

Antimicrobial Activity of Temu Blenyeh (*Curcuma purpurascens* Blume) Ethanol Extract Against on *Streptococcus mutans* and *Candida albicans*

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ABSTRACT

Introduction: Temu blenyeh (*Curcuma purpurascens* Blume) is a species of *Curcuma* belonging to the Zingiberaceae family. This plant contains secondary metabolites such as flavonoids, alkaloids, terpenoids, saponins, tannins, and polyphenols, which function as antimicrobials. **Aims:** The aims of this study was to determine the antimicrobial activity of the ethanol extract of intersection blenyeh against *Streptococcus mutans* and *Candida albicans*. **Methods:** The extraction process was carried out by maceration, and the antimicrobial activity test was carried out using paper disc diffusion. The negative control used in this study was 5% DMSO, while the positive control was 0.2% Chlorhexidine. **Result:** The results of phytochemical screening using the TLC method showed that the ethanol extract of intersection blenyeh contained flavonoids, alkaloids, terpenoids, saponins, tannins, and polyphenols. The results of the antimicrobial activity test of the ethanol extract of the temu blenyeh at concentrations of 30; 40; 50; 60; and 70% against *Streptococcus mutans* produced an inhibition zone belonging to the medium category with an average inhibition zone value of 6.75; 7.00; 7.08; 7.16; and 8.26 mm, while *Candida albicans* produced an inhibition zone that belonged to the very weak category with an average inhibition zone value of 2.75; 3.16; 3.33; 3.60; and 3.67 mm. **Conclusion:** The conclusion of this research is that the ethanol extract of intersection blenyeh has antimicrobial activity against *Streptococcus mutans* and *Candida albicans*.

KEYWORDS: Antimicrobial, *Candida albicans*, *Streptococcus mutans*, temu blenyeh, TLC.

INTRODUCTION

Based on the 2016 global burden of disease study, oral and dental health problems affect almost 3.58 billion people worldwide (Marthinu & Mustapa, 2020). The results of basic health research data (Riskesdas) in 2013 amounted to 25.9% of Indonesia's population experiencing oral and dental health problems. Traditional medicine derived from medicinal plants native to Indonesia is still often used by Indonesians. A traditional plant that has medicinal properties and can be used as

medicine is Intersection blenyeh. Temu blenyeh contains compounds such as flavonoids, alkaloids, triterpenoids or steroids, saponins, and tannins (Pramiastuti & Murti, 2022), according to Rasyid (2012), steroid compounds and saponins are secondary metabolites that have antibacterial activity, then research conducted by Sukmawati (2013) found that flavonoid and alkaloid compounds also have antibacterial activity (Rasyid, 2012; Sukmawati, 2013). Secondary metabolites such as tumeron, gemcron, bisabalone, curlone

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curcumin, arturmeron, flavonoids, quinones, tannins, terpenoids, sesquiterpenes, alkaloids, and essential oils have pharmacological, anticancer, antioxidant, gastroprotective, apoptogenic, hepatoprotective, antimicrobial, antifungal, antiproliferative, and cytotoxic effects (Pramiastuti, Wahyuono, Fakhrudin, & Astuti, 2023). In a study conducted by Astuti (2022), the terponoid compounds contained in the rhizome of the temu benyeh rhizome have antibacterial activity against *Staphylococcus aureus* bacteria (gram-positive bacteria) and *Escherichia coli* (gram-negative bacteria) (Astuti, 2022). Then, in a study conducted by Anggraini *et al.*, (Anggraini, Salim, & Elinda, 2013), the results showed that Intersection blenyeh has antibacterial activity against the bacteria *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* (gram-positive bacteria), and *Escherichia coli* (gram-negative bacteria).

Streptococcus mutans is a normal flora that exists in the human oral cavity, but if the population increases, this bacterium can turn into a pathogen. This bacterium has the ability to form plaque on teeth, which can cause dental caries, bad breath, and other oral and dental health problems (Susanti, Isbiyantoro, & Simanjuntak, 2019). Apart from *Streptococcus mutans*, there are other microorganisms that grow in the oral cavity, namely *Candida albicans*. *Candida albicans* is a fungus that causes canker sores in the oral cavity. This is due to the excessive growth of the *Candida albicans* fungus (Qhorina,

Prasetya, & Ardana, 2021). However, research on the comparison of antimicrobial activity against *Streptococcus mutans* and *Candida albicans* as causes of dental caries, bad breath, and mouth ulcers has never been carried out; therefore, it is necessary to conduct research on testing antimicrobial activity against *Streptococcus mutans* bacteria and *Candida albicans* fungi.

MATERIAL AND METHODS

Materials

The tools used in this study were measuring cups (pyrex), Erlemmeyer (pyrex), test tubes (pyrex), analytical balances (aeADAM), stirring rods, dropping pipettes, funnels, ovens (Gentra), mixers (Mitochiba), beaker glass (pyrex), petri dish (pyrex), petri dish (Normax), autoclave (Alamerican), incubator (Mammert IN 55), loop needle, micro pipette (ecopipette), mortar and steamer, L rod, hot plate (nesco lab), water bath (dss), tweezers, cotton, chamber, homogienizer, porcelain krush, centrifugation, spirit, flannel, plastic wrap, disc paper, label paper, filter paper, pH meter, temu blenyeh, ethanol 96%, H₂SO₄, BaCl₂H₂O, glacial acetic acid, water, butanol, methanol, distilled water, degrendrof reagent, chloroform, ethyl acetate, butanol, sulfuric acid, N-Hexane, 5% DMSO, 0.2% *Chlorhexidine*, sodium agar (NA), potato dextrose agar (PDA), MC solution Farland, *Streptococcus mutans*, and *Candida albicans*, ATC 0231.

Extraction

500 grams of Intersection blenyeh powder were extracted by the maceration method; the powder was put into the maserator, then 1.5 L of 96% ethanol was added, and the mixture was let stand for 3 x 24 hours in a closed vessel, protected from direct sunlight, and stirred periodically. After 3 x 24 hours filtered with Whattman No. 1 filter paper, the residue was remacerated five times with the same solvent, namely 96% ethanol, and the result was evaporated over a water bath to obtain a thick extract. After that, the yield obtained was calculated and ready to be used for extract standardization tests, phytochemical screening tests, and antimicrobial activity tests.

Standardization Extract

Intersection blenyeh extract was standardized by testing specific parameters and non-specific parameters. Tests for specific parameters were carried out organoleptically by observing the color, shape, smell, and taste of the ethanol extract of intersection blenyeh (RI, 2000). Furthermore, non-specific parameter tests were carried out, which included water content tests, total ash content tests, dry shrinkage tests, and ethanol extract-free tests (RI, 2000). According to the Farmakope Herbal Indonesia (2017), the extract is said to be ethanol-free if it has no ester odor (RI D. , 2017). In research conducted by Ballo *et al.* (2021), it was also

Antimicrobial activity of temu blenyeh stated that the extract can be said to be ethanol-free if it does not smell of esters (Ballo, Indriarini, & Shinta, 2021).

Phytochemical Screening

Phytochemical screening includes the flavonoid, alkaloid, terpenoid/steroid, polyphenol, tannin, and saponin (Yuda, Cahyaningsih, & Winarianthi, 2017; Sawiji, La, & Yuliawati, 2020; Maulana, Triatmoko, & Nugraha, 2020; Sopianti & Sary, 2018).

