In vitro anti-Candida activity of Melaleuca cajuputi extracts

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ABSTRACT

Aims: The rise of drug-resistant infectious diseases worldwide has spurred experts’ interest in developing safe and effective alternative medicine. Melaleuca cajuputi extracts have been shown to exhibit antimicrobial activity in vitro against various bacterial species. This study evaluated the antimicrobial activity of local M. cajuputi leaf extracts (MCEs) against Candida albicans.

Methodology and results: Phytoconstituents of aqueous and ethanolic MCEs were screened conventionally using chemical tests. Broth microdilution assay and scanning electron microscope (SEM) were performed to study the anti-Candida activity of the extracts. Both MCEs contained terpenoids, phenols, flavonoids and tannins. Aqueous and ethanolic MCEs showed good fungicidal activity against the tested organism with minimum inhibitory concentration (MIC) values of 50 mg/mL and 25 mg/mL, respectively and a minimum fungicidal concentration (MFC) to MIC ratio of less than 2. Scanning electron micrographs revealed yeast cell surface morphology alterations when treated with both MCEs at 1 × MIC.

Conclusion, significance and impact of study: In conclusion, MCEs have anti-Candida properties and thus, M. cajuputi extract could be an excellent potential source of natural antimicrobial agents for disease remedies.

Keywords: Anti-Candida activity, cell morphology, Melaleuca cajuputi extract (MCE), phytoconstituents, scanning electron microscope (SEM)

INTRODUCTION

Traditional or herbal medicine has been used to maintain physical and mental health for a long time. Herbal medicine may include various herbal preparations and finished herbal products that contain active ingredients extracted from different parts of the plants or their combinations (WHO, 2020). In this era of globalization, the complementary use of herbal medicine in developing countries keeps increasing due to its accessibility and affordability. Moreover, it also alleviates concerns about the adverse effects of conventional synthetic drugs, addresses the demand for more personalized health care and facilitates more access to health information for the public. Furthermore, herbal medicine is widely perceived as natural, safe and non-toxic, which corresponds more closely to the ideology of consumers (Benzie and Wachtel-Galor, 2011).

Melaleuca cajuputi is commonly known as "Gelam" or "Kayu Putih" in Malaysia (Figure 1). The plant belongs to the Myrtaceae family, widespread in Australia, Southeast Asia, New Guinea and the Torres Strait islands. It is a medium to tall tree with a spongy trunk, white and flaky bark, silvery new growth, white or greenish flower spikes, and dull green, leathery leaf blades with distinct longitudinal veins and elliptic lance shapes. The tree can grow up to 40 meters high and is mainly found in low swampy and regularly flooded coastal plains (Flora and Fauna Web, 2019). Melaleuca cajuputi is a source of cajuput oils or extracts commercially available in industries. Their phytoconstituents are known for their antibacterial, antifungal and antiparasitic activity (Bua et al., 2020). Candida albicans is a yeast and has been the most common cause of opportunistic mycoses worldwide. It is also a member of normal flora and a frequent colonizer of human skin and mucous membranes such as the mouth, throat, gut and vagina. Candidiasis is a fungal infection caused by Candida spp. in which C. albicans accounts for most of the isolates (Aboualigalehdari et al., 2013). There are several types of candidiasis, such as oropharyngeal candidiasis, invasive candidiasis and vulvovaginal candidiasis. Candidiasis is associated with treatment failure and a high mortality rate due to antibiotic resistant Candida spp., which could arise from the extensive use of a few major classes of antifungal drugs (Zaidi et al., 2016).

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Hence, research and development to explore safe and effective alternative therapeutic medicine are necessary to overcome these problems. Herbal alternatives to conventional antibiotics in treating infections are becoming more common, with promising and effective treatment outcomes. Studies have revealed the antibacterial activity of *M. cajuputi* extracts (MCEs) against Gram-positive bacterial strains such as *Staphylococcus aureus*, *S. epidermidis* and *Bacillus cereus* (Al-Abd et al., 2015). The use of MCEs as an alternative or complementary approach to conventional antibiotics is not only natural-based with fewer adverse effects but also potentially cost-effective in treating infections.

A standard plant extraction to extract these bioactive compounds from plant materials must be adequately performed to ensure that the potential phytoconstituents are not lost or destroyed. This study used the maceration technique to extract bioactive compounds from *M. cajuputi* leaves because the method preserves the chemical structure of the phytoconstituents without destruction (Altemimi et al., 2017). Thus, the targeted bioactive compounds need to be identified as the extraction of bioactive compounds depends on the solvent system (Sasidharan et al., 2011). Studies on the antifungal activity of MCEs are scarce as there was limited published report on the antifungal effect of MCEs against fungal strains. This study aimed to evaluate the antifungal activity of ethanolic and aqueous MCEs against *C. albicans in vitro*.

**MATERIALS AND METHODS**

**Plant extraction**

The leaves of *M. cajuputi* were collected from Pasir Puteh, Kelantan, Malaysia and identified based on their physical appearance. The authentication of the plant was done in our previous study with voucher specimen number PIUUM 0304. The leaves were cleaned a few times thoroughly using tap water to wash off the attached dirt, air-dried and then transferred into an incubator for complete drying at 50 °C. The dried leaves were grounded into powders using an electric grinder.

Approximately 200 g of *M. cajuputi* powder was immersed in 1 L of distilled water and absolute ethanol (ratio of 1:5) in a glass bottle, respectively and incubated in a water bath at 50 °C for 72 h. The mixtures were filtered through filter papers to separate the undissolved *M. cajuputi* powder from the filtrates. The aqueous and ethanolic filtrates were transferred into separate boiling flasks and concentrated under reduced pressure using a rotary evaporator at 40 °C. The concentrated aqueous MCE was freeze-dried at −50 °C until a crystal-like crude extract was obtained. Meanwhile, the concentrated ethanolic MCE was transferred into Petri dishes and air-dried under a fume hood.

The crude aqueous and ethanolic MCEs were then weighed using an analytical balance to determine the percentage yields of extraction. The percentages of aqueous and ethanolic MCEs yielded were calculated using the following formula:

\[
\text{Percentage yield} (\%) = \frac{\text{Final weight of crude plant extract (g)}}{\text{Initial weight of plant powder (g)}} \times 100
\]

The crude extracts were stored at 4 °C in air-tight containers until further use.

**Phytochemical analysis**

Phytochemical analysis of aqueous and ethanolic MCEs was performed using the standard procedures to determine phytoconstituents such as terpenoids, phenols, flavonoids, tannins, phlobatannins, cardenolides, cardiac glycosides, saponins, anthraquinones and volatile oils (Solihah et al., 2012; Gul et al., 2017). A test for terpenoids was performed by mixing 5 mL of the extract with 2 mL of chloroform and added with 3 mL of sulphuric acid (H₂SO₄). The presence of terpenoids was shown by the reddish-brown colour produced at the interface. The determination of phenols was done by adding 2 mL of 3% ferric chloride (FeCl₃) solution to 2 mL of the pre-warmed leaf extracts. A colour of dark green or blue suggested the presence of a phenolic compound. Flavonoids were determined by adding 2 mL of 2% sodium hydroxide (NaOH) to 2 mL of leaf extracts. The presence of flavonoids was indicated by the formation of a yellow colour solution. For the tannins screening test, 10 mL of bromine water was added to the 0.5 g aqueous extract. Decoloration of bromine water showed the presence of tannins. Then, 1 mL of extract was boiled with 2 mL of 1% hydrochloric acid (HCl) to detect the presence of phlobatannins by the formation of red precipitates.

Fifty milligrams of the extract were dissolved in 1 mL glacial acetic acid containing one drop of 3% FeCl₃. Then it was under-layered with 1 mL of concentrated H₂SO₄.
The formation of a brown ring at the interface indicated the presence of the de-oxy sugar characteristic of cardenolides. Two mL of acetic acid and 2 mL of chloroform were added to the plant extracts. The mixture was then cooled and concentrated H$_2$SO$_4$ was added. The green colour showed the entity of aglycone, the steroidal part of the glycoside. The screening of saponins was done by mixing 5 mL of distilled water with the plant extracts in a test tube and mixed properly. The frothing was mixed with a few drops of olive oil and mixed vigorously, and the foam appearance showed the presence of saponins. Ten milliliters of benzene were added to 3 g of the plant sample in a conical flask and soaked for 10 min, and then filtered. Further 10 mL of 10% ammonia solution was added to the filtrate and shaken vigorously for 30 sec and pink, violet or red colour indicated the presence of anthraquinones in the ammonia phase. A spot test was performed by pressing a small amount of crude extract in between two filter papers. The oil stain on the paper indicated the presence of volatile oils. The findings of screening tests were qualitatively expressed as positive (+) or negative (-) for the presence or absence of the phytoconstituents, respectively.

**Inoculum preparation**

*Candida albicans* American type culture collection (ATCC) 10231 (Virginia, USA) was grown and maintained by subculturing the organism on Sabouraud dextrose agar (SDA) at 30 °C for 24 h. A fresh inoculum was prepared before the antimicrobial assay by inoculating the colonies of *C. albicans* into a tube containing Mueller Hinton broth (MHB). A nephelometer adjusted the suspension's optical density to 0.5 McFarland standard (equivalent to 1.5 × 10$^8$ CFU/mL).

**Antimicrobial assay**

The minimum inhibitory concentration (MIC) values of aqueous and ethanolic MCEs against *C. albicans* were determined using the broth microdilution method as previously described by Baharuddin et al. (2014). The assay was performed in a sterile 96-well microtitre plate with extract concentrations ranging from 0.78 mg/mL to 100 mg/mL. One hundred microlitres of MHB was pipetted into wells 2 to 8, 9 and 10. Meanwhile, 200 μL of MHB was pipetted into well 11, which served as sterility control. Then, 100 μL containing 100 mg/mL of MCEs were added into wells 1 and 2, respectively. Two-fold serial dilutions of the extract were performed by transferring 100 μL from well 2 to well 8 and 100 μL was discarded from the last well. Then, 100 μL of *C. albicans* inoculum (7.5 × 10$^4$ CFU/mL) was pipetted into well 1 to well 10, making a final volume of 200 μL in each of the wells.

The inoculum suspensions in broth supplemented with 2 μg/mL amphotericin B (well 9) served as a positive control or reference for the growth inhibition or killing of the organism. Meanwhile, the inoculum in broth without the antifungal drug and extracts (well 10) was used as a negative or growth control to ensure the addition of fungal inoculums in wells and to monitor the organism’s viability to grow in the broth. The growth was indicated by the turbidity of the wells in the microtitre plate. The 96-well microtitre plate was then incubated at 30 °C for 24 h. The lowest concentrations of aqueous and ethanolic MCEs in the wells that showed no turbidity (clear broth) were recorded as MIC values. The assay was performed in triplicate.

The minimum fungicidal concentration (MFC) values of aqueous and ethanolic MCEs against *C. albicans* were determined by subculturing the wells with clear broth onto a Mueller Hinton agar (MHA) plate. Ten microlitres of the clear broth in wells with growth inhibition were inoculated onto the MHA plate using a sterile cotton swab, and the plate was incubated at 30 °C for 24 h. The lowest concentrations of the crude extracts that kill up to 99.9% of the cells were taken as the MFC values (Baharuddin et al., 2014).

**Scanning electron microscopic study**

A scanning electron microscope (SEM) was used to study the morphological changes of *C. albicans* following its treatment with aqueous and ethanolic MCEs. *Candida albicans* at a logarithmic phase in Roswell Park Memorial Institute (RPMI) 1640 medium was treated with 1× MIC of aqueous and ethanolic MCEs for 24 h at 30 °C. Inoculums treated with 2 μg/mL amphotericin B and 1% dimethyl sulfoxide (DMSO) were used as positive and negative controls, respectively.

The microcentrifuge tubes containing the cell treatments were centrifuged at 4,500 rpm for 10 min to obtain the pellets at the bottom of the microcentrifuge tubes. The supernatants were discarded and the pellets were fixed with McDowell-Trump fixative for 2 h. Then, the pellets were washed with 0.1 M phosphate buffer saline, followed by another centrifugation at 4,500 rpm for 10 min. The washing process was repeated twice. Subsequently, the pellets were post-fixed with 1% osmium tetroxide for 1 h and washed with distilled water twice.

The pellets were dehydrated using ascending acetone concentrations, starting with 50%, 75%, 95% and 100%. After adding different acetone concentrations, centrifugation was carried out at 4,500 rpm for 10 min and the supernatants were discarded. For 100% acetone, the dehydration process was repeated twice before 100% hexamethyldisilazane (HMDS) was added twice at 10 min intervals. Finally, the pellets were air-dried at room temperature for a few days. The dried pellets were then mounted onto the SEM specimen stub with double-sided carbon tape and coated with gold using a sputter coater. The mounted specimens were viewed and examined under the SEM and the micrographs of *C. albicans* were taken with an accelerating voltage of 5.00 kV (Chen et al., 2018).
RESULTS

Percentage yield of extracts

The crude aqueous MCE appeared fine, crystal-like, rough and brown-coloured powder, while the crude ethanolic MCE was coarse, rough and dull, green-coloured powder. Both the aqueous and ethanolic MCEs had a pleasant odour, but their smells were not very pungent. The percentage yield of aqueous and ethanolic MCEs obtained were 8.58% and 11.1%, respectively.

Phytochemical contents

The results of phytoconstituents analysis for the presence of terpenoids, phenols, flavonoids, tannins, phlobatannins, cardenolides, cardiac glycosides, saponins, anthraquinones and volatile oils are tabulated in Table 1. Both aqueous and ethanolic MCEs showed the presence of terpenoids, phenols, flavonoids and tannins. Meanwhile, cardenolides and saponins were detected in aqueous and ethanolic MCEs, respectively.

Anti-Candida activity

The MIC and MFC values of aqueous MCEs against C. albicans were 50 mg/mL, respectively, while the MIC and MFC values of ethanolic MCEs were 25 mg/mL and 50 mg/mL, respectively (Table 2 and Table 3). The results of the broth microdilution assay for both MCEs against C. albicans (Figure 2) are summarised in Table 4.

Morphological changes of extract-treated cells

The effects of aqueous and ethanolic MCEs on the surface morphology of C. albicans during its logarithmic growth phase were observed under the SEM after 24 h of incubation at 30 °C and the results were shown in the micrographs (Figure 3). The cells of C. albicans treated with 2 μg/mL amphotericin B (positive control) showed oval-shaped, appeared rough and uneven with a distorted cell surface (Figure 3A). While C. albicans treated with 1% DMSO (negative control) was oval, with a smooth and even surface (Figure 3B). When the fungal cells were exposed to both extracts at 50 mg/mL (aqueous MCE) and 25 mg/mL (ethanolic MCE), the cell shape was slightly deformed, with bleb-like structures and rough surfaces (Figure 3C). Some of the cells appeared distorted (Figure 3D) compared to those in positive and negative controls.

DISCUSSION

Over the years, M. cajuputi plant has been widely used for vast applications, including nutrition, personal care, home cleaning and cosmetics products. Nowadays, the antimicrobial properties of M. cajuputi have attracted much attention within the scientific community due to the worldwide emergence of multidrug-resistant (MDR) microorganisms. The conventional, chemically

Table 1: Qualitative phytochemical analysis of aqueous and ethanolic MCEs.

<table>
<thead>
<tr>
<th>Phytoconstituent</th>
<th>Aqueous</th>
<th>Ethanol</th>
</tr>
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<tbody>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cardenolides</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Volatile oils</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+): Presence of phytoconstituent; (-): Absence of phytoconstituent.

Table 2: MIC determination of aqueous and ethanolic MCEs against C. albicans.

<table>
<thead>
<tr>
<th>MCE concentration (mg/mL)</th>
<th>Aqueous</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>50</td>
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<td>-</td>
</tr>
<tr>
<td>25</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>12.5</td>
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<td>6.25</td>
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<td>3.13</td>
<td>+</td>
<td>+</td>
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<tr>
<td>1.56</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.78</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Positive control</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Negative control</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sterility control</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 3: MFC determination of aqueous and ethanolic MCEs against C. albicans.

<table>
<thead>
<tr>
<th>MCE concentration (mg/mL)</th>
<th>Aqueous</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>12.5</td>
<td>ND</td>
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<td>6.25</td>
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<tr>
<td>3.13</td>
<td>ND</td>
<td>ND</td>
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<td>1.56</td>
<td>ND</td>
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<tr>
<td>0.78</td>
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<tr>
<td>Positive control</td>
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<td>-</td>
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<tr>
<td>Negative control</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sterility control</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+): Growth of C. albicans; (-): No growth of C. albicans; Positive control: Fungal inoculum and MHB supplemented with 2 μg/mL amphotericin B; Negative control: Fungal inoculum and MHB; Sterility control: MHB only.
Figure 2: Anti-Candida activity of MCEs in a 96-well microtitre plate. The MIC values of aqueous (A1–A3) and ethanolic extracts (E1–E3) (in triplicate) against C. albicans were 50 mg/mL and 25 mg/mL, respectively. PC: Positive control (amphotericin B), NC: Negative control (growth reference) and SC: Sterility control.

Figure 3: Scanning electron micrographs of C. albicans observed at 15,000× magnification. A: Treatment of cells with 2 μg/mL amphotericin B (positive control); B: Treatment of cells with 1% DMSO (negative control); C: Treatment of cells with aqueous MCE at 1× MIC (50 mg/mL); D: Treatment of cells with ethanolic MCE at 1× MIC (25 mg/mL).

Table 4: MIC and MFC values of aqueous and ethanolic MCEs against C. albicans.

<table>
<thead>
<tr>
<th>MCE</th>
<th>MIC value (mg/mL)</th>
<th>MFC value (mg/mL)</th>
<th>MFC/MIC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>50</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>Ethanolic</td>
<td>25</td>
<td>50</td>
<td>2</td>
</tr>
</tbody>
</table>
synthesized antibiotics may no longer be effective in treating infectious diseases caused by the MDR microorganisms such as methicillin-resistant S. aureus (MRSA), Streptococcus pneumoniae, extended spectrum beta-lactamase Escherichia coli, Pseudomonas aeruginosa and fluconazole-resistant C. albicans (Koulenti et al., 2020). The essential oils and extracts derived from M. cajuputi can be applied as an alternative medicine to deal with certain infectious diseases. Based on these facts, the present study focuses on the MCEs screening for the presence of phytoconstituents and the antifungal potential of the crude extract against a selected fungal strain.

In this study, the targeted bioactive compounds from M. cajuputi leaves were phenolic compounds such as phenols, tannins and flavonoids. Phenolic compounds are the most important secondary plant metabolites and exhibit various physiological properties, including antioxidant, anti-inflammatory, anti-carcinogenic and antimicrobial activities. The antimicrobial activity of phenolic compounds has been linked to the inactivation of cellular enzymes by altering cell membrane permeability (Mandal et al., 2017). Suitable solvents for extracting phenolic compounds include water and ethanol (Azmir et al., 2013). In polar solvents, these compounds can be dissolved easily because of their solubility (Tiwari et al., 2011). Due to their excellent ability to extract phytoconstituents with antimicrobial characteristics, the solvents were used in this study.

Solvents with a low polarity index might result in low to moderate antimicrobial effects, while moderately to highly polar solvents like water (10.2) and ethanol (5.2) might have good antimicrobial potentiality due to their ability to extract many bioactive compounds (Ghosh et al., 2012).

The percentage yield of ethanolic MCE in this study was higher than aqueous MCE, supporting the assumption that organic solvents, such as ethanol, are more efficient in extracting phenolic chemicals than water (Sun et al., 2015). However, water is more polar than ethanol, contradicting the assumption that the extraction yield improves with increasing polarity of the solvent employed in the extraction (Do et al., 2014). This could be owing to insufficient filtration of the MCEs during the extraction procedure.

The essential characteristic related to MCEs' pharmacological action is their bioactive components. Both aqueous and ethanolic MCEs revealed the presence of terpenoids, phenols, flavonoids and tannins in the present study. This finding is comparable to those reported by Al-Abd et al. (2015), which discovered terpenoids (22.91%), phenolic compounds (14.01%) and flavonoids (7.07%) from the M. cajuputi methanolic leaf extract. Terpenoids have been shown to have potential antibacterial activity, inhibiting two critical microbial biological activities: oxygen absorption and oxidative phosphorylation, both of which are necessary for microbial survival (Mahizan et al., 2019). In addition, cardenolides were found in aqueous MCE but not ethanolic MCE, while saponins were found in ethanolic MCE but not aqueous MCE. Both cardenolides and saponins are produced from a class of terpenoids known as triterpenoids which have been shown to inhibit microbial growth and work synergistically with antibiotic therapy (Mahizan et al., 2019). MCE chemical constituents have been shown to vary based on climate change, geographical factors, growth regulators and harvest seasons (Dahiya, 2016).

In this study, the aqueous and Ethanolic MCEs were assessed for their antifungal activity against the C. albicans strain (ATCC 10231). The inhibitory and fungicidal effects of ethanolic MCE were more potent than aqueous MCE. This could be because ethanolic MCE has a higher phenolic content, contributing to its antifungal activity (Mandal et al., 2017). In a previous study, M. cajuputi methanolic leaf extract showed better inhibitory and bactericidal effects than aqueous and ethanolic MCEs against S. aureus and S. epidermidis with MIC and MBC values of 12.5 μg/mL and 25 mg/mL, respectively (Al-Abd et al., 2015). The MFC/MIC ratio is a significant analysis and it is used to describe the type of antimicrobial activity against a particular pathogen (Ana et al., 2017). This study determined the MFC/MIC ratios of aqueous and ethanolic MCEs against C. albicans to classify their antifungal activity, either as fungistatic or fungicidal. The fungistatic activity of an extract or agent is determined when the MFC/MIC ratio is more than four, while the fungicidal activity is considered when the MFC/MIC ratio is less than or equal to 4 (Freire et al., 2017). The results showed that both MCEs have fungicidal activity against C. albicans.

Scanning electron microscopy is a technique used to inspect specimens’ surface topographies at very high magnification under SEM. A specimen to be examined is irradiated with a finely focused beam of high-energy electrons, which may be static or swept in a raster across the specimen's surface to generate various signals (Goldstein, 2012). The signals derived from the electron-specimen interactions will create high-resolution images to reveal information about the specimen, including external morphology, chemical composition, crystalline structure and orientation of materials making up the specimen (Swapp, 2017). In microbiology, the introduction of SEM as a tool brought about a complete reappraisal of the micro-anatomy of microorganisms. SEM can be used to visualize microorganisms that have been fixed, dehydrated and dried. This helps to understand and analyze the morphological structures of microorganisms, which can be correlated with their pathogenesis toward particular diseases (Kaláb et al., 2008).

This study provides information on the morphological changes of C. albicans after treatment with aqueous and ethanolic MCEs using SEM. The external surface of the aqueous MCE-treated cells appeared irregular and the deformities of cell exposed to ethanolic MCE was much more prominent, most likely due to the malformation of the cell structure. The effects can be attributed to the disruption of cell membrane permeability, leading to structural damage (Kabir and Ahmad, 2012). The results
of the SEM analysis in this study were comparable to those described by Septiana et al. (2019). The cajuput candy reduced biofilm-biomass and viable cells and altered the morphology of C. albicans, as observed under the SEM (Septiana et al., 2019).

CONCLUSION

In conclusion, this study provides an overview of the in vitro antifungal activity of aqueous and ethanolic MCEs. The inhibitory and fungicidal effects of both MCEs against C. albicans were evident in the current findings based on the surface morphology of the treated test organism. Different types of MCEs have the potential to be explored as efficacious antifungal agents in the treatment of C. albicans and non-albicans infections. In-depth research is needed to determine the effectiveness of MCEs when combined with currently available antibiotics or antifungals to understand their therapeutic applications better.

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