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Research Article

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_{50} and $MBIC_{50}$. 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_{50} and $MBIC_{50}$. Therefore, the nanoemulsion formulation of *S. littoralis* extract could inhibit the growth of *C. albicans* in the oral cavity.

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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[1]. *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

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conditions[2]. 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[3]. 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[4]. 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[3]. The infection factor of *C. albicans* is caused by hydrolytic enzymes: phospholipase and proteinase[5]. 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[3]. Yeast cells, hyphae, and pseudohyphae can be involved in the formation of biofilms that are usually found in the oral cavity, skin, and vagina[2]. *Candida albicans* consumes glucose as a carbon source and amino acids as a nitrogen source[6].

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[7]. 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[8].

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[9]. 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[10,11]. 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%[12]. 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[12-14].

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[12-14]. *Spatholobus littoralis* is used by the local community by drinking boiled water from the trunk of *S. littoralis*[15]. 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[16]. Phytochemical screening results showed that the ethanol extract of *S. littoralis* contained saponins, tannins, and flavonoids[17]. Research by Kumar *et al.*[18] 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[19-21].

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

analyzer (Microtac, Germany), pH meter (Ionix, Thailand), autoclave (All American, USA), incubator, microplate 96 well flat bottom (Iwaki, Japan), microplate reader (HiPo, Germany) and oven (LabTech, Hungary). The ingredients include *S. littoralis* skin (**Figure 1**) collected from Loa Kulu Forest, Samarinda, East Borneo, Indonesia. Determination was carried out at the Faculty of Forestry, Universitas Mulawarman, with the identification number 04/UN17.4.08/LL/2022, identified as *Spatholobus littoralis* Hassk. Other materials include Listerine® Mouthwash, tween 80, PEG 400, virgin coconut oil (VCO; Al Afiat), peppermint oil, sorbitol, sodium benzoate, 96% ethanol, potato dextrose agar (Oxoid), potato dextrose broth (HiMedia), pure culture of *C. albicans* ATCC 10231 from the Microbiology Laboratory of the Medical Education Study Program, Universitas Islam Negeri Maulana Malik Ibrahim, sterile distilled water, crystal violet, Mayer's, Wagner's, and Dragendorff's reagents, NaOH, concentrated H_2SO_4, concentrated Mg-HCl, as well as 1% and 5% $FeCl_3$.

Figure 1. *Spatholobus littoralis* skin.

Methods

Nanoemulsion mouthwash formulation

The procedure of mouthwash nanoemulsion preparation resulted from a combination of previous research[22,23] 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[24]. 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[25,26].

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[27]. The polydispersity index indicates uniform particle size in the mouthwash nanoemulsion formulation. The lower the polydispersity index, the more homogeneous the resulting nanoemulsion[28].

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_{50} (minimal inhibition concentration)[29].

$$\%\ inhibition = \frac{\text{Average OD of negative control} - \text{Average OD of test samples}}{\text{Average OD of negative control}} \text{x} 100\% \quad [1]$$

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 $MBIC_{50}$ (minimal biofilm inhibition concentration)[29].

$$\%\ inhibition = \frac{\text{Average OD of negative control} - \text{Average OD of test samples}}{\text{Average OD of negative control}} \text{x} 100\% \quad [2]$$

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_{50} and $MBIC_{50}$ values are determined by looking at % inhibition of >50% with the smallest concentration; the MIC_{50} and $MBIC_{50}$ 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[30]. The ultrasonication method has the advantage of a simple, fast, and more efficient process in producing nanoparticles than using conventional methods[31].

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

Ingredients	Formulations (in %)			
	F1	F2	F3	F4
Spatholobus littoralis skin extract	0	0.5	1	2
VCO	2	2	2	2
Tween 80	20	20	20	20
PEG 400	10	10	10	10
Sorbitol	10	10	10	10
Natrium benzoat	0.02	0.02	0.02	0.02
Peppermint oil (drops)	5	5	5	5
Distlled water	ad 100	ad 100	ad 100	ad 100

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[32]. According to Numberi *et al*[33], 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[34]. Then changes in pH can also be caused by temperature, poor storage, and it could also be due to the preparation process[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[36]. 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.

Formulation	Testing time (week)				
	0	1	2	3	4
F1	6.3	6.2	6.1	6.0	5.8
F2	5.9	5.9	5.8	5.8	5.7
F3	6.0	6.0	5.9	5.9	5.5
F4	5.9	5.8	5.8	5.7	5.7

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[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[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*.[39] that the droplet size in the nanoemulsion has a size of <200 nm and is by the statement of Sonneville-Aubrun *et al*.[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[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

size. Then, the ultrasonication method required fast and relatively long stirring and treatment to get the maximum nanoparticle size. This is to the research of Delmifiana and Astuti[41] that the longer the stirring, the smaller the size of the nanoparticles because the more particles that break into nano. The sonification time in the optimal range will provide a more homogeneous and stable droplet size[42].

The polydispersity index indicates uniform particle size in the mouthwash nanoemulsion formulation. The lower the polydispersity index value, the higher the uniform particle size in the mouthwash nanoemulsion formulation[28]. The polydispersity index obtained from three repetitions in a row on F2 has a polydispersity index of 0.088, 0.0561, and 0.0789, while F4 has a polydispersity index of 0.1477, 0.1127, and 0.364. This shows that the two formulas tested, F2 and F4, produced a more uniform particle size. This is by the statement of Prihantini *et al.*[43] 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[44].

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

Formulation	Nanoparticle size duplication			Average
	1	2	3	
F2	26.97	27.41	27.54	27.30
F4	26.20	25.22	25.67	25.69

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

Formulation	Polydispersity index duplication			Average
	1	2	3	
F2	0.088	0.0561	0.0789	0.0743
F4	0.1477	0.1127	0.364	0.2081

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[45]. 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.*[46] that the nanoemulsion preparation is declared to have a higher degree of stability if the charge is more than +/- 30 mV. that the zeta potential value >+30 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[47].

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[48] 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[49].

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

Formulation	Zeta potential value (mV)			Average
	1	2	3	
F2	38.2	28.6	30.7	32.5
F4	16.2	11.1	11.6	12.96

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*.[50], 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 assays[50].

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*.[29] 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, MIC_{50} of the nanoemulsion formulation of *S. littoralis* skin extract was a concentration of 1% with % inhibition of 64.75%. Determination of the MIC_{50} 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*.[29] that the sample content that can inhibit at least 50% of fungal growth is considered a MIC_{50}. If the antifungal test is known as MIC_{50}, it differs from the antibiofilm test known as $MBIC_{50}$.

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 walls[53]. 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. albicans*[54]. 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 growth[55]. Terpenoids work by inhibiting the growth of fungi through the cytoplasmic membrane or by interfering with the growth and development of *C. albicans* spores[56]. And lastly, alkaloids work by damaging the cell walls of microbes[57].

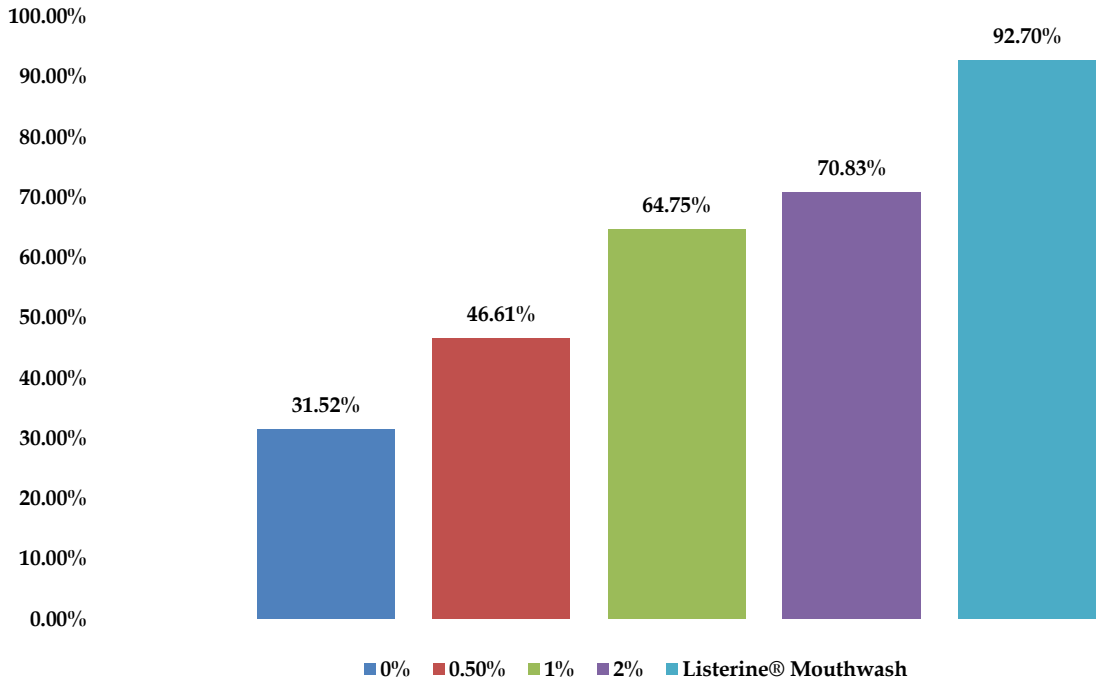

Figure 2. % inhibition of *C. albicans* from nanoemulsion of *S. littoralis* skin extract.

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, $MBIC_{50}$ from the nanoemulsion formulation of *S. littoralis* skin extract was a concentration of 1% with a percentage of inhibition of 58.81%. $MBIC_{50}$ is the lowest concentration of the test sample that can inhibit biofilm growth with a percentage of >50%[58]. Hamzah *et al.*[29] state that the test sample concentration that can inhibit at least 50% of biofilm formation is $MBIC_{50}$.

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 damaged[59,60].

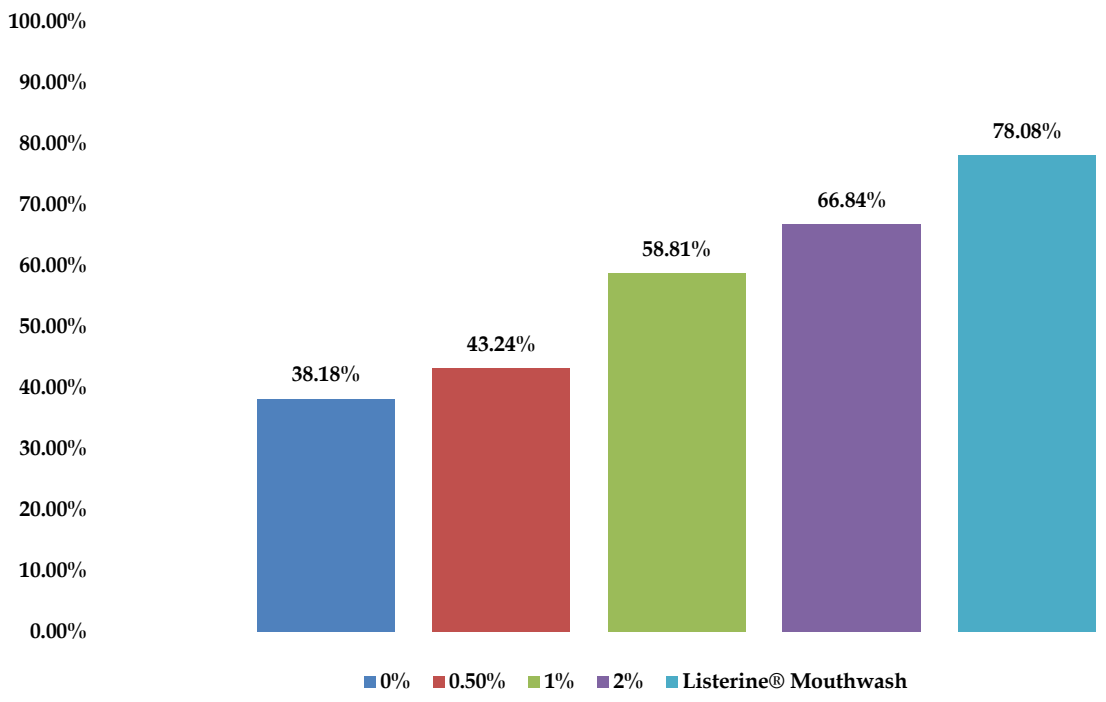

Figure 3. % inhibition of *C. albicans* biofilm from nanoemulsion of *S. littoralis* skin extract.

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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DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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