Evaluation of Antibacterial Activity of Essential Oils of *Melaleuca cajuputi* Powell

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**Abstract**

*Melaleuca cajuputi* Powell is a tree species belonging to the family Myrtaceae and is widely used in traditional medicine. This study was conducted to investigate the antibacterial activities of essential oils of *M. cajuputi* Powell. Antibacterial activity was tested against Gram positive and Gram negative bacteria using the agar disc diffusion method. The essential oils of *M. cajuputi* were found to exert antibacterial activity against all of the tested bacteria, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *methicillin-resistant Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, and *Escherichia coli*. The zones of inhibition for *S. aureus*, *S. pyogenes*, MRSA, *E. coli*, and *K. pneumoniae* were 12.7 mm, 10.7 mm, 10.0 mm, 8.7 mm and 9.3 mm respectively, against 0.714% (w/w) of the essential oils. These results highlighted that Gram negative bacteria are less susceptible to the essential oils of *M. cajuputi*. A large zone of inhibition might be a sign of a leaching antimicrobial agent. These findings suggest that *M. cajuputi* is a potential natural antibacterial agent.

**Keywords:** *Melaleuca cajuputi* Powell, essential oils, antibacterial, minimum inhibitory concentration

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INTRODUCTION
Essential oils are highly concentrated, unstable substances found in plants. They have a distinctive fragrance with a high refractive index owing to ethers, aldehydes, terpenes, esters, ketones, phenols, and alcohols. Most essential oils are colorless or pale yellow in color and are liquid at room temperature. Essential oils are a mixture of volatile compounds that exclude saponins, flavonoids, tannins, steroids, terpenoids, and alkaloids. Notably, essential oils are significant in folk herbal medicines, cosmetic products, aromatics, perfumes, and phototherapy. Moreover, they are well known for their antimicrobial properties and are also effective in treating diseases such as carcinoma, Alzheimer’s disease, cardiovascular disease, discomfort, pregnancy, and insomnia. The secondary metabolites in essential oils play a critical role in plant protection, as they regularly have antimicrobial characteristics.

Melaleuca cajuputi Powell is a member of the Myrtaceae family and is prominently known as gelam, white tree, cajeput oil tree, tea tree, or paper bark tree. M. cajuputi is commonly present in swampy ground close to the coasts and is frequently found in tropical countries, such as Malaysia, Indonesia, Vietnam, Thailand, Myanmar, and northern Australia. In Malaysia, it can be found in mangrove swamps, particularly in Peninsular Malaysia. A few Melaleuca species harbor essential oils that are broadly utilized as therapeutic products, insecticides, and body care products. Adult M. cajuputi grows up to 33 m in height and are characterized by a slender crown. The tree is normally an unattached stem but may develop into collective stems. It can be easily recognized by a pure-white papery bark and thin strips as an outer layer. The leaves are gray-green in color, 4-10 cm long and 2 cm wide, firm, and have a pleasant scent. The flowers are whitish-pink or purple in color when bloom. The seeds are firmly encircled and joined. Stems are enclosed in grayish-brown woody capsules. M. cajuputi essential oils are isolated from the leaves via simple steam distillation. These essential oils are either colorless or pale yellow in color and release camphor (menthol)-like aroma with a moderately bitter taste. This odor makes M. cajuputi essential oils anti-insecticidal because the aroma expels the mosquitoes. They are also prescribed as mucus expectorant and as medication for bronchitis. In Australia, the leaves are used for various types of ill-treatment for centuries. In Asia, its oil is customarily used to diminish joint discomfort, stiff joints, and rheumatism, and used as a mosquito repellent. Interestingly, water from the boiled leaves could relieve pain and jaundice. Meanwhile, the shoots can be eaten as a salad.

According to a study by Hyldgaard et al, p-cymene is the most abundant compound in Cajaput essential oils. This compound might potentially act as a substitutional impurity, which partly disturbs the cytoplasmic membrane of bacteria. Phytochemical analysis revealed that α-pinene, limonene, aterpinene, and 4-terpineol extracted from M. cajuputi leaves have antibacterial, anti-inflammatory, anodyne, and insecticidal properties. These phytochemical compounds are also used as cooking seasonings and aromatic agents in soaps, body care products, cleansers, and fragrances. M. cajuputi essential oils are also used to ease dental pain, headaches, seizures, and rheumatoid arthritis, and screen insects. In the present study, we focused on the antibacterial properties of the essential oils of M. cajuputi against five bacteria.

MATERIALS AND METHODS
Plant material
Information regarding plant collection is listed in Table 1. Plant authentication was performed by a competent botanist from the Universiti Sultan Zainal Abidin. In this study, 21 essential oils of different geographical origins were used.

Extraction of essential oils
The extraction yield of essential oils ranges from 0.2 to 0.3 %. Briefly, the essential oils were extracted from the fresh leaves via steam distillation for 4 h. By utilizing water vapor at atmospheric pressure, the oil are refined from the leaves at a temperature below 100 °C, and the 4-h extraction allows for the isolation and production of essential oil from the crude leaf samples. This process was followed by mixing the sample with distilled water and boiling at 100 °C in a distillation flask. The emulsion of oil and water was permitted
Table 1. Place of plant collection

| Voucher No. | Location                  | latitude, longitude | Types of soil | Soil area | States       | District        |
|-------------|---------------------------|---------------------|---------------|-----------|--------------|-----------------|
| UniSZA P1   | Kg. Gong Badak            | 5.395461, 103.086865| Bris Village  | Terengganu| Kuala Terengganu |
| UniSZA P2   | Kg. Merabang Panjang      | 5.487304, 102.950802| Bris Village  | Terengganu| Kuala Terengganu |
| UniSZA P3   | Kg. Merang                | 5.522293, 102.965654| Bris Village  | Terengganu| Setiu         |
| UniSZA P4   | Kg. Pulai Baru            | 5.373320, 103.065907| Bris Village  | Terengganu| Setiu         |
| UniSZA P7   | Sg. Merang                | 5.524582, 102.941955| Bris Village  | Terengganu| Merang        |
| UniSZA P8   | Kg. Lembah Bidong         | 5.490218, 102.988754| Bris Village  | Terengganu| Setiu         |
| UniSZA P9   | Kg. Telaga Papan          | 5.533344, 102.911413| Bris Village  | Terengganu| Kuala Terengganu |
| UniSZA P10  | Kg. Sekeping, Penarek     | 5.570487, 102.846866| Bris Village  | Terengganu| Kuala Terengganu |
| UniSZA P11  | Kg. Rhu Tapai             | 5.515039, 102.978965| Bris Village  | Terengganu| Kuala Terengganu |
| UniSZA P12  | Kg. Beris Tok Ku         | 5.590235, 102.426125| Bris Village  | Terengganu| Setiu         |
| UniSZA P13  | Pantai Bachok             | 5.920937, 102.462125| Peaty Waterlogged| Kelantan| Bachok       |
| UniSZA P14  | Cherang Ruku              | 5.886560, 102.487515| Peaty Waterlogged| Kelantan| Pasir Puteh |
| UniSZA P15  | Kg. Pendas                | 1.3764218, 103.6366624| Peaty Waterlogged| Johor Bharu| Tanjung Kupang |
| UniSZA P16  | Damai laut                | 4.2590101, 100.591164| Peaty Waterlogged| Perak| Lumut |
| UniSZA P20  | Sg. Jawi                  | 5.192505, 100.504226| Bris Village  | Pulau Pinang| Seberang Perai |
| UniSZA P21  | Rantau Panjang            | 5.9475900, 101.9570540| Peaty Waterlogged| Kelantan| Pasir Mas |
| UniSZA P22  | Kerubong                  | 2.2809800, 102.2340030| Peaty Waterlogged| Melaka| Melaka Tengah |
| UniSZA P23  | Port Dickson              | 2.4284720, 101.8946300| Clay Hill| Negeri Sembilan| Port Dickson |
| UniSZA P24  | Wangsa Maju               | 3.224553, 101.728333| Clay Hill| Kuala Lumpur| Kuala Lumpur |
| UniSZA P25  | Sintok                    | 6.459666, 100.498843| Clay Hill| Kedah| Sintok |
| UniSZA P26  | Shah Alam                 | 3.065988, 101.491633| Peaty Waterlogged| Selangor| Shah Alam |
for 4 h to guarantee the segregation of oil layers and water. The oil yield was transferred into an amber bottle. The proportion of essential oils was determined and stored at room temperature until further use. The oil samples were then analyzed using FTIR and GCMS according to Zainon et al. with some modifications.

**Determination of the extraction yield of essential oils**

Chemometric analysis was performed using the spectroscopic data to assess the spatial variations of the 21 *M. cajuputi* essential oils. Spectroscopy techniques are used for phytochemical identification and provide significant information regarding the qualitative and quantitative composition of essential oils, as well as their pattern recognition using chemometrics. Hierarchical cluster analysis was used to differentiate the samples. To evaluate the samples, the similarity between the spectral fingerprints was determined using similarity analysis (SA), which is based on correlation coefficients *r*.

**Test organisms**

The bacterial species used as test organisms were *Staphylococcus aureus* (ATCC 11632), *Streptococcus pyogenes*, and clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* (ATCC 10536), and *Klebsiella pneumoniae* (ATCC 10031). All stock cultures were obtained from the Faculty of Medicine, Microbiology Laboratory, Universiti Sultan Zainal Abidin.

**Preparation of microbial cultures**

All tested bacterial strains were cultured in nutrient agar and broth. Bacterial suspensions were prepared by inoculating the nutrient broth with each of the bacterial cultures and incubated overnight at 37 °C.

**Antibacterial assay**

Antibacterial activity assay was performed using the disc diffusion method. The test organisms were cultured on sterile Petri dishes containing nutrient agar for 18–24 hours at 37 °C. On the next day, the cultures were adjusted to match 0.5 McFarland standards using normal saline. Then, a sterile cotton swab was dipped into each bacterial suspension and streaked onto MHA plates. Blank discs that were already impregnated with essential oil and distilled water (negative control) were placed on the surface of the agar using forceps. A chloramphenicol disk was used as a positive control. The three discs were placed on each plate and labelled correctly. The plates were incubated at 37 °C for 24 h. A clear zone indicated growth inhibition, and the diameter of the zone was measured in millimeters using a ruler. The test was performed in triplicate.

**Minimum inhibitory concentration (MIC)**

The minimum inhibitory concentration (MIC) of the *M. cajaputi* essential was determined by performing MIC using a 96-well microtiter
The highest concentration of essential oils used was 0.714% (w/w). For the positive control, 1 mg/L to 512 mg/L chloramphenicol was prepared. The negative control was prepared using 100 µL of MHB inoculated with the bacteria and 100 µL of 10% methanol. Then, the microtiter plate was incubated for 24 h at 37 °C.

**RESULTS**

A total of 19 compounds were identified from the essential oils of *M. cajuputi* leaves (Fig. 1). Moreover, the essential oils exhibited antibacterial activity against *S. aureus*, *S. pyogenes*, MRSA, *K. pneumoniae*, and *E. coli* (Table 2), with inhibition zones ranging from 8.7 to 12.7 mm. Chloramphenicol disk was used as a positive control, and distilled water was used as a negative control. The largest inhibition zone (12.7 mm) was observed against *S. aureus*, followed by *S. pyogenes* (10.7 mm), MRSA (10.0 mm), *K. pneumoniae* (9.3 mm), and *E. coli* (8.7 mm).

Next, MIC was used to measure the efficacy of the extracts against the tested bacterial strains. MIC was for the tested bacteria, which showed a zone of inhibition and were susceptible to the essential oils of *M. cajuputi* in the earlier antibacterial assay using the disc diffusion method. Results showed that the essential oils of *M. cajuputi* showed promising antibacterial activities. The MIC of the essential oils against *E. coli* and *K. pneumoniae* was 0.714%. Similarly, the MICS against Gram negative bacteria (*S. aureus*, *S. pyogenes*, and MRSA) were also 0.714% (Table 3).

**DISCUSSION**

The phytochemical compounds found in *M. cajuputi* essential oils were determined using FTIR and GC-MS. Nineteen compounds, namely p-cymene, linalool, carvophellene, terpinolene, alpha-pinene, terpinene-4-ol, (+)-4-carene, D-limonene, alpha-copaene, alpha-cubebene, beta-thujene, beta-pinene, guiaol, eucalyptol, azulenemethanol, phellandrene, aromadendrene, (+)-3-carene, and gamma-elemene, were identified. The highest compounds found in *M. cajuputi* essential oils were p-cymene, followed by linalool and carvophellene. P-Cymene is the main antimicrobial compound in *M. cajuputi* essential oils. Several studies have suggested that this monoterpene possesses antibacterial, antiviral, and antifungal activities. Furthermore, previous studies have shown that linalool has anxiolytic, anti-cholesterol, and antibacterial activities. Aelenei et al. demonstrated that linalool alone or in combination with antibiotics showed antibacterial activity against Gram positive bacteria.
and Gram negative. Meanwhile, the antimicrobial effect of β-caryophyllene was previously examined against human pathogenic bacterial and fungal strains. The results showed that β-caryophyllene demonstrated selective antibacterial activity against S. aureus and had a more pronounced antifungal activity than kanamycin. Therefore, our results showed that M. cajuputi essential oils were effective in inhibiting Gram positive and Gram negative bacteria.

Essential oils can inhibit the growth of various types of pathogens because of the existence of natural substances produced by plants. The phytochemical composition of essential oils is heterogeneous and comprises 20–60 different bioactive compounds. Essential oils and their components are hydrophobic, which makes them promising antimicrobial agents. This characteristic allows them to be separated from lipids, which are constituents of the cell membrane of bacteria and mitochondria. Thus, the disruption of cell structures renders the cell membrane more permeable, which causes the leakage of critical molecules and ions from the bacterial cell. As a result, the bacteria eventually die. Some compounds aim for the efflux mechanisms in Gram negative bacteria to regulate drug resistance.

In this study, we determined the antibacterial activities of essential oils of M. cajuputi against Gram positive (S. aureus, S. pyogenes, and MRSA) and Gram negative (K. pneumoniae and E. coli) bacteria using the disc diffusion and MIC assays. The results revealed that the essential oils of M. cajuputi intensively inhibited Gram positive bacteria compared to Gram negative bacteria, wherein the inhibition zones against Gram positive bacteria were greater than those in Gram negative bacteria. This is due to the more comprehensible cell walls of Gram positive bacteria. These findings are of great significance, especially in cases of S. aureus and clinical isolates of MRSA that are prominent for being resistant to some antibiotics. In addition, these organisms have the ability to produce several types of enterotoxins that can cause serious infections, leading to sepsis or death. The efficacy of essential oils depends on the structure of the target bacteria. According to a study by Swamy et al. essential oils easily penetrate the bacterial cell membranes and destabilize cellular architecture. The disruption of the membrane integrity is caused by an increase in bacterial cell membrane permeability, resulting in the leakage of cellular components, loss of ions, and disruption of many cellular activities. Gram negative bacteria have an advanced tolerance toward hydrophobic antimicrobial substances because their outer membrane encloses hydrophilic lipopolysaccharides, which block macromolecules and hydrophobic substances like those found in essential oils. This differentiating character of the cell wall makes Gram positive bacteria more sensitive to distinctive substances than Gram negative bacteria. Therefore, Gram negative bacteria are ordinarily less sensitive than Gram positive bacteria. Interestingly, our results were similar to that reported by Al-Abd et al., wherein M. cajuputi flower and leaf extracts were found to have a wide range of antimicrobial potential against Gram positive bacteria. However, they did not observe inhibition zones against the Gram negative bacteria tested. The differences in these findings are probably due to the distinctive solvent types that were used to extract phytocomponents from plant materials. Bioactive phytocomponents present in plants are proven and confirmed to be simulated using extraction approaches and extraction solvent systems.

Conventional medication practices in old-world human cultures worldwide have shown that plants are beneficial sources of potent antimicrobial agents. For this reason, scientific studies have been conducted on the antimicrobial activities of plant extracts against various types of microorganisms, which have arisen in the evolution of alternative plant-based antimicrobial pharmaceutical medicines. Extracts and some natural phytoconstituents found in the Myrtaceae family have been reported to have anticancer, antimicrobial, antioxidant, and anti-inflammatory properties. Qualitative determination of phytochemicals showed the presence of flavonoids, saponins, and condensed tannins in all parts of this plant. The monoterpene content of essential oils is mainly composed of melaleucol, β-caryophyllene, terpinolene, g-terpinene, and plathyllol. Therefore, the antibacterial activity of the essential oils M. cajuput used in this study corresponded with their phytochemical contents.
CONCLUSION

Essential oils of *M. cajuputi* possess antibacterial properties against diverse clinical isolates and can be used as a medication for several bacterial diseases. Nonetheless, further studies are required to explore their efficiency in suppressing the growth of pathogenic microorganisms.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

AUTHORS’ CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analysed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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