

Research Article
Antifungal Activity of *Jasminum sambac* against *Malassezia* sp. and Non-*Malassezia* sp. Isolated from Human Skin Samples

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*Malassezia* sp. causes skin diseases such as pityriasis versicolor, folliculitis, and atopic dermatitis. The present study aims to evaluate the antifungal activity of *J. sambac* or Arabian jasmine, a flowering plant abundant in the Southeast Asia against *Malassezia* sp. using disc diffusion and broth microdilution method. The methanol extract and essential oil from the flowers and leaves of *J. sambac* were, respectively, prepared using solvent extraction and hydrodistillation process. Skin samples from individuals with dandruff were cultured on Sabouraud dextrose agar overlaid with olive oil. The fungi that grew were observed microscopically, tested with Tween assimilation test, and cultured on CHROMagar (the chromogenic media pioneer) to identify *Malassezia* sp. Out of 5 skin samples, only 2 *Malassezia* sp. isolates were identified based on morphology and their ability to assimilate Tween. The inhibition zones of methanol extract of flowers and leaves of *J. sambac* and essential oil of flowers showed potential for antifungal activity withinhibition zones of 11.10 ± 1.92, 12.90 ± 1.68, and 13.06 ± 0.26 mm, respectively, and minimum inhibitory concentration (MIC) values of 80 mg/mL to 160 mg/mL and 50%, respectively. In conclusion, *J. sambac* may be used as an alternative treatment against *Malassezia*-associated skin infections.

1. Introduction

*Malassezia* sp. is a type of glabrous fungus which causes infections of superficial layer of the skin. *Malassezia* species are normal flora found on the skin of 7 billion humans but they can be pathogenic causing the common skin disorder which includes pityriasis versicolor, folliculitis, and atopic dermatitis in humans [1]. In immunocompromised hosts, *Malassezia* can also cause systemic infections. *Malassezia* are lipophilic or lipid-dependent fungi requiring long chain fatty acids, essential for the growth of most *Malassezia* species, and are being supplied from the human skin lipids [2]. This lipid requiring property causes the highest density of *Malassezia* in the sebaceous areas such as the scalp, face, and upper trunk and the lowest density on the hands. Being a lipophilic yeast, therefore in vitro growth must be stimulated by natural oils or other fatty substances. The most common method used is to overlay Sabouraud dextrose agar with olive oil [3]. Reproduction of *Malassezia* sp. is by budding from a broad base present on the same cell pole (monopolar budding) [4]. Prolonged use of topical antifungals such as Itraconazole, Fluconazole, and Terbinafine to treat infections caused by *Malassezia* sp. has its drawbacks by causing side effects such as burning, stinging, or redness when applied to the skin. The main problem with oral antifungals is nausea and dizziness on continuous use [5]. Other drawbacks of commercial antifungals, whether topical or oral, are their adverse drug interaction with other medications and high cost in pharmacies [6].

In an effort to find an alternative from a more natural and safer source, it is very applicable to utilize the use of natural products. Recent study [7] on *Jasminum sambac*, a flowering plant abundant locally in Southeast Asia and traditionally used as remedies for skin problems such as acne, whitehead, and blackhead, has proven successful. This Arabian jasmine plant is distinctive for its flower's white petals which emit a fragrant odour and bloom throughout the year. The leaves of *J. sambac* contain the secoiridoid glycosides, jasminin, quercitrin, and rutin [8]. The preliminary phytochemical study of the antimicrobial activity of ethanolic callus extracts
of jasmine focused on two species of Jasminum, *J. grandiflorum* and *J. sambac*, showed that the plants can be used as medicine for skin disorders [9]. As *J. sambac* possesses antibacterial property, it can be used to treat acne and skin infections. Essential oil and extracts of *J. sambac* have been shown to exhibit antibacterial and antifungal properties [10]. Jasmine oil has been proven to reduce skin inflammation, tones the skin by repairing skin cells by encouraging cell growth, and increases skin elasticity [11].

*Jasminum sambac* has also been known to inhibit the growth of *Alternaria* sp. and dermatophytes [12]. The callous extracts of *J. sambac* were also reported to display antimicrobial activity against *Staphylococcus albus*, *Proteus mirabilis*, *Salmonella typhi* [13], and *Propionibacterium acnes* [14]. The use of *J. sambac* extract can be a potential additive in skin and hair products to prevent *Malassezia* sp. infection altogether [15]. The present study was therefore undertaken to confirm isolated from the skin samples of individuals with dandruff.

### 2. Materials and Methods

#### 2.1. Plant Materials

Fresh flowers and leaves of *Jasminum sambac* were purchased from a nursery located in Sungai Buloh, Selangor, Malaysia. Prior to analysis, the flowers and leaves were taken out of the freezer, thawed at room temperature, and then washed thoroughly under running tap water. The leaves and the flowers were then spread on the tray and allowed to dry at room temperature for several days. The dried flowers and leaves were grinded into powder using an electrical blender. Another batch of the flowers was left fresh for hydrodistillation process to obtain the essential oil.

#### 2.2. Extraction of Plant

Two methods [16], namely, hydrodistillation and methanol extraction, were employed to produce essential oil and the crude methanol extract, respectively. In the preparation of essential oil, about 100 g of powdered leaves and 100 g of fresh flowers were added to a 500 mL round-bottom flask and filled half full with distilled water. The 500 mL distilling flask was rested on the heating mantle and was heated slowly while adding water sparingly from the separatory funnel to prevent the plant from drying out and burning. The distillation was stopped when the distillate reached 100 mL. The distillate was extracted three times with 15 mL aliquot of dichloromethane. The combined dichloromethane layers were then extracted three times with 15 mL aliquot of saturated NaCl. Magnesium sulphate (MgSO₄) was used as a drying agent to dry the organic layer. The filtrates were collected and left to evaporate in a beaker on a hot plate [17].

The crude extract was prepared from the dried powder of the leaves and flowers using methanol as the extraction solvent. In the ratio of 1:5, 100 g of the powdered *J. sambac* leaves and flowers was soaked in 500 mL methanol, respectively. The mixture was then subjected to agitation using magnetic stirrer for 24 hours at room temperature. The mixture was then filtered using the Whatman filter paper No. 1 whereby the filtrate obtained was collected. The process was repeated using the remaining residue with 250 mL methanol. Both filtrates were then mixed and concentrated under reduced pressure using a rotary evaporator. The extracts obtained were finally poured to dryness under the fume hood in order to produce a crude methanol extract [18].

The essential oil and methanol extract were dissolved with 10% DMSO whereas the aqueous extract was dissolved in sterile distilled water to final concentration of 100%, 75%, 50%, and 25% for essential oil and 10 mg/mL, 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL, 160 mg/mL, and 320 mg/mL for methanol extract. All the extracts were sterilized by passing through a 0.45 μm pore membrane filter.

#### 2.3. Preparation of Microorganism Strain

The skin samples were collected from the subjects under the approval of the Faculty Research Committee Code NN-2014-027. Using a sterile scalpel blade, the samples were placed in sterilized plastic bags for storage and transport to the laboratory. The fungal strains isolated from skin samples were mounted in 10% KOH, glycerol, Parker ink solution, and lactophenol cotton blue to identify the samples. It was also tested with 10% H₂O₂ to investigate the presence of catalase which is an indicator of *Malassezia* sp. It was then inoculated on Sabouraud dextrose agar (SDA) plates overlaid with 1 mL olive oil as a source of lipid to which penicillin and streptomycin combination (PenStrep) was added to inhibit bacterial growth. The plates were incubated for 48 hours at 32°C after which the colonies were examined and studied. The colony was identified as *Malassezia* sp. after undergoing macroscopic colony test on SDA, microscopic observation by Gram staining, and Tween assimilation test [19].

#### 2.4. Inoculum Preparation

The inoculum preparation of yeast suspension was standardized using a spectrophotometer. The optical density of the yeast suspension was adjusted to turbidity at absorbance (A) reading within the range of 0.08 at 625 nm which corresponded to 10⁸ CFU/mL whereas for the filamentous fungi, the same inoculum size was equivalent to absorbance (A) reading adjusted within the range of 0.09 to 0.13 at 530 nm. Tween 80 was added as wetting agent to facilitate the preparation of inoculum [20].

#### 2.5. Screening of Antimicrobial Activity

The extracts from the flowers and leaves of *J. sambac* were subjected to antifungal screening by agar disc diffusion method [21]. SDA plates which were overlaid with 1 mL of olive oil were inoculated with the test isolate by spreading the standardized inoculum on the surface of the agar plate with sterile swab. Holes of diameter 5 mm were punched onto the sterile filter paper and soaked with 20 μL of each of the extract solutions at 25 mg/mL, 50 mg/mL, 75 mg/mL, and 100 mg/mL. Fluconazole disc (40 mg/mL) served as positive control whereas the disc containing 10% DMSO alone was used as a negative control for antifungal assay. The discs were dried for 8 hours and placed onto the agar plates. All the plates were incubated at 32°C for 48 hours in moist condition because this is the optimum growth temperature for all the fungi tested.
The antimicrobial activity was assessed by measuring the diameter of the zone of inhibition in mm from observation of the clear zones formed surrounding each disc. The bioassay was performed in triplicate in order to calculate the mean value.

2.6. Determination of Minimum Inhibitory Concentration. The methanol extract from the flowers and leaves of J. sambac was subjected to antifungal sensitivity testing by broth microdilution method [22]. The 96-microtitr well was prepared by dispensing 95 μL of SDA broth overlaid with 1 mL of olive oil and left for 15 minutes before adding 5 μL of the yeast suspension into each well. One hundred μL from the stock solution of J. sambac essential oil at 100% concentration was added into the first well, then followed by 100 μL of 75%, 50%, and 25% essential oil added to the next three wells consecutively. The plate was covered with a sterile plate shaker at 300 rpm for 20 seconds and then incubated at 32°C for 48 hours. At the end of the incubation period, the plates were evaluated for the presence or absence of growth. MIC is the lowest concentration of the antifungal agent showing no turbidity after 48 hours, where the turbidity is interpreted as visible growth of the fungi. The bioassay was performed in triplicate in order to calculate the mean value.

3. Results

The skin samples obtained from the patients showed cell morphology bearing the hyphae that resembled the shape of spaghetti and meatball. The samples were tested positive for catalase due to the presence of gas bubbles in reaction to 10% hydrogen peroxide (H_2O_2). Out of the five skin samples, 3 isolates were shown to be similar to the morphology of Malassezia sp. The isolates were named M1, M2, and M3, respectively, with M1 and M2 growing on SDA with olive oil and M3 without olive oil. The first isolate (M1) showed a creamy white and smooth colony, while the second (M2) isolate showed a creamy pink and smooth colony, both on SDA with olive oil. The third (M3) isolate showed a creamy white and smooth colony on SDA without olive oil. All isolates were then observed microscopically by Gram staining using a microscope. The morphology of cells according to isolates was presented in Figures 1–3. All isolates showed budding in the cells with M1 (Figure 1) and M2 (Figure 2) showing broad based budding while M3 (Figure 3) showing narrow based budding. Tween assimilation test indicated a positive growth on Tween 40, Tween 60, and Tween 80 for M1 (Figure 4) and positive growth on all the Tween 20, Tween 40, Tween 60, and Tween 80 for M2 isolate (Figure 5). M3 isolate, however, showed negative growth for all the Tween concentration as observed in Figure 6.

Table 1 showed the percentage yield of methanol extract and essential oil from both the leaves and flowers of the plant. The extraction yield using methanol showed that the flowers of J. sambac produced 20.69% crude extract whereas the yield from leaves of J. sambac was only 11.49%. On the other hand, it was the leaves of the plant which produced a higher yield of essential oil (8.20%) compared to its flowers, whereby only 2.12% of the essential oil was obtained using hydrodistillation technique.

Table 2 showed the result of antifungal activity of the methanol extract from the leaves and flowers of J. sambac. All the isolates were susceptible towards the methanol extract from both parts of the plant with mean inhibition zone ranging from 9.10 ± 1.92 mm to 12.90 ± 1.68 mm. M1 isolate showed the highest susceptibility towards the methanol extract from both the leaves and flowers with inhibitory zone of 11.10 ± 1.92 mm and 12.90 ± 1.68 mm, whereas the methanol extract from J. sambac leaves and flowers exhibited the lowest inhibitory zone of 9.10 ± 1.92 mm and 10.17 ± 0.38 mm, respectively against M3 isolate. Results of MIC value determination using microdilution assay showed that both the extracts from J. sambac have equal antifungal efficacy towards the M1 and M2 isolates with MIC values of the leaves extract and flower extract at, respectively, 160 mg/mL and 80 mg/mL. This indicated that methanol extract of flowers exhibited twice inhibitory effect against Malassezia sp. compared to leaves. On the other hand, M3 isolate displayed weaker activity with MIC value of methanol extract from J. sambac leaves and flowers of 320 mg/mL and 160 mg/mL, respectively.

Table 3 showed the result of antifungal activity of the essential oil from the leaves and flowers of J. sambac. Disc diffusion screening as measured by zone of inhibition showed that essential oil from J. sambac flowers produced mean inhibition zone ranging from 9.36 ± 1.22 mm to 13.06 ± 0.26 mm.
No antifungal activity was observed from the essential oil from the leaves of *J. sambac* as it did not produce any inhibitory effect against all isolates tested. The MIC values of essential oil from *J. sambac* flower against all isolates were in a range of 50% to 75% out of 100% in 100 μL of essential oil. It was also clearly demonstrated that *J. sambac* flowers have the same antifungal efficacy towards both M1 and M2 isolates with MIC value of 50% strength of the essential oil.

### 4. Discussion

Although *Malassezia* is an opportunistic fungus in normal flora, it can be pathogenic and causes a variety of skin disorders which utilizes the lipid produced among humans due to the increasing population and global temperature [23]. Although these diseases were treatable with commercial antifungals, the need to continually use them over a long period of time results in many side effects and can be the cause of increased resistance [24, 25].

The search for antimicrobial from natural sources has received much attention and effort has been put in to identify compounds that can act as suitable antimicrobial to replace synthetic agent. Natural plants have been used in many countries following remedies from tradition and custom due to its abundance and availability. Phytochemicals derived from plant product serve as a prototype to develop more effective medicine in controlling the growth of microorganism with less toxicity [26]. Throughout the history of mankind, many infectious diseases have been treated with plant extract. The extraction of extract from medicinal plant has shown that some of the screened plants are potentially rich source of antibacterial and antifungal agent [27, 28].

In the present study, the skin samples obtained from five subjects were cultured to isolate *Malassezia* sp. From the five samples, only three isolates of fungi were obtained which showed resemblance to *Malassezia* sp. Microscopical observation showed broad based budding of cells in M1 and M2 isolates whereas M3 showed narrow based budding. The broad based budding of M1 and M2 isolates mimicked the morphology of cells represented by *Malassezia* sp. [29].
Table 2: Antifungal activity of methanol extract from the leaves and flowers of *J. sambac* against *M. sympodialis*, *M. dermatitidis*, or *M. furfur* and non-*Malassezia* species.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Parts of the plants</th>
<th>Zone of inhibition (mm ± SD)</th>
<th>MIC value (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>Leaves</td>
<td>11.10 ± 1.92</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>Flowers</td>
<td>12.90 ± 1.68</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Positive control</td>
<td>15.67 ± 1.68</td>
<td>40</td>
</tr>
<tr>
<td>M2</td>
<td>Leaves</td>
<td>10.87 ± 1.28</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>Flowers</td>
<td>11.17 ± 0.38</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Positive control</td>
<td>16.67 ± 1.11</td>
<td>40</td>
</tr>
<tr>
<td>M3</td>
<td>Leaves</td>
<td>9.10 ± 1.92</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>Flowers</td>
<td>10.17 ± 0.38</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>Positive control</td>
<td>15.88 ± 1.11</td>
<td>40</td>
</tr>
</tbody>
</table>

Positive control used was Fluconazole at 40 mg/mL. The diameter of inhibition zone was presented as a mean of 3 replicates. The isolates M1, M2, and M3 represented *M. sympodialis*, *M. dermatitidis*, or *M. furfur* and non-*Malassezia* species, respectively.

Table 3: Antifungal activity of essential oil from the leaves and flowers of *J. sambac* against *M. sympodialis*, *M. dermatitidis*, or *M. furfur* and non-*Malassezia* species.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Parts of the plants</th>
<th>Zone of inhibition (mm ± SD)</th>
<th>MIC value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>Leaves</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Flowers</td>
<td>13.06 ± 0.26</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Positive control</td>
<td>15.92 ± 1.57</td>
<td>25</td>
</tr>
<tr>
<td>M2</td>
<td>Leaves</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Flowers</td>
<td>12.36 ± 1.33</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Positive control</td>
<td>15.87 ± 1.72</td>
<td>25</td>
</tr>
<tr>
<td>M3</td>
<td>Leaves</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Flowers</td>
<td>9.36 ± 1.22</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Positive control</td>
<td>15.71 ± 1.68</td>
<td>25</td>
</tr>
</tbody>
</table>

Positive control used was Fluconazole at 40 mg/mL. The diameter of inhibition zone was presented as a mean of 3 replicates. — denotes no inhibition of bacterial growth. The isolates M1, M2, and M3 represented *M. sympodialis*, *M. dermatitidis*, or *M. furfur* and non-*Malassezia* species, respectively.

All isolates were tested with Tween assimilation test which acts as a lipid provider for the fungus. M1 isolate showed positive growth towards Tween 40, Tween 60, and Tween 80 whereas positive growth was recorded by M2 isolate in all Tween. This indicated that M1 isolate could possibly be *M. sympodialis* while M2 isolate showed the possibility of either *M. dermatitidis* or *M. furfur* species. M3 isolate showed negative growth towards all Tween and is therefore possibly *M. globosa* or a non-*Malassezia* species. However, since *M. globosa* needed lipid for its growth and isolation and M3 is isolated from SDA without olive oil, M3 can be confirmed as a non-*Malassezia* species. From these observations, M1 and M2 isolates were confirmed to be *Malassezia* sp. whereas M3 was identified as a non-*Malassezia* species.

The result of extraction yield of the *J. sambac* showed that methanol was capable of extracting the high quantity of constituents from the flowers of *J. sambac* whereas most of the essential oil was produced by the leaves of the plant. The high percentage yield of methanol extract indicated that methanol was the best solvent that can extract many of the polar active compounds found in the flowers compared to the leaves of *J. sambac*. The polarity of methanol and the solubility of plant secondary metabolites in methanol could be the probable reason for the high extractive value of methanol extract [30]. Hydrodistillation however produced higher yield of essential oil from the leaves of *J. sambac* compared to the flowers. This is supported by the finding that an essential oil is distilled from the fermented and dried leaves of *Camellia sinensis*, a source of the popular beverage tea [31].

Results obtained from the screening of antifungal activity indicated that the methanol extract from both the leaves and flowers of *J. sambac* showed inhibitory effect against all isolates tested. However, *Malassezia* sp. represented by M1 and M2 isolates appeared to be more susceptible towards the effect of *J. sambac* flowers which yield more phytocomponents extracted by methanol. The methanol flower extract showed higher yield and higher antifungal activity than the methanol leaves extract. Hence, it can be noted that as for methanol extraction the higher the extractive potential, the stronger the antifungal activity. However this is not the case for the hydrodistillation method because despite the high yield of essential oil from *J. sambac* leaves compared to its yield from the flowers, the former showed no inhibitory effect against all three isolates studied. This means that *Malassezia* sp. represented by M1 and M2 isolates was only susceptible to the essential oil from *J. sambac* flowers which might contain a promising antifungal phytoconstituent against *Malassezia* sp. The inability of essential oil of leaves to exhibit any inhibitory effect could mean that hydrodistillation method was unable to extract the active antifungal compound efficiently from the leaves of the plant [32]. In other words, this method of essential oil extraction is slightly ineffective if used on other parts of the plant other than the flowers. This is supported by [33] on multiple plants, which stated that hydrodistillation on other parts of a plant besides flowers produces less antimicrobial activity. However, there is no past research in order to make comparison that reports the antimicrobial potential of other parts of *J. sambac* besides flowers and therefore, this finding is interesting because it was shown that essential oil from other parts of *J. sambac* besides flowers cannot be exploited as a source of antimicrobial agent.

The presence of antifungal activity in the methanol extract from both the leaves and flowers of *Jasminum sambac* may be due to the presence of tannin. *J. sambac* flowers and leaves were reported to contain the polyphenol tannin and sambacin which are known to possess antimicrobial property [34]. However the essential oil from *J. sambac* flowers which comprise mainly α-farnesene, benzyl acetate,
and linalool [35] could contribute to its anti-Malassezia activity.

5. Summary

This study demonstrated that both the methanol and essential oil from the flowers of Jasminum sambac showed promising inhibitory effect against Malassezia sp. isolated from the skin samples of patients with dandruff. In conclusion, J. sambac flowers can be developed as potential phytotherapeutic source of treatment against Malassezia-associated skin infections and possibly as an additive ingredient in the development of medicated shampoo against dandruff as well as skin and scalp infections.

6. Recommendation

However, further investigation to evaluate the antifungal activity of J. sambac in mice infected with Malassezia sp. is necessary to correlate the effectiveness of its methanol extract and essential flowers in vivo.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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