Antimicrobial activity test

Sodium agar (NA) media (*Streptococcus mutans*) and potato dextrose (PDA) agar media (*Candida albicans*), which had been cooled in a petri dish, were then added to the bacterial suspension by flattening it with a sterile gauze swab. Furthermore, the discs that had been soaked with various extract concentration solutions of 30; 40; 50; 60; and 70%, a negative control (5% DMSO), and a positive control (0.2% *Chlorhexidine*) were placed on a petri dish and incubated for 24 hours at 37°C. The presence of antimicrobial activity can be seen if a clear area is seen on the edge of the disc after incubation for 24 hours. This is because the growth of microbes is inhibited, so the inhibition is measured in mm. Antimicrobial activity testing was carried out three times (triplo) (Nurbianti, Alhawaris, & Yani, 2021; Gurning, Dicky, Okpri, & Zuraida, 2018).

Table 1. test results for non-specific parameters of temu blenyeh extract

Non-specific extract parameter test	Literature requirements	Results	Description	Literature
Test the moisture content	<10%	5,54%	Qualify	(RI, 2000)
Test total ash content	<10%	3%	Qualify	(RI, 2000)
Drying shrinkage test	<10%	5,36%	Qualify	(RI, 2000)
Ethanol free test	No ester smell	No ester smell	Qualify	(RI, 2017)

RESULTS AND DISCUSSION

The intersection blenyeh extract obtained was 166.87 g with a yield percentage of 33.37%. The results of the specific extract parameter test are in accordance with previous research conducted the organoleptic results of the ethanol extract test of Intersection blenyeh have a thick shape that smells of turmeric and temulawak, has a slightly spicy, bitter taste, and is blackish brown in color (Pramiastuti & Murti, 2022). The results of the non-specific parameter test can be seen in Table 1.

From Table 1, it can be concluded that the temu blenyeh extract meets the specific parameter test requirements where the resulting extract is ethanol-free and has a water content, shrinkage, and total ash content of less than 10%. A non-specific parameter test was carried out using the TLC method.

Furthermore, in Figure 1 and Tables 2, it can be seen that the ethanol extract of Intersection blenyeh contains flavonoids, alkaloids, terpenoids, saponins, tannins, and polyphenols based on the color of the stains produced in each test. The results of research conducted by Pramiastuti & Murti (2022), the ethanol extract of Intersection blenyeh also contains flavonoids, alkaloids, tannins, steroids, saponins, and triterpenoids

(Pramiastuti & Murti, 2022). However, there are differences in the R_f values between this study and previous studies. These differences are usually influenced by several factors, including the compatibility of the solvent with the compound to be searched for in the extract, the amount of extract weighed, the selection of an inaccurate visualization method, and the chromatography system in the stationary phase and mobile phase (Saifudin, Rahayu, & Teruna, 2011).

Testing the antimicrobial activity of the ethanol extract of Intersection blenyeh was carried out by the diffusion method. The advantages of this method are that it is easy to do, does not require special equipment, and is relatively inexpensive, while the weaknesses are the size of the clear zone formed depending on the inoculum, preincubation, prediffusion, medium thickness, and incubation conditions. If these four factors do not match, then the results will be difficult to interpret. In addition, this method cannot be used on types of bacteria that grow slowly and have obligate anaerobic properties (Prayoga, 2013). 24 hours later, the disc paper that has been soaked with the sample material is the ethanol extract of intersection blenyeh, with a concentration of 30; 40; 50; 60%; and 70% for 15 minutes.

