeutic substances. The purpose of this study was to determine the anti-microbial effects of an EtOAc extract of Memecylon edule. Furthermore, the compound's mode of action must be examined by extracting the extract and analyzing the active element that plays the most important function. Different spectrum analyses, including The functional groups in the extract of memecylon edule study were also identified by further UV, FT-IR, NMR, and GC-MS analysis, were used to determine the structure of the isolated chemical analysis. The findings of this study imply that bioactive chemicals can be used in the pharmaceutical sector to produce plant-based medications to treat illnesses caused by microbes.

Keywords: Memecylon edule, Flower, Ethyl acetate, Anti-microbial, NMR, GC-MS

Introduction

Memecylon edule, a small tree or shrub belonging to the Melastomataceae family, possesses medicinal properties. Within the Melastomataceae family, Memecylon edule is a tiny tree or shrub having therapeutic qualities [1- 4]. It is also referred to as puvai kaya in Tamil and ironwood tree in English [5]. Medicinal substances found in plants are referred to as secondary metabolites or biologically active compounds. A few of the bioactive substances found in M. edule include triterpenes, tannins, and flavonoids [6]. A previous investigation of the phytochemistry of the whole Memecylon genus revealed the presence of glucose, carotenoids, fatty acids, 12 methyltetradeconate, amino acids, and perhaps undefinable saponins [7]. Memecylon edule endophytic bacteria were used to isolate putative antibiotic compounds, which were then studied in this paper. The little evergreen shrub Memecylon edule which is found growing in tropical and subtropical areas of the world, has been utilized in traditional medicine. There is sample evidence of its biological characteristics, including its antibacterial, apoptogenic, and antioxidant effects [8-11], We discovered, isolated, and examined endophytic bacteria for their potential antibacterial action against pathogenic microorganisms. GC-MS was used to identify the chemical components of the active fractions that were separated from an isolate's EtoAc extract. Next, NMR was used to investigate the active fraction's initial mechanism of action produced on an industrial basis as a potential novel anti-microbial medication options.

Materials and Methods

Plant material Flowers of Memecylon edule (Melastomataceae) **(Figure 1)** were collected from the Kottiyal Village (Ariyalur dt). A voucher specimen (KP008) is deposited in, Sri Indu Pharmacy College, Hyderabad. The flowers of Memecylon edule were dried under airflow between 41 and 52 ◦C and pulverized (sieve number 60). Following the crushed material's 385 g, solvents (2 L each) with varying polarity were macerated at room temperature hexane (Hex), then parts of 500 mL each of ethyl acetate (EtOAc), methanol (MeOH), and 50% aqueous methanol (MeOH50) for two weeks while being stirred every day. Each solvent's parts were mixed. A rotary evaporator was used to mix and evaporate portions of each solvent until they were

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dry. Before being used, each portion of Memecylon edule that was so produced was stored in tightly sealed containers in a refrigerator.

Figure 1. Pictorial representation of flowers of Memecylon edule

Microbial Strains

Bacterial strains used for testing included Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris, Vibrio cholera, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas chloroform extracts of flowers of Memecylon edule

Antibacterial Assay

The disc diffusion technique was used to assess the antibacterial activity of the aforementioned chloroform extracts of Memecylon edule flowers against a set of 10 chosen strains of bacteria [11, 12]. Following a 24-hour incubation period at 35˚ C for each of the 10 bacterial strains, the nutrient agar plates were allowed to dry [13, 14]. 200 µl of the ready-made inoculum was applied to the sterile Petri plates to inoculate the nutrient agar. To test the infusions, each extract was made at a concentration of 50 mg/ml. For the antibacterial assay, blank discs with a 6 mm diameter and DMSO impregnation were utilized as negative controls, and discs containing ampicillin (10 mg) as positive controls [15, 16]. The disc was filled with 20 microliters of 50 milligrams per milliliter of crude extract, left to air dry, and then Whatman filter paper No. 1 filter paper discs were gently placed on the surface of an agar plate that had been seeded. After the plates were inspected the width of the ensuing zones of inhibition (mm) was determined after 24 h. Standard ampicillin at a dosage of 10 mg/ml was compared to the effects of plant extracts containing 50 mg/ml of the zone of inhibition. Calculating the average values of the antibacterial activity of the chloroform and ethyl acetate extracts of seeds included experimenting three times in triplicate.

Antibiofilm Activity Assay

The method described below was used to assess the biofilm inhibitory capacity against four pathogenic microbial strains: P. aeruginosa and E. coli were Gram-negative bacteria, while S. aureus and Bacillus subtilis were Gram-positive bacteria [17, 18].

GC-MS Analysis for Chemical Characterization

The described protocols were followed for conducting the GC-MS test [19]. With a 30 m, 0.251 mm, and 0.1 mm film thickness, Thermo Scientific Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column was utilized. For GC/MS identification, an electron ionization device with an ionization energy of 70 eV was used. Helium gas was utilized as the carrier gas, with a steady flow rate of one milliliter per minute. The injector and MS transfer line temperature was set to 280°C. The oven was set to start at 50°C and hold it there for two minutes. It was then instructed to increase the temperature to 150°C at a rate of 7°C per minute, 270°C at a rate of 5°C per minute, and finally, 310°C, which was the final temperature, at a rate of 3.5°C per minute (hold it for ten minutes). The quantitative determination of each discovered chemical was examined using a percent relative peak area. Using the Wiley library data from the GC-MS technique and the irrelative retention time and mass spectra from the National Institute of Standards and Technology (NIST), a preliminary identification of the components was made.

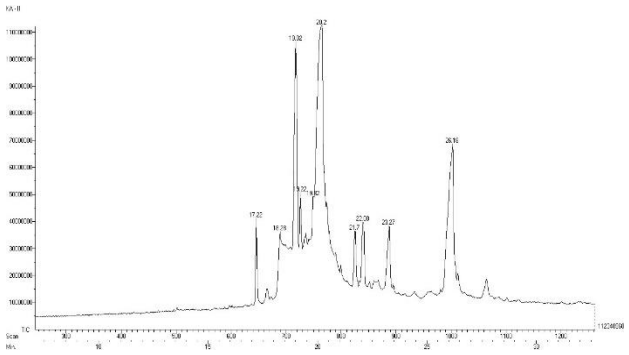

Figure 2. GC-Chromatogram analysis of the fraction of memecylon edule folwers extract.

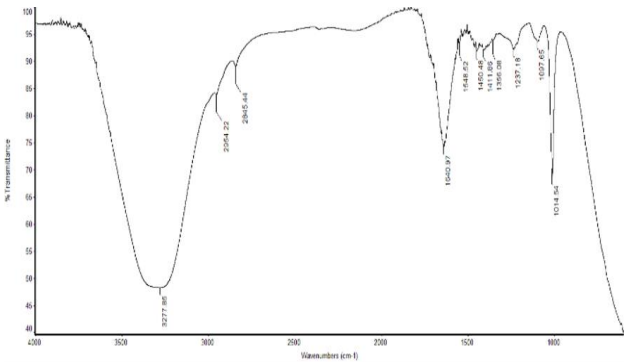

Figure 3. FT-IR analysis of the fraction of memecylon edule folwers ext

Table 1. FTIR peak values and functional groups of ethyl acetate flower extract of memecylon edule

	Wavenumber (cm-1)	Functional group/modeof vibration
1	3277	-NH_2,NH_2Symmetric stretch
2	2954	-CH and–CH_2-, -CH_3- Antisymmetric and symmetric stretching

3	2845	-CH_3, CH Stretch
4	1640	N-H,NH Deformation
5	1548	C = C, C = C Stretch
6	1450	C=C stretch
7	1411	–CH2 bending centering
8	1350	t-butyl groups, CH3 deformations
9	1237	C-N, C-N Stretch
10	1097	C-O stretch

Table 2. Characteristics of the GCMS analysis of the fraction of memecylon edule

S.No	RT	M.weight	Identified compounds
1	17.21	198.31	Undecanoic acid 10 -methyl- methyl ester
2	19.12	326.4	Dasycarpidan-1-methanol, acetate(ester)
3	20.3	282.46	Oleic acid
4	21.7	356.2	9octadecenoic acid (z)-2-hydroxy-1-(hydroxymethyl)ethyl ester
5	23.27	581.0	Hexadecanoic acid 1-(hydroxymethyl)-1 2-ethanediyl ester

GC-MS investigation of the Ethyl acetate extract of memecylon edule.

Results and Discussion

Table 2 displays the chemical formula, molecular weight, and retention duration of the bioactive substances identified by GC-MS **(Figure 2)**. This analysis was used to identify unique components in the fermented product. One of the main compounds in the FP is the alkaloid dasycarpidan-1-methanol (acetate). Previous research found that dasycarpidan-1-methanol possesses antibacterial properties, which eluted at a retention time of 19.12 min [20, 21]. 9-octadecenoic acid (z)-2-hydroxy-1-(hydroxymethyl)ethyl ester reportedly possesses antibacterial properties. It had an eluted time of 21.07 minutes [22]. Undecanoic acid 10-methyl- methyl ester was selected for the lipid portion as undecenoic acid derivatives are powerful bioactive substances. It took 17.21 minutes to elute [23, 24]. The antibacterial properties of oleic acid, a prevalent monounsaturated fatty acid found in a variety of plant, animal, and vegetable oils and fats. was identified, with a maximum RT of 20.3 min [25, 26].

Table 1 present the results of the vibration spectroscopy (FTIR) analysis of the ethyl acetate floral extract of Memtecylon edule. is well known for its capacity to identify crucial functional groups concealed in biological, synthetic, and plant extracts. The FTIR spectra of the ethyl acetate extract, displayed in **Figure 3**, unraveled twelve peaks that corresponded to ten different types of bonds, or functional groups, in compounds. The range of peaks for the ethyl acetate extract is 3277.85, 2954.22, 2845.44, 1640.97, 1548.52, 1450.48, 11.66, 1350.08, 1237.18, and 1097.65 cm-1. The Ethyl Acetate Extract's 10 functional groups include phenols and alcohols, amino acids, aliphatic compounds, hydrohalides, isonitriles, β-lactones, vinyl ethers, t-butyl groups, sulfones, and primary amines. The following vibrational functional groups result in the production of the OH stretch, =CH-H stretch, and CH stretch, respectively: alcohols/phenols, pathogenic microbial strains aromatic/unsaturated hydrocarbons, ether/amine, and so on. The OH group's capacity to form hydrogen bonds is probably what gives rise to the inhibitory activity against pathogenic microorganisms shown in both methanol and ethanol extract [16, 27, 28].

Antibacterial and Antibiofilm Activities

Memeticylon edule clam ethyl acetate flower extract was tested in vitro against a variety of pathogenic microorganisms, including four Gram-negative, one Gram-positive, and one yeast. According to our research, the studied extract's antibacterial efficacy varied when tested against different microbial strains in the (blank/tested extract) manner: (0.552/0.232), (0.452/0.369), (1.148/0.774), (1.077/0.221), (1.267/0.282), and (0.892/0.385), respectively, for E. coli, P. aeruginosa, P. vulgaris, K. pneumoniae, S. aureus, and C. albicans **(Table 3)**. Conversely, the Media Transfer Protocol (MTP) assay was employed to assess the antibiofilm activity **(Table 4)** at a concentration of 100 µg/ml. This experiment evaluated the tested extract's degree of malice as observed from the standpoint of biofilm growth, as well as its capacity to suppress the formation of biofilms against a few pathogenic bacteria strains under research. According to the data collected, **Table 3** shows the extract's strong inhibitory percentage against S. aureus, which was 94.48%. The percentages against B. subtilis, P. aeruginosa, and E. coli are 66.51%, 48.10%, and 37.40%, respectively. In conclusion, ethyl acetate floral extract clams from Memeticylon edule are likely a great choice for inhibiting cell adhesion and biofilm growth.

Table 3. Memecylon edule mussels' ethyl acetate floral extract has in vitro antibacterial action against a few harmful microbial

	Pathogenic microbial strains					
Test extract/ blank	**Escherichia coli**	**Pseudomonas aeruginosa**	**Proteus vulgaris**	**Klebsiella pneumoniae**	**Staphylococcus aureus**	**Candida albicans**
Blank	0.552	0.452	1.148	1.077	1.267	0.892
Methanol extract	0.232	0.369	0.774	0.221	0.282	0.385

Table 4. Antibiofilm activity of the ethyl acetate flowers extract of memecylon edule against some pathogenic microbial strains

	Microbial strains/biofilm inhibitory (%)			
Tested extract	Staphylococcus aureus	Bacillus subtilis	Escherichia coli	Pseudomonas aeruginosa
Methanol extract	94.48%,	94.48%,	48.10%,	37.40%,

Conclusion

The obtained results demonstrated that the extract has remarkable antibacterial and antibiofilm activities. It also revealed that the ethyl acetate flower extract of the memecyclon edule contains a variety of bioactive molecules, including Dasycarpidan-1-methanol, acetate(ester), 9-octadecenoic acid (z)- 2-hydroxy-1-(hydroxymethyl) ethyl ester, and Hexadecanoic acid 1-(hydroxymethyl)-1 2-ethanediyl ester. The current findings may be useful to pharmaceutical businesses when they create natural supplements made from these clams. Using sophisticated chromatographic and spectroscopic instruments, it is advised to subject the extract to further chromatographic isolation and identification in order to determine its primary constituents.

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