Nanoemulsion Mouthwash Formulation of Bajakah Tampala (Spatholobus littoralis Hassk.) Skin Extract Against Candida albicans

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Abstract

Candida albicans can cause two infections in humans: superficial and systemic. The ability of C. albicans to infect the host is influenced by virulence factors and character changes so that it can fool the immune system. From the character change factor, C. albicans can form a biofilm. This study aims to determine the good concentration in inhibiting and determine the antifungal and antibiofilm activity of nanoemulsion mouthwash formulation of bajakah tampala (Spatholobus littoralis Hassk.) skin extract against C. albicans. This research was conducted with an experimental method. The formulation used a spontaneous magnetic stirrer technique to make nanoemulsion preparations. Antifungal and antibiofilm tests were carried out by dilution method using a 96-well plate and a microplate reader with a wavelength of 620 nm to determine the percentage inhibition against C. albicans and determine MIC and MBIC. The results showed that the nanoemulsion mouthwash formulation of S. littoralis inhibited the planktonic and biofilm of C. albicans. The concentration of 1% is expressed as MIC and MBIC. Therefore, the nanoemulsion formulation of S. littoralis extract could inhibit the growth of C. albicans in the oral cavity.

INTRODUCTION

Based on the results of the Basic Health Research in 2018, the percentage of Indonesian people who have problems with their teeth and mouth increased from 25.9% in 2013 to 45.3% in 2018, meaning that in 5 years, the percentage of dental and oral problems is still experiencing problems enhancement, one of which is caused by Candida albicans infection. Candida albicans is one of the microorganisms that can form biofilms to protect themselves from external attacks the formation of this biofilm is influenced by saliva and food eaten daily. Candida albicans form mycelia in a transformed environment in stem cells, then adapt to the ecological microenvironment. The three forms differ in cell morphology, function, and growth

conditions. The potential of C. albicans to alter yeast morphology and filamentous form is a condition that influences its pathogenic potential on the mucosal surface of the host. The ability of C. albicans to infect various hosts is influenced by virulence factors and character changes so that it can escape the immune system. Candida albicans can cause two main infections in humans: superficial infections such as oral and vaginal candidiasis and systemic infections that endanger life. Some characteristics of C. albicans are changes in the shape between yeast and hyphae, expression of adhesins and invasions on the cell surface, thigmotropism, formation of biofilms, and secretion of hydrolytic enzymes, which are infectious factors. The infection factor of C. albicans is caused by hydrolytic enzymes: phospholipase and proteinase. Of these two factors, there are other enzymes: extracellular hydrolytic enzymes SAPs (Secreted aspartyl proteinases) are one of the leading infectious factors that contribute to the proliferation of C. albicans because these enzymes provide an entry point for adhesion, penetration, and invasion of tissues. Yeast cells, hyphae, and pseudohyphae can be involved in the formation of biofilms that are usually found in the oral cavity, skin, and vagina. Candida albicans consumes glucose as a carbon source and amino acids as a nitrogen source. Biofilm is a complex network of several types of microorganisms surrounded by an extracellular polymeric matrix consisting of nucleic acids, proteins, and carbohydrates. This structured arrangement of cells provides various advantages for C. albicans, such as protection against environmental stressors, manipulation of the immune system stem cells, and the central part can increase resistance to antimicrobial drugs. Protection of C. albicans can be through the formation of biofilms. The ability of C. albicans to form biofilms can be formed through three steps: adhesion and colonization of cells on the host surface; cell growth and proliferation; and formation of a basal layer, hyphae, and pseudohyphae with the secretion of extracellular matrix.

Ordinary emulsion preparations have a shape that is less pleasing to the eye because they have a larger particle size. Meanwhile, nanoemulsion preparations have low turbidity, so they are excellent for mouthwash because they look like water. Nanoemulsion is also a solution for making clear, stable mouthwash and making it easier for substances to be absorbed into the mouth because of their small particles. Several studies have also shown that nanoemulsions enhance antimicrobial activity. Currently, the use of mouthwash as a form of nanoemulsion still uses a lot of chemical-based ingredients. Seeing from this, the risk of drug side effects is quite considerable. Some mouthwashes have an alcohol percentage of 25% or more; this can cause the risk of mouth, throat, and pharyngeal cancer with a percentage of 50%. This can be prevented with natural ingredients. Therefore, research related to herbal medicines must continue to be carried out to obtain safe treatment and minimal side effects.

The use of mouthwash against fungi in the oral cavity still needs to be improved. Therefore, researchers are interested in using the bajakah tampala (Spatholobus littoralis) as an alternative to mouthwash made from natural ingredients. Spatholobus littoralis is a plant that grows in the Kalimantan region. Spatholobus littoralis is used by the local community by drinking boiled water from the trunk of S. littoralis. Several species of the genus Spatholobus are found in the interior of the tropical forests of Indonesia. Spatholobus littoralis is often found in the interior forests of Kalimantan and usually propagates on tall and large wooden trees. Phytochemical screening results showed that the ethanol extract of S. littoralis contained saponins, tannins, and flavonoids. Research by Kumar et al. reported that the active substance above can be an antifungal against C. albicans. The compounds above have the potential as antifungals, especially alkaloids, saponins, and flavonoids, which are also helpful as antioxidants, antiinflammations, and antibacterials. Researchers are actively investigating the formulation of a nanoemulsion mouthwash utilizing S. littoralis skin extract as a potent antifungal and antibiofilm agent against C. albicans. This pioneering research aims to develop a natural and effective nanoemulsion mouthwash capable of inhibiting the growth of C. albicans in the oral cavity. The study is breaking new ground as, until now, research has yet to be conducted on this specific formulation.

MATERIALS AND METHODS

Materials

The tools used include a pycnometer (Iwaki, Japan), Erlenmeyer (Iwaki, Japan), Laminar airflow (LAF), water bath (Faithful, Australia), rotary vacuum evaporator (Buchi, Switzerland), digital scale (Ohaus, USA), Ostwald viscometer (Iwaki, Japan), vortex mixer (DLab, China), magnetic stirrer (DLab, China), hot plate (Maspion), micropipette (DLab, China), Particle size
Hamzah H, Gunawan DR, Pratiwi SUT, Bakhtiar MI, Pratama VY, Subhan M, et al. 2024. Nanoemulsion Mouthwash Formulation of Bajakah. ...

Figure 1. Spatholobus littoralis skin.

Methods

Nanoemulsion mouthwash formulation

The procedure of mouthwash nanoemulsion preparation resulted from a combination of previous research with modifications. The oil phase was made with VCO mixed with tween 80, put into a 250 mL beaker glass, and stirred with a magnetic stirrer for 2 minutes at 800 rpm. The oil phase mixed with surfactant was then added with PEG 400 as a co-surfactant and stirred for 10 minutes at 1000 rpm. Both S. littoralis skin extract and sodium benzoate were dissolved in 10 mL of distilled water. The aqueous phase was made with S. littoralis skin extract into another 250 mL beaker glass, mixed with the remaining distilled water, and stirred for 2 minutes at 1000 rpm. The aqueous phase formed was mixed with sorbitol and sodium benzoate, then five drops of peppermint oil were added and stirred for 10 minutes at 1000 rpm. The aqueous phase was added to the oil phase drop by drop using a dropper until it ran out, then stirred for 10 minutes at 1000 rpm so that a mouthwash nanoemulsion of S. littoralis skin extract would be formed.

pH test

The test was carried out with a pH meter. The pH meter was calibrated using pH 4 and 7 buffer liquid. Then, the lower end of the pH meter was immersed in the test sample, remained until the pH on the instrument was constant, and recorded the results obtained. The pH test is part of the mouthwash preparation's physical and chemical examination criteria and must be based on the quality requirements of the herbal mouthwash, which are 5 to 7.

Particle size distribution, polydispersity index, and zeta potential

Particle size distribution, polydispersity index, and zeta potential were measured using the particle size analyzer. The nanoemulsion formula should have an average droplet size of <100 nm. The polydispersity index indicates uniform particle size in the mouthwash nanoemulsion formulation. The lower the polydispersity index, the more homogeneous the resulting nanoemulsion.
**Candida albicans antifungal test**

The antifungal test was carried out by the microdilution method. The test was carried out on microplate 96 well flat bottom with 0%, 0.5%, 1%, and 2% concentrations. Positive control was Listerine® Mouthwash, and fungal suspension was used for negative control. Subsequently, incubation was carried out at 37°C for 72 hours. Then, the absorbance value test was carried out with a microplate reader at a wavelength of 595 nm. The data obtained was in optical density (OD) values; the data was then calculated as % inhibition in the Equation 1. The sample concentration inhibiting at least 50% biofilm formation is expressed as MIC<sub>50</sub> (minimal inhibition concentration)<sup>29</sup>.

\[
\% \text{ inhibition} = \left( \frac{\text{Average OD of negative control} - \text{Average OD of test samples}}{\text{Average OD of negative control}} \right) \times 100 \%
\]  

**Candida albicans antibiofilm test**

In the antibiofilm test, 100 L of fungal suspension was added to each hole of the required 96 wells plate. For the attachment phase, the suspension was incubated at 36-37°C for 90 minutes. Then, the plate was washed thrice with 150 L of sterile distilled water to remove nonadherent cells. A total of 100 L samples with concentrations of 0%, 0.5%, 1%, and 2% were added to the required wells and washed. Fungal suspension was used as a negative control. The drug was given a microbial suspension added with Listerine® Mouthwash as a positive control. Furthermore, incubation is carried out at 36-37°C for 24 hours for the middle phase and 48 hours for the ripening phase. Then, the plate was washed with distilled water three times, rinsed, then dried at room temperature for 5 minutes. A total of 125 L of 1% crystal violet was inserted into each well, which was used to color the biofilm formed. Then, the plate was incubated at room temperature for 15 minutes. After that, it was washed with running water three times to remove crystal violet on the plate and then added with 200 L of 96% ethanol to each well used to dissolve the biofilm. The absorbance value was read using a microplate reader with a wavelength of 595 nm. The test was carried out with three duplications. The data obtained was in the form of OD values; the data was then calculated as % inhibition in the Equation 2. The sample concentration inhibiting at least 50% biofilm formation is expressed as MBIC<sub>50</sub> (minimal biofilm inhibition concentration)<sup>29</sup>.

\[
\% \text{ inhibition} = \left( \frac{\text{Average OD of negative control} - \text{Average OD of test samples}}{\text{Average OD of negative control}} \right) \times 100 \%
\]  

**Data analysis**

Data analysis in this study used Microsoft Excel software and the Statistical Package for the Social Sciences (SPSS). The OD value was obtained in the microplate reader antifungal and antibiofilm test results, which were later calculated in Excel and SPSS. The absorbance value results were analyzed using the Shapiro-Wilk method and the Levene test on SPSS to determine the distribution of normal and homogeneous data. The results were normal and homogeneous if the significance value was >0.05. Furthermore, the one-way ANOVA test was carried out to test for differences in fungal growth after being given the test solution, indicated by a p-value of <0.05. Then, a follow-up test was carried out using post-hoc Tukey to determine the significant difference in the resistance of each test solution. The percentage of inhibition in the antifungal and antibiofilm tests was calculated using Excel. Both MIC<sub>50</sub> and MBIC<sub>50</sub> values are determined by looking at % inhibition of >50% with the smallest concentration; the MIC<sub>50</sub> and MBIC<sub>50</sub> values.

**RESULTS AND DISCUSSION**

**Nanoemulsion mouthwash formulation**

The formulation used in manufacturing mouthwash nanoemulsion of *S. littoralis* skin extract is a modification of the existing formulation. The formulation of the ethanol extract of *S. littoralis* skin can be seen in Table I. Mixing with a magnetic stirrer is a spontaneous nanoemulsion (spontaneous emulsification) method in which the energy used is low so that the particle size is less uniform. After being magnetic, it should be continued with ultrasound using a sonicator to reduce the particle size of the nanoemulsion further and make it more stable, but due to limited tools, only use a magnetic stirrer to make nanoemulsions. The mixing method using magnetic alone is not optimal in making nanoemulsions because the droplet size...
and polydispersity index during storage have increased\textsuperscript{39}. The ultrasonication method has the advantage of a simple, fast, and more efficient process in producing nanoparticles than using conventional methods\textsuperscript{31}.

### Table I. Nanoemulsion mouthwash formulation of S. littoralis skin extract.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1 (in %)</th>
<th>F2 (in %)</th>
<th>F3 (in %)</th>
<th>F4 (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spatholobus littoralis skin extract</td>
<td>0</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>VCO</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Tween 80</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>PEG 400</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Natrium benzoat</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Peppermint oil (drops)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Distilled water</td>
<td>ad 100</td>
<td>ad 100</td>
<td>ad 100</td>
<td>ad 100</td>
</tr>
</tbody>
</table>

### pH test

The pH testing has been performed using a pH meter. The results of the pH test of the mouthwash nanoemulsion can be seen in Table II, which was observed at weeks 0, 1, 2, 3, and 4. We are examining the pH of the mouthwash nanoemulsion preparation and obtained data on F1 to F4 in 4 weeks. From weeks 1 to 4, the pH range in F1 is 6.3 – 5.9; F2 is 5.9 – 5.7; F3 is 6.0 – 5.5; F3; and F4 is 5.9 – 5.7. Changes in the pH value that occur during storage indicate a reaction in the components that make up the preparation so that it can increase or decrease pH\textsuperscript{38}. According to Numberi et al\textsuperscript{35}, changes in pH during storage indicate a less stable preparation. Changes in pH value are influenced by sorbitol, with an acidity level of 4.5\textsuperscript{34}. Then changes in pH can also be caused by temperature, poor storage, and it could also be due to the preparation process\textsuperscript{35}.

The formulations given the extract with a predetermined concentration experienced a decrease in pH at the beginning of the test, and after being tested for four weeks, the four formulas experienced changes in pH, but the pH obtained was still within the standard quality of herbal mouthwash. The quality requirement for herbal mouthwash is pH 5 to 7\textsuperscript{26}. Based on the results in Table II, the mouthwash nanoemulsion formulation has met the standard requirements for herbal mouthwash.

### Table II. pH of nanoemulsion of S. littoralis skin extract.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>6.3</td>
<td>6.2</td>
<td>6.1</td>
<td>6.0</td>
<td>5.8</td>
</tr>
<tr>
<td>F2</td>
<td>5.9</td>
<td>5.9</td>
<td>5.8</td>
<td>5.8</td>
<td>5.7</td>
</tr>
<tr>
<td>F3</td>
<td>6.0</td>
<td>6.0</td>
<td>5.9</td>
<td>5.9</td>
<td>5.5</td>
</tr>
<tr>
<td>F4</td>
<td>5.9</td>
<td>5.8</td>
<td>5.8</td>
<td>5.7</td>
<td>5.7</td>
</tr>
</tbody>
</table>

### Particle size distribution, polydispersity index, and zeta potential

The results of the measurement of the particle size distribution and the polydispersity index of the mouthwash nanoemulsion can be seen in Tables III and IV. The particle size distribution (droplet) test was carried out to determine whether the nanoemulsion preparation of S. littoralis skin mouthwash had a particle size that was by the ideal standard of nanoemulsion particle size, which was <100 nm\textsuperscript{37}. It is necessary to know the particle size of the nanoemulsion tested to determine drug absorption and release rate—the smaller the particle size, the faster the absorption process and the resulting pharmacological effects\textsuperscript{38}. Tests were only carried out on F2 and F4 because F1 and F3 used the same ingredients, only differing in the concentration of the extract used and expected to have particle sizes that are not much different from F2 and F4 because of the use of the same ingredients. Based on the particle size distribution carried out in three repetitions, the results from the first to the third test in a row were 26.7, 27.41, and 27.54 nm for F2 and 26.2, 25.22, and 25.67 nm for F4. The data obtained are by the theory of Kumar et al\textsuperscript{39} that the droplet size in the nanoemulsion has a size of <200 nm and is by the statement of Sonneville-Aubrun et al\textsuperscript{24} that the ideal standard of nanoemulsion particle size is <100 nm. To get the results above, a reasonably high focus is needed in the manufacture because, at the time of mixing, it should not be too slow; this will affect the nanoemulsion’s homogeneity level. This is to the statement of Nirmalayanti\textsuperscript{40} that mixing using a magnetic stirrer should not be too slow or too fast because if it is too slow, it will not form a nanoemulsion preparation, and if it is too fast, it can cause turbulence so that the particle size is not evenly dispersed and this results in the formation of a larger particle.
Listerine® Mouthwash showed inhibition of C. albicans antifungal test results of the mouthwash nanoemulsion found that all the formulations have a positive charge, so there is a repulsive force between particles, indicated by a zeta potential value. The zeta potential data repeated three times showed that the zeta potential value in F2 is 38.2, 28.6, and 30.7, while F4 is 16.2, 11.1, and 11.6. These results indicate that the nanoemulsion of S. littoralis mouthwash has good stability at F2 because it has a value of +30 mV, while at F4, it has poor stability because the potential zeta value falls into the range of -30 to -30 mV. This is by the statement of Nugroho et al. that the nanoemulsion preparation is declared to have a higher degree of stability if the charge is more than +30 mV or <30 mV has a higher degree of stability. The positive-negative sign indicates that the particles in the nanoemulsion formulation have a charge. From the results of the zeta potential, which got a positive value, it means that most of the nanoemulsion formulations have a positive charge, so there is a repulsive force between the particles. The repulsion that occurs makes the nanoemulsion formulation not settle quickly. Several factors, such as the type of surfactant, medium concentration, particle size, and pH, can influence zeta potential value. This is to the research of Huda and Wahyuningsih that the difference in the type of surfactant and the volume of the surfactant is one of the factors causing the difference in the zeta potential value. Tween 80 was used as a surfactant in the formulation. Tween 80 is a non-ionic surfactant, so it has no charge on the hydrophobic groups.

The measurement of the zeta potential value of the mouthwash nanoemulsion can be seen in Table V. Zeta potential is a repulsive force between particles, indicated by a zeta potential value. The zeta potential value is used to determine the charge and stability of nanoparticles. The polydispersity index has a value range from 0 to 1; the lower the polydispersity index value, the higher the uniform particle size. This is by the statement of Prihantini et al. that the polydispersity index has a value range from 0 to 1; the droplet size is declared uniform if the polydispersity index value obtained is close to a value of 0, which indicates a homogeneous dispersion. The polydispersity index can affect drug delivery and release and the stability of nanoparticles. The polydispersity index provides information about the physical stability of the dispersion system, which is more stable in the long term.

### Table III. Particle size distribution of nanoemulsion of S. littoralis skin extract.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Nanoparticle size duplication</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>F2</td>
<td>26.97</td>
<td>27.41</td>
</tr>
<tr>
<td>F4</td>
<td>26.20</td>
<td>25.22</td>
</tr>
</tbody>
</table>

### Table IV. Polydispersity index measurement of nanoemulsion of S. littoralis skin extract.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Polydispersity index duplication</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>F2</td>
<td>0.088</td>
<td>0.0561</td>
</tr>
<tr>
<td>F4</td>
<td>0.1477</td>
<td>0.1127</td>
</tr>
</tbody>
</table>

### Table V. Zeta Potential value of nanoemulsion of S. littoralis skin extract.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Zeta potential value (mV)</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>F2</td>
<td>38.2</td>
<td>28.6</td>
</tr>
<tr>
<td>F4</td>
<td>16.2</td>
<td>11.1</td>
</tr>
</tbody>
</table>

**Candida albicans antifungal test**

The antifungal test results of the mouthwash nanoemulsion found that all the concentrations tested and the positive control Listerine® Mouthwash showed inhibition of C. albicans, as shown in Figure 2. The wavelength used is 620 nm. This is to the
research of Maghfirah et al.⁹, in which absorbance was measured using a microplate reader with a wavelength of 620 nm. They also used the same wavelength for antifungal and biofilm assays⁹.

The value of OD indicates the high and low growth of C. albicans in the media. The value seen is the OD value of 620 nm; the smaller the OD value of 620 nm, the better the sample inhibits the growth of C. albicans. From the three replications, the average OD value of 620 nm at a concentration of 0% was 0.90156, 0.5% of 0.692767, 1% of 0.4573, 2% of 0.3785, the negative control was 1.2976, while Listerine® Mouthwash was 0.094667. Calculations using Equation 1 obtained that the % inhibition of C. albicans with control at a concentration of 0% was 31.52%, 0.5% was 46.61%, 1% was 64.75%, 2% was 70.83%, while Listerine® Mouthwash by 92.70%. At a concentration of 0%, there was an inhibition of C. albicans because the VCO and sodium benzoate have antifungal activity. Based on research conducted by Burhanuddin et al.⁵¹, VCO has the potential to be used as an alternative treatment for C. albicans infection. VCO can be used orally or applied directly to the infected skin with fungi. According to Mroz et al.⁵², benzoate is a natural element found in some plants and is used as an antibacterial and antifungal to preserve food.

The results obtained are by the statement of Hamzah et al.⁵⁰ that the higher the concentration the more remarkable the inhibition given. The percentages obtained at the given concentration variations were still under positive control, but at concentrations of 1% and 2%, the percentages were above 50%, indicating a relatively significant inhibition of C. albicans. Then, MIC₅₀ of the nanoemulsion formulation of S. littoralis skin extract was a concentration of 1% with % inhibition of 64.75%. Determination of the MIC₅₀ was carried out to determine the minimum concentration of S. littoralis skin extract that was able to inhibit the growth of C. albicans. This is to Hamzah et al.⁵⁰ that the sample content that can inhibit at least 50% of fungal growth is considered a MIC₅₀. If the antifungal test is known as MIC₅₀, it differs from the antibiofilm test known as MBIC₅₀.

Several compounds that inhibit C. albicans are flavonoids, saponins, tannins, terpenoids, and alkaloids. Flavonoids increase protein denaturation, disrupting the fat layer and causing damage to cell walls⁵³. Saponins work by lowering the surface tension of the sterol membrane of the cell wall of C. albicans so that its permeability increases, which can cause intracellular fluid to come out of the cell so that later enzymes, proteins, nutrients, and metabolic substances come out of C. albicans, causing death in C. albicans⁵⁴. Tannins work by inhibiting the synthesis of chitin, which is used in forming cell walls in C. albicans, and damaging cell membranes to disrupt fungal growth⁵⁵. Terpenoids work by inhibiting the growth of fungi through the cytoplasmic membrane or by interfering with the growth and development of C. albicans spores⁵⁶. And lastly, alkaloids work by damaging the cell walls of microbes⁵⁷.

**Figure 2.** % inhibition of C. albicans from nanoemulsion of S. littoralis skin extract.
Candida albicans antibiofilm test

The mouthwash nanoemulsion antibiofilm test found that all the concentrations tested and Listerine® Mouthwash inhibited C. albicans biofilm growth, as shown in Figure 3. From the three replications, the average OD value of 620 nm at 0% concentration was 0.0908, 0.5% of 0.083367, 1% of 0.0605, 2% of 0.0487, the negative control was 0.1469, and Listerine® Mouthwash was 0.0322. After getting the average of OD 620 nm, the percentage of inhibition of the biofilm produced is calculated using the formula for the % inhibition (Equation 2). From the calculation, the % inhibition of C. albicans biofilm at concentrations of 0% of 38.18%, 0.5% of 43.24%, 1% of 58.81%, 2% of 66.84%, and Listerine® Mouthwash of 78.08%.

The % inhibition obtained at the given concentration variations was still under positive control, but at concentrations of 1% and 2%, the percentages were above 50%, indicating a relatively significant inhibition of the C. albicans biofilm. Then, MBIC₅₀ from the nanoemulsion formulation of S. littoralis skin extract was a concentration of 1% with a percentage of inhibition of 58.81%. MBIC₅₀ is the lowest concentration of the test sample that can inhibit biofilm growth with a percentage of >50%. Hamzah et al.⁹ state that the test sample concentration that can inhibit at least 50% of biofilm formation is MBIC₅₀. Several compounds that act as antibiofilms are flavonoids, phenols, and tannins. Flavonoids, phenols and tannins have a biofilm inhibition mechanism by inhibiting intercellular adhesion (icaA and icaD). Both icas mediate the formation of polysaccharide intercellular adhesin (PIA), an essential component in forming biofilms. Intercellular adhesion, after being inhibited, will also have an inhibitory effect on the formation of PIA, and this causes the formation of biofilms to be disrupted or damaged⁶⁸⁶⁹.

CONCLUSION

In summary, the nanoemulsion mouthwash of S. littoralis skin extract demonstrates excellent pH stability, small nanoparticle size, and homogeneity. Both formulations show antifungal and antibiofilm efficacy against C. albicans, with the 2% concentration inhibiting C. albicans by 70.83% and biofilm formation by 66.48%. The 1% concentration also inhibits fungal growth and biofilm formation (>50% inhibition). This underscores the promising antimicrobial potential of S. littoralis skin extract nanoemulsion mouthwash.
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Methodology: Hasyrul Hamzah, Sylvia Utami Tunjung Pratiwi
Project administration: Dede Reza Gunawan, Hasyrul Hamzah
Resources: -
Software: -
Supervision: Hasyrul Hamzah, Sylvia Utami Tunjung Pratiwi
Validation: Hasyrul Hamzah, Dede Reza Gunawan
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DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES