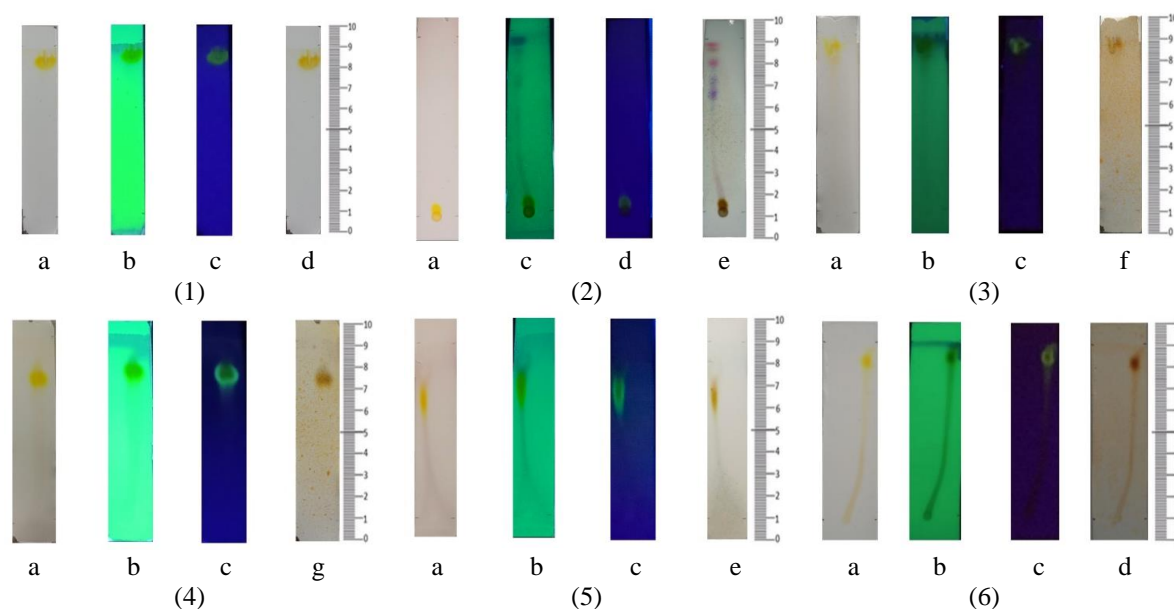


Figure 1. Phytochemical screening of temu blenyeh extract. (1) flavonoid; (2) terpenoid; (3) alkaloid; (4) tannin; (5) saponin; (6) polifenol. (a) visible; (b) 254 nm; (c) 366 nm; (d) ammonia; (e) vanilin + sulfuric acid; (f) dragendorff; (g) FeCl_3 5%. Mobile phase: (1) glacial acetic acid- water-butanol (1:5:4); (2) n-hexane-ethyl acetate (4:1); (3) Ethyl acetate-methanol-water (200:23.5:10) (4) ethyl acetate:methanol (1:4); (5) butanol:water (1:1); (6) chloroform:ethyl acetate (1:9).

Table 2. The results of the phytochemical screening of temu blenyeh extract using the KLT method

Phytochemical	Visible results	UV 254 mm	UV 366 mm	Stain Viewer	RF	References	Desc.
Flavonoid	Yellow	Yellow	Yellow fluorescence	Yellow	0,90	Yellow, blue or green (Yuda <i>et al.</i> , 2017)	(+)
Terpenoid	Colorless	Dark purple	Does not fluoresce yellow	Red	0,98	Blue, green, yellow-brown & red-violet (Wardhani & Sulistyani, 2012)	(+)
Alkaloid	Yellow	Green	Yellow fluorescence	Brown, there are black spots	0,92	Black spots (Sawiji <i>et al.</i> , 2020)	(+)
Tanin Test	Yellow	Greenish yellow	Yellow fluorescence	Dark brown	0,77	Black spots (Sopianti <i>et al.</i> , 2018)	(+)
Saponin	Yellow	Yellow	Does not fluoresce yellow	Red	0,73	Red, Yellow, Dark blue, purple, dark green, or brown yellow (Khasanah & Nastiti, 2021)	(+)
Polifenol	Yellow	Greenish orange	Yellow fluorescence	Brown, there are black spots	0,97	Black spots (Maulana <i>et al.</i> , 2020)	(+)

The extract solution is prepared by dissolving the ethanol extract of intersection blenyeh using Dimethylsulfoxide (DMSO) 5 solvent%, vortexed for 10 minutes, and sonicated at 40°C for 30 minutes with a frequency of 40 kHz (Susanti, Isbiyantoro, &

Simanjuntak, 2019). Then the disc paper was placed on the media and incubated for 24 hours at 37°C. The inhibition zone can be seen after 18–24 hours of incubation (Prayoga, 2013).

DMSO used as a negative control, is a compound that is able to dissolve polar and

Table 3. Antimicrobial activity of the ethanol extract of intersection blenyeh *Streptococcus mutans* and *Candida albicans*

Concentration of extract (%)	Inhibition zone	
	<i>Streptococcus mutans</i>	<i>Candida albicans</i>
30	6,75	2,75
40	7,00	3,16
50	7,08	3,33
60	7,16	3,60
70	8,06	3,67
<i>Chlorhexidine</i> 0,2%	14,73	8,92
DMSO 5%	-	-

non-polar compounds in various organic solvents and water (Fajrina, Bakhtra, Eriadi, Putri, & Wahyuni, 2021). In addition, according to DMSO, it is not bactericidal, so it will not affect the results of antimicrobial activity testing (Huda, Putri, & Sari, 2019). While the positive control used was 0.2% *Chlorhexidine*. The use of 0.2% *Chlorhexidine* as a positive control is recommended because it is known to have bactericidal and bacteriostatic activity against oral bacteria (Kurniawati, 2018). Another alternative positive control that can be used is amoxicillin for *Streptococcus mutans* (Khairani, Busman, & Edrizal, 2017) and nystatin for *Candida albicans* (Yanti, Samingan, & Mudatsir, 2016). it can be seen in Table 3.

The inhibition zone category to be very weak if an inhibition zone of 2–5 mm, moderate at 5–10 mm, strong at 10–20 mm, and very strong at >20 mm (Davis & Stout, 1971). From Table 3, it can be seen that the antimicrobial activity of the ethanol extract of Intersection blenyeh at concentrations of 30; 40; 50; 60%; and 70% has an average diameter of the medium category of inhibition zone

against *Streptococcus mutans* with an average inhibition zone value of 6.75; 7.00; 7.08; 7.16; and 8.06 mm, and *Candida albicans* is classified as very weak with an average inhibition zone value of 2.75; 3.16; 3.33; 3.60; 3.67; and 8.92 mm. From these results, it can be concluded that the ethanol extract of Intersection Blenyeh has greater antimicrobial activity against *Streptococcus mutans* compared to *Candida albicans*.

The results of the treatment on the negative control used, namely 5% DMSO, had no inhibitory power. This is evidenced by the absence of a clear zone around the disc paper. According to Fajrina (2021), While the test results on the positive control of *Chlorhexidine* 0.2% showed relatively strong inhibition with an average inhibition zone of 14.73 mm on *Streptococcus mutans* and moderate inhibition with an average inhibition zone of 8.92 nm on *Candida albicans*, Research conducted by Andriyani & Udin, (2015) found that the n-hexane extract of Intersection blenyeh produced a minimum inhibitory concentration (MIC) for *Bacillus subtilis* of 1549.59 ppm and 0.00119%, *Eschericia coli* of 14.65 ppm and

0.000192%, and the bacterium *Pseudomonas aeruginosa* of 2508.15 ppm and 0.000998%. whereas in Astuti (2022) study, the inhibition zone produced by the n-hexane fraction isolate temu blenyeh for *Staphylococcus aureus* bacteria was 1.5 mm and for *Eschericia coli* bacteria it was 2.2 mm, which was classified as very weak. Based on these results, the ethanol extract of intersection blenyeh has a larger inhibition zone compared to the n-hexane extract and the n-hexane fraction isolate.

Concentration variations were carried out with the aim of seeing the differences in the inhibition zones produced at each concentration (Wahyuni & Karim, 2020). *Streptococcus mutans* is a gram-positive bacterium that has a thick peptidoglycan layer ranging from 20 to 80 nm, making it difficult to be damaged by secondary metabolites such as flavonoids, alkaloids, tannins, