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ORIGINAL STUDY

Antimicrobial Properties of *Jasminum sambac* Linn Leave Extract on Selected Oral Associated Pathogen

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ABSTRACT

Traditional medicine using *Jasminum* leaves to treat oral health problems is expected due to easy access. Furthermore, due to the rising issue of antibiotic resistance, discovering a natural antibiotic that can effectively replace synthetic antibiotics would be valuable as natural antibiotics can cover the benefits, challenges, and future development. This study aims to assess the antimicrobial properties of ethanolic and aqueous extracts of *Jasminum sambac* L. leaves at the concentration of 50, 150, and 250 mg/mL against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 10145, *S. aureus* ATCC 33591 and *B. cereus* ATCC 11778, *C. albicans* ATCC 10231 and *A. niger* ATCC 16404 using disk diffusion test and 96-well plate. The ethanolic extract showed antimicrobial activity against *E. coli* ATCC 25922 and *A. niger* ATCC 16404 with inhibition zones 9.33 ± 0.67 mm (50 mg/mL), 9.67 ± 0.58 mm (150 mg/mL), 10.00 ± 0.33 mm (250 mg/mL) and 12.33 ± 0.33 mm (50 mg/mL), 13.33 ± 1.67 mm (150 mg/mL), 19.67 ± 0.33 mm (250 mg/mL), respectively. Meanwhile, aqueous extract only showed antimicrobial activity against *A. niger* ATCC 16404 with the zone of inhibition 15 ± 1.15 mm (50 mg/mL), 23.0 ± 1.15 mm (150 mg/mL), and 24.0 ± 0.58 mm (250 mg/mL). The MIC value for ethanolic extract against *E. coli* ATCC 25922 was 50 mg/mL, and the MBC value was 250 mg/mL while, against *A. niger* ATCC 16404 was 18.75 mg/mL, whereas the MFC value was 250 mg/mL. The results showed that the antimicrobial properties of *J. sambac* leaf extract were still shown in the maceration method, which justifies the tribal uses of *J. sambac* leaf against oral-associated pathogen infections.

Keywords: Jasminum sambac L., Leaf extract, Antimicrobial, Oral pathogens

1. Introduction

Oral problems have become an emerging issue around the globe. Oral infections that result from dental caries, pulpal necrosis, trauma, and periodontal diseases can cause severe consequences on the soft and hard tissues of the oral cavity. Antibiotic usage is applied in cases such as prophylaxis for local and focal infections and odontogenic and nonodontogenic infections [1]. Since antibiotic therapy is also applied to treat oral problems, it is vital to note that antibiotic resistance among oral microbes might emerge. Antimicrobial resistance (AMR) is threatening since it will lower the efficiency of treating bacterial disease and other microbes-causing diseases [2]. Since modern medicine has turned the table, scientists are now discovering the use of herbal medicine obtained from nature, such as plants, to study the compounds it holds that can replace modern antibiotics. In addition, many reports have disclosed that dental surgeons frequently prescribe antimicrobials improperly, leading to the development of antimicrobial resistance in many oral pathogens [2], thus leading to a significant global health challenge, which is antibiotic resistance. A variety of synthetic antimicrobials are sold on the market to treat illnesses.

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However, synthetic antimicrobials' cost, safety, and effectiveness are subjects of growing concern today. As a result, many people are shifting to herbal remedies made up of medicinal plants.

There are two reasons why clinical microbiologists are intrigued by the topic of antimicrobial plant extracts. First, these phytochemicals will likely find their way into the arsenal of antimicrobial medications recommended by physicians; several are already being investigated in humans, and secondly, the public is becoming more aware of the issues linked to conventional antibiotic abuse and overprescribing [3].

Herbal medicine has been studied to assure the public of its safety and benefits from consuming it. The use of plants has been proven to contain antioxidants, anti-inflammatory, antibacterial, antifungal, and more. Phytochemicals are bioactive compounds in plant parts like fruits, nuts, leaves, and flowers. For example, bioactive compounds such as flavonoids, alkaloids, phenols, and more can act as antioxidants and antimicrobials [4]. The phytochemicals are extracted using organic solvents like ethanol, methanol, and hexane based on polarity principles [5].

Several herbs have been studied for their impact on oral problems—for instance, ginger, garlic, aloe vera, and miswak [6]. There are about 200 species of *Jasminum* sp. in Asia, especially in China, Malaysia, and Thailand [7]. However, little research is carried out on its ability to soothe oral problems. Moreover, some folk cultures, like those in northern India, especially Himachal Pradesh, already practiced using *Jasminum officinale* L. (flower) and *Jasminum arborescens* Roxb. (leaves) to treat mouth rashes [8]. Hence, without scientific proof, the public will feel less convinced to practice it. Nevertheless, few studies have been conducted on oral problems related to the *Jasminum* genus plant. However, some studies have shown the Jasminum plant's antimicrobial activities [9]. Moreover, several folk cultures use jasmine for traditional purposes, such as in cases of aphthous, stomatitis, toothache, and oral ulcers, where leaves are eaten. Conventional medicine also employed *Jasminum officinale* to treat urinary tract infections. In South China, *Jasminum officinale* L. var. *grandiflorum buds* were used as a traditional medicine to cure duodenitis, stomatitis, hepatitis, and dysmenorrhea [10]. Alkaloids, flavonoids (rutoside), terpenes, phenols, and iridoid glucosides are common phytoconstituents in diverse *Jasminum* species [11].

Several studies showed antimicrobial properties of parts of the *Jasminum* sp. plant, such as flowers, essential oils, leaves, and roots. Nonetheless, there are few reports on the effectiveness of *Jasminum sambac* L. leaves on oral pathogens, although they have been proven to have antimicrobial properties. Hence, this study was focused on the investigation of antimicrobial properties of *J. sambac* L. leaf and to determine the minimum inhibitory concentration (MIC), minimum bacterial concentration (MBC), and minimum fungal concentration (MFC) of *J. sambac* L. against selected oral-associated pathogens.

2. Methodology

2.1. Preparation of Jasminum sambac L. leaf extraction

The fresh leaves collected were extracted based on the method by previous researchers with slight modifications [12]. The leaves from *J. sambac* L. were rinsed with water to wash off unwanted material and dried under shade. About 26 g of air-dried *J. sambac* L. leaves were crushed until they became powder. Since it was extracted using the maceration method, the leaf powder was placed in two Erlenmeyer flasks with different solvents (water and ethanol) with a ratio of 1:20 (10 g in 200 mL of solvent). The flasks were placed on the table at room temperature. The process took three days. The mixture in the Erlenmeyer flasks was filtered through No.1 Whatman filter paper to obtain the supernatant. Then, the concentrated liquid of the jasmine leaf extract was obtained using a rotary evaporator. Each extract was run twice using the rotary evaporator to ensure the solvent used to extract the leaf was properly evaporated. The yield of the extract was calculated. The extracts were placed in a universal bottle and stored in the chiller at 6°C for further use.

2.2. Preparation of bacterial inoculum

The cultures were obtained from the Faculty of Applied Sciences Microbiology Laboratory in UiTM Shah Alam, Selangor, Malaysia. It was streaked on slanted agar tubes and then incubated at 37°C for 24 h. Then, one loop from the colony was transferred to the nutrient broth and incubated at 37°C overnight to obtain a fresh culture that is in the log phase where bacteria were active and good to test for antimicrobial testing [13]. The UV-Vis spectrophotometer was used to get the equivalent of 1×10^8 CFU/mL at the wavelength of 600 nm with an optical density (OD) of 0.08–1.0 [14].

2.3. Preparation of fungal inoculum

The fungi (*A. niger*) was subcultured on PDA (Potato Dextrose Agar, Oxoid CM0139, United Kingdom) plate agar and incubated at 35°C for two days. To

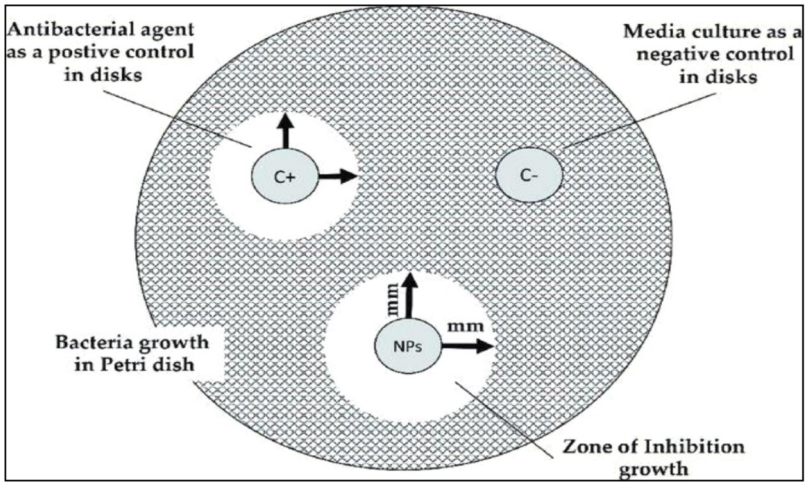

Fig. 1. Disk diffusion test. Source: Vega-Jiménez et al. (2019) [18].

obtain the conidia, the colonies were covered with 5 mL of distilled water. The conidia were scrapped using a sterile spatula and transferred to a sterile tube to vortex it until it was homogenized. Then, standardised using a UV-Vis spectrophotometer with 530 nm wavelength with 0.2 OD to achieve 1–5 $\times$ 10^6 CFU/mL [15]. For *C. albicans*, it was cultured on potato dextrose agar and incubated at 35°C for 24 h [16]. About 5 colonies from the plate were suspended in sterile distilled water. Then, using a vortex mixer for 15 s, the inoculum was evenly suspended. The suspension was adjusted to the wavelength of 600 nm with 0.15 OD to achieve 1–5 $\times$ 10^6 CFU/mL using a UV-Vis spectrophotometer [17].

2.4. Antimicrobial activity using disk diffusion test

The disk diffusion test was conducted by using the MHA (Mueller Hinton agar, Oxoid CM0337, United Kingdom) plate [16]. Once the surface of the agar on the plates solidified, 0.1 mL of standardized inoculum was spread on the MHA plate uniformly. The excess inoculum was discarded and dried for 5 min. The disk (Oxoid antimicrobial susceptibility test disc, United Kingdom) used to test the antimicrobial activity was impregnated with 20 μL of extract solution with the concentrations of 50, 150, and 250 mg/mL by using a micropipette. Positive control disks including commercial clindamycin, penicillin G, and erythromycin were added to the plates of tested bacteria. The positive control for fungi was a commercial fluconazole disk, and distilled water was for the negative control. The experiments were done in triplicate. The plates were incubated at 37°C for 24 h for bacteria and 35°C for 24–48 h for fungi. To observe the antimicrobial activities of the extract, the plate containing positive control was compared to study the formed clear zone. The clear zone measurement was measured using a ruler and recorded in a table. Fig. 1 shows the inhibition zone in the disk diffusion test if the leaf extract contains antimicrobial properties.

2.5. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) test was used to determine the lowest concentration that can inhibit the growth of bacteria and fungi [19]. This test was used with 96-well plates. For the MIC test, based on Fig. 2, bacteria were adjusted to 0.08–1.0 OD with 600 nm, and fungi were adjusted to 0.2 OD with 530 nm (*A. niger*) and 0.15 OD with 600 nm for *C. albicans*. The MIC test must be done within 30 minutes after it is standardized. Each well of the rows was added with 100 μL of sterile nutrient broth. The ratio of NB was 1:1 the amount of plant extract 100 μL and the amount of inoculum 100 μl. The eleventh well was used as growth control, which contains antibiotics/antifungals. The negative control was used on the 12th well. Then, serial two-fold dilutions were performed in the wells with *J. sambac* Linn leaf extract on all wells on the plate except for the positive control well. Lastly, 100 mL of each inoculum was added, and mixed in the well. After 24 h at 37°C (bacteria) and 35°C for 24–48 h (fungi) of incubation.

2.6. Minimum bactericidal concentration (MBC)

Minimum bactericidal concentration (MBC) determines the lowest concentration of extract that can kill the microorganisms. MBC is a continuation of

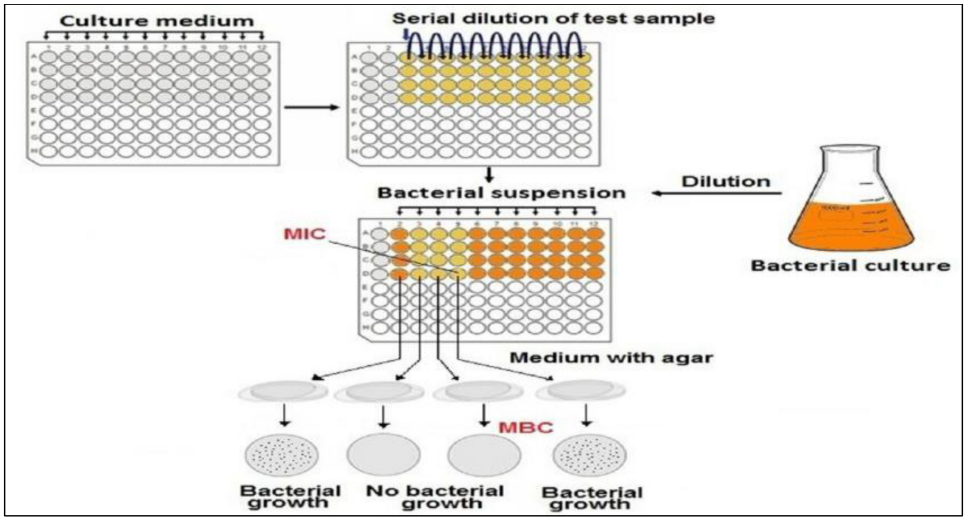

Fig. 2. Minimum inhibitory concentration (MIC) test flow. Source: Microbe Investigate Switzerland [20].

MIC. The content of the well from MIC that shows no growth (clear well) of microbes was streaked on the NA agar plate and incubated overnight at 37°C. The plate with the lowest concentration of bacteria growth was determined to be MBC.

2.7. Minimum fungicidal concentration (MFC)

The minimum fungicidal concentration (MFC) test showed the least concentration of antifungal activities when streaked on a plate free from test agents, and it showed no growth. MFC was a continuation of MIC. The content of the well from MIC that showed no growth (clear well) of microbes was streaked on the PDA agar plate, and it was incubated for 24–48 h at 35°C. The plate of the lowest concentration that showed np growth of microbes was determined as MFC.

2.8. Statistical analysis

The data of disk diffusion obtained was expressed as the mean ± SD. The statistical analysis compared the mean zone of inhibition using one-way ANOVA and post-hoc test (Tukey test) using SPSS software version 27 at $p < 0.05$ at different concentrations and microorganisms.

3. Results and discussion

3.1. The yield of Jasminum sambac L. leaves extraction

Table 1 shows the colour of the extract and the total yield of each extract from different solvents.

Table 1. Percentage yield for leaf extract of *J. sambac* Linn.

Solvent system	Colour of extract	Percentage yield (w/w%)
Distilled water	Dark brown	7.21%
70% Ethanol	Dark green	20.01%

The ratio (%) of the theoretical yield to the actual yield is termed the percent yield. It was calculated as the theoretical yield multiplied by 100% divided by the experimental yield. Moreover, yield percentage is crucial due to many parameters that affect the efficiency of the plant extract, such as temperature and extraction time [21]. Typically, the percentage yield is lower than 100% due to loss of samples during recovery, whereas if the percentage yield is above 100%, more samples are gained during the recovery, or incomplete removal of solvent may cause the miscalculation of the overall weight. Based on Table 1, the percentage yield (w/w%) for aqueous extract was 7.21%, while for ethanolic extract was 20.01%.

Most of the studies related to *Jasminum* sp. extract used the Soxhlet method [12, 22, 23]). This study applied the maceration method to observe the extracts' effectiveness in antimicrobial activities. In most of the studies, after they obtained the crude or product using the rotary evaporator, they stored the leaf extract in powder form using freeze-drying. The extract is favored to be kept in powder form as it can preserve the extracted compound longer [24]. In this study, the final form of the extract was liquid form. Since the freeze-drying instrument was unavailable, the extract solution was left to dry in a fume hood for two days in a petri dish covered with aluminum foil to prevent contamination. However, the extract remained the same as it showed no differences in weight and physical changes. Hence, the extracts obtained were

Table 2. Zone inhibition of microorganisms for the ethanolic and aqueous extraction.

Microorganisms	Mean zone of inhibition (mm) and Concentration (mg/mL)					
	Ethanol			Aqueous		
	50	150	250	50	150	250
S. aureus	ND	ND	ND	ND	ND	ND
B. cereus	ND	ND	ND	ND	ND	ND
P.aeruginosa	ND	ND	ND	ND	ND	ND
E. coli	9.33 ± 0.67^{a}	9.67 ± 0.58^{a}	10.00 ± 0.33^{a}	ND	ND	ND
A. niger	12.33 ± 0.33^{a}	13.33 ± 1.67^{a}	19.67 ± 0.33^{b}	15.0 ± 1.15^{a}	23.0 ± 1.15^{b}	24.0 ± 0.58^{b}
C. albicans	ND	ND	ND	ND	ND	ND

*ND means not detected.
Results are the means of inhibition zone values followed by standard deviations; Different letters within the same row indicate means at different concentrations for the same species are significantly different ($p \leq 0.05$).

tightly kept in a universal bottle, and the next step was antimicrobial testing using disc diffusion.

3.2. Antimicrobial activity of J. sambac leaf

This study studied the ethanolic and aqueous extract of *J. sambac* leaf against selected bacteria and fungi. The bacteria used were *S. aureus*, *B. cereus*, *E. coli* and *P. aeruginosa*. On the other hand, the fungi used were *C. albicans* and *A. niger*. As a result, the ethanolic extract of *J. sambac* leaf worked against *E. coli* and *A. niger*. As for aqueous extract, it worked against *A. niger* only. Nonetheless, previous studies showed outstanding antimicrobial properties with different solvents used to extract the leaf of *J. sambac* and *Jasminum* species against diverse microorganisms by using various extraction methods.

3.2.1. Antimicrobial activity against ethanolic extraction of J. sambac L.

The ethanolic extract of *J. sambac* leaf only showed antibacterial activities against *E. coli*. Based on the concentrations that had been tested, 50, 150, and 250 mg/mL, all showed zone of inhibition. Based on Table 2, the inhibition zones recorded for *E. coli* were 9.33 mm, 9.67 mm, and 10.00 mm for 50, 150, and 250 mg/mL concentrations, respectively. Other bacteria tested were recorded as not detected (ND) as the zone of inhibition had no clear zone, and some were too small to measure. The ANOVA analysis was conducted with a significance level of 5% ($\alpha = 0.05$). As determined by One-Way ANOVA, there was no significant difference between the concentrations tested ($p = 0.702$). Moreover, Table 2 generated the standard error of the mean (SE) for each concentration. The inhibition zone of *E. coli* for each concentration was small, based on Table 2. Hence, there was no significant difference between the concentrations. Other bacteria tested showed no inhibition zone in either ethanolic or aqueous extract. Nevertheless, previous studies did show antibacterial activity. Based on previous studies [25], on *J. sambac* leaf, showed the zone of inhibition for ethanolic extract of *E. coli* was 27 mm. Moreover, the leaf ethanolic extraction of *Jasminum flexile* Vahl against *E.coli* showed outstanding antibacterial when tested with eluents and fractions obtained from the leaves [26] using refluxing. However, in another study, extracting *J. sambac* leaf with ethanol using the Soxhlet method, the size of the inhibition zone obtained was 8.9 mm, 10 mm, and 15 mm with concentrations of 50, 100, and 150 mg/mL, respectively [25].

For fungi in the ethanolic *J. sambac* leaf extract, only *A. niger* showed a zone of inhibition. The zone measured was 12.33 mm, 13.33 mm, and 19.67 mm, with concentrations of 50, 150, and 250 mg/mL, respectively. The *C. albicans* was recorded as ND as it did not show any sign of a clear zone. One-Way ANOVA analysis of the inhibition zone against ethanolic leaf extract of *J. sambac* against *A. niger* with a significance level of 5% ($\alpha = 0.05$) shows there was a significant difference in the concentration tested ($p = 0.004$). Tukey test revealed a significant difference between 50 mg/mL with 250 mg/mL ($p = 0.005$) and 150 mg/mL with 250 mg/mL ($p = 0.010$). However, the concentration of 50 mg/mL with 150 mg/mL showed no significant difference ($p = 0.768$). In previous studies, both *A. niger* and *C. albicans* did show a zone of inhibition but using different methods of extraction. Following the previous study, the ethanolic extract (using the Soxhlet method) showed antifungal activities against *C. albicans* and *A. niger*. As reported by previous researchers [12], the zone of inhibition for *A. niger* with concentrations of 50, 100, and 150 mg/mL were 7.3, 8.5, and 10 mm, respectively. Whereas for *C. albicans*, the inhibition zone was 8.5, 9.0, and 10.5 mm using 50, 100, and 150 mg/mL concentrations, respectively.

The antimicrobial test results in this study were different from other studies probably due to the different

extraction methods applied. The zone of inhibition appearance was due to the secondary metabolites that were successfully extracted from the *J. sambac* leaves. Thus, other secondary metabolites were extracted when the maceration method was applied. Alkaloids and phenolic compounds can disrupt the cell membrane of Gram-negative [27]. Regardless, the biochemical tests for ethanolic extract showed the presence of flavonoids but the absence of alkaloids [12]. Moreover, saponin and phenolic compounds may contribute to the antibacterial and antifungal properties. Saponin showed good effectiveness on Gram-positive rather than Gram-negative [28]. Despite that, *S. aureus* and *B. cereus* did not show any antibacterial activities. Furthermore, using the Soxhlet method and ethanol as a solvent to extract it showed maximum activity for both antibacterial and antifungal [12]. In addition, studied on *Jasminum azoricum* leaf with maceration as an extraction method, aqueous extract succeeded in extracting tannins, saponins, flavonoids, phenols, coumarins, alkaloids, steroids, and betacyanin [29]. On the other hand, ethanolic extract can be used to extract tannins, cardio glycosides, terpenoids, steroids, and alkaloids. The result was astonishing in aqueous extract as it contains coumarins, which, due to their planar molecules and lipophilic structures, aided in penetrating the cell membrane or wall, thus leading to antibacterial activity.

3.2.2. Antimicrobial activity against aqueous extraction of J. sambac L.

The aqueous extract of *J. sambac* leaf in this study showed that only *A. niger* had a zone of inhibition (Fig. 3). The inhibition zone displayed was 15 mm ± 1.15, 23 mm ± 1.15, and 24 mm ± 0.58 with the tested concentration of 50, 150, and 250 mg/mL, respectively. Other microorganisms tested were recorded as ND due to not showing any clear zone or little zone that cannot be measured. The aqueous extract showed better antifungal activity in *A. niger* compared to the ethanolic extract of *J. sambac* leaf. One-Way ANOVA analysis of the aqueous extract *J. sambac* leaf against *A. niger* with a significance level of 5% ($\alpha = 0.05$) demonstrates there was significance in the concentrations tested ($p = 0.001$). Tukey test displayed a significant difference in concentrations of 50 mg/mL and 150 mg/mL ($p = 0.003$) and concentrations of 50 mg/mL and 250 mg/mL ($p = 0.002$). Nevertheless, the 150 mg/mL concentration and 250 mg/mL displayed no significance as $p = 0.768$.

Despite little research being done using distilled water as the solvent, there was a study highlighting that aqueous extract of *J. sambac* showed zone of inhibition when tested on clinical isolates such as

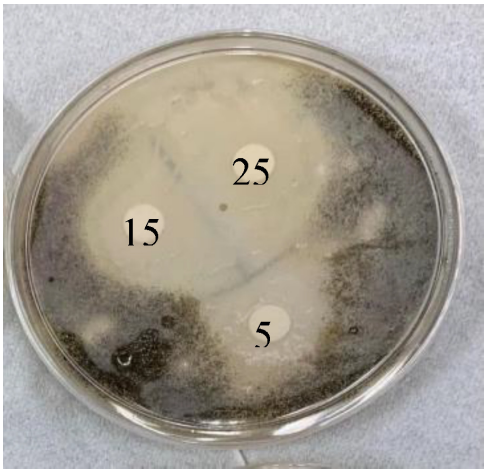

Fig. 3. Disc diffusion result for aqueous extract against *A. niger.*

Staphylococcus mutans and *Lactobacillus casei* [30]. In this study, the bacteria tested showed no zone of inhibition. Although there was a study showing that *P. aeruginosa*, *S. aureus,* and *E. coli* did display no zone of inhibition using the Soxhlet method. Despite getting the same result, future research can still be conducted, such as longer soaking days and increasing the concentration tested (>250 mg/mL), as the maceration method might turn the table. Furthermore, since there was no study yet to test *A. niger* using aqueous extract with the maceration method, there must be compounds that could show the inhibition zone against *A. niger* but not on *C. albicans* despite there being a study stating that with the use of leaf of *J. sambac* using Soxhlet method, it displayed that *C. albicans* showed zone of inhibition [30]. Based on the previous studies, little research was done with distilled water as a solvent to extract as it rarely showed a zone of inhibition [31]. However, using the Soxhlet method, *C. albicans* and *S. aureus* showed antimicrobial activities with 12 mm ± 0.50 zone inhibition [30]. Most of the studies that used distilled water as the solvent for extraction showed more significant gains of secondary compounds compared to other extracts that used solvents such as chloroform, acetone, and petroleum ether. In addition, the extract from *J. grandiflorum* (extracted by Soxhlet method), which was expressed in μg/mL unit, presented that the aqueous extract had antibacterial activity against *E. coli*, *P. aeruginosa,* and *S. aureus*. Nevertheless, based on past studies, *S. aureus* usually needed higher concentration compared to *E. coli* and *P. aeruginosa* to show antibacterial activity [23].

As mentioned earlier, different solvents also will exhibit different antimicrobial activities. For example, methanolic and petroleum ether extract of *J. grandiflorum* leaf showed better activity against

P. aeruginosa, *S. aureus*, *B. subtilis,* and *E. coli* [23]. In addition, this study also observed that chloroform extract was effective against *B. subtilis* and *P. aeruginosa* while acetone extract was effective against *P. aeruginosa* and *E. coli*. That study used maceration as the extraction method. Hence, the choice of solvent is crucial to perform the extraction for better antimicrobial activity.

3.2.3. Minimum inhibitory concentration (MIC)

The MIC test was conducted only on the plate that showed a zone of inhibition on the disc diffusion test. Hence, the MIC and MBC test for ethanolic extract of *J. sambac* leaf against *E. coli* has been conducted. However, the MIC and MFC test for ethanolic and aqueous extract against *A. niger* have been proceeded. Table 3 shows the MIC and MBC value of the ethanolic extract of *J. sambac* leaf against *E. coli*, whereas Table 4 shows the MIC and MFC value of the ethanolic extract of *J. sambac* leaf against *A. niger*. Based on the ANOVA result, all the concentrations tested were insignificant against *E. coli* for ethanolic extract. Thus, the lowest concentration was tested to identify the MIC value. The MIC value for the *E. coli* was 50 mg/mL, meaning the lowest concentration that can inhibit the growth of *E. coli* was 50 mg/mL.

The MIC value for the ethanolic extract of *J. sambac* leaf against *A. niger* was 18.75 mg/mL. Referring to the Duncan test in Appendix 4 and Appendix 8, the concentration that was chosen to test for MIC value was 150 mg/mL for both extracts. However, only the ethanolic extract showed MIC value whereas none for the aqueous extract of *J. sambac* leaf against *A. niger* despite showing better antimicrobial activities in the disc diffusion test compared to the ethanolic extract. This is probably due to the compounds that aid in the antifungal activities of *A. niger* degraded or lost to the environment. The secondary metabolites also might be degraded due to improper storage of extracts, as secondary metabolites can be unstable and easily metabolized during storage [32]. Higher concentrations were tested; however, it still showed no result. Previous study [30] observed that the MIC value for the methanolic extract of *J. sambac* leaf against *S. aureus* and *C. albicans* was 3.12 mg/mL and 25 mg/mL. There was little record of MIC value for the leaf extract of *J. sambac*. Regardless, the essential oil extracted from the flower of *J. sambac* showed that the MIC values for *S. aureus* (>12.5% v/v), *E. coli* (1.56% v/v) and *C. albicans* (0.39% v/v) [9]. Furthermore, a study using *Murraya paniculate* leaf extract, which has similar characteristics to *J. sambac* leaves, presented that the MIC value was indeterminable by using the same method as in this study but using 40% ethanol [33]. However, when tested with methanol and aqueous (freeze-drying was applied for drying purposes), there was antibacterial activity [34]. Hence, this shows the concentration of solvents used and the proper elimination of solvent used from the extract played a crucial role in obtaining the extracted metabolites. In addition, there was a study stated that the ethanolic extract of *J. sambac* flower that was macerated for 48 h showed, the MIC value for *S. aureus* was 250 μg/mL [35]. Whereas for *E. coli* and *B. cereus*, both MIC values were 1000 μg/mL. In other studies, *J. sambac* flowers macerated for 3 days to obtain essential oil showed it can inhibit the growth of *E. coli* at a concentration of 25% by using ethyl acetate as a solvent [36]. In addition, because the ethyl acetate extract has a hydrophilic and lipophilic balance, which made it more effective in damaging the bacterial cell wall component, it was the most effective solvent of extract at preventing the growth of *E. coli*.

Table 3. Result for MIC and MBC for ethanolic extract of *J. sambac* leaf.

Microorganisms	MIC (mg/mL)	MBC (mg/mL)	Ratio MBC/MIC	Bactericidal/ Bacteriostatic
E. coli	50	250	5	Bacteriostatic

Table 4. Result for MIC and MFC for ethanolic extract of J. sambac leaf.

Microorganisms	MIC (mg/mL)	MFC (mg/mL)	Ratio MFC/MIC	Fungicidal/ Fungistatic
A. niger	18.75	250	13.33	Fungistatic

3.2.4. Minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC)

The minimum bactericidal (MBC) and fungicidal (MFC) tests were done to determine at which concentration the bacteria or the fungi are killed. Based on Tables 3 and 4 above, the MBC for ethanolic extract against *E. coli* was 250 mg/mL and the MFC value for *A. niger* was 250 mg/mL. In addition, in Tables 3 and 4, the ethanolic extract of *J. sambac* leaf showed bacteriostatic and fungistatic effects. This indicates the extract was able to inhibit the growth of bacteria and fungi, not killing them. The bacteriostatic effect was calculated based on the ratio of MBC/MIC. If the value is more than 4, it is considered bacteriostatic and vice versa [37]. This calculation can also determine fungistatic [38]. A previous study using the ethanolic extract of the *J. sambac* flower, the MBC for *S. aureus* and *E. coli* was >4000 μg/ml. For *B. cereus*, the MBC value was 2000 μg/mL [35]. Moreover, neither MIC nor MFC were detected for C. albicans. In addition, the inhibition zone for *S. aureus* was 7.33 mm. By referring to the data of *S. aureus*

of the study, the MIC value was 250 μg/mL, whereas the MBC value was >4000 μg/mL. This showed that the size of the zone of inhibition played a role in determining the MIC and MBC/MFC values. Since the zone of inhibition for *S. aureus* was small, hence the MIC and MBC values were higher to inhibit or kill the bacteria.

The aqueous extract of *J. sambac* against *A. niger* showed a greater measurement of the inhibition zone compared to ethanolic leaf extract. However, when tested for MIC, it was not detected. As mentioned earlier, this was probably due to the secondary compounds that contribute to the presence of an inhibition zone lost to the environment or degraded. Hence, the MFC value was not able to be determined since the MIC value was indeterminable.

In the present study, the data gained differed from the previous research, which mainly used the Soxhlet and other extraction methods. Nevertheless, the use of the maceration method showed antimicrobial activities, but further research is needed such as in terms of days of soaking, temperature, and more. The characteristics of the leaves also required to be considered as the penetration of the solvent into the leaves played a significant role in extracting the secondary metabolites [33]. The choice of solvent is also vital as each solvent has different capabilities for extracting the secondary metabolites [39]. In addition, the effect of the material/solvent ratio also played a role in studying the efficiency of extraction. A previous study [39] showed that a ratio of 1:30 showed the optimum material/solvent ratio.

Nevertheless, a study that used the ratio 1:20 with the Soxhlet method produced a result but not on aqueous extract [22]. However, there was a report that by using the ratio of 1:3, aqueous extract managed to produce results for both clinical isolates of oral pathogens, bacteria, and fungi [30]. It is also worth mentioning that the concentrations to be tested should be less than the commercial antibiotics or antifungals since lower dosages can have minimal effects on humans. Lastly, it was also due to the further extraction, and adjustments are needed so that the extraction will be optimum.

4. Conclusion

In summary, ethanolic extract of *J. sambac* L. leaf against selected associated oral pathogens only showed better antimicrobial properties (for bacteria) in *E. coli*) than aqueous extract. Whereas for fungi, the aqueous extract showed better antimicrobial properties against *A. niger* only. The MIC value for *E. coli* was 50 mg/mL, whereas the MBC value was 250 mg/mL. The MIC value for *A. niger* was 18.75 mg/mL and the MFC value was 250 mg/mL.

Regardless, in future studies, a few recommendations can be added. First, more advanced methods to detect the phytochemical compound in *J. sambac* L. through maceration, such as the use of Liquid Chromatography-Mass Spectrophotometry (LCMS), should be established to identify any other compounds that may lead to such results. For example, as mentioned earlier, saponin is one of the secondary metabolites that act as antibacterial and antifungal however, the result only showed excellent antimicrobial properties against *A. niger* only and moderate antimicrobial properties against Gram-negative bacteria. This indicates that there must be another compound that may boost the antifungal activity instead of saponin. The biochemical test can lead to false positive-negative results. Hence, higher sensitivity technology should be applied to enhance the information obtained.

Next, as stated previously, other factors like temperature, pH, and days of maceration should be studied so that the maceration method can have an outstanding result as good as the Soxhlet method. Lastly, storing the extract is crucial to further studies for an extended period. The extract can be stored either in powder form through lyophilization or stored in the freezer at −80°C in separate containers. This will reduce the probability of the compounds in the extract being degraded or lost to the environment. Keeping the extracts in individual containers with desired amounts in each container will take out only the amount of extracts needed. Thus, the compounds can be preserved for a long time by reducing the defrosting process.

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Conflicts of interest

The authors declare that there are no competing interests.

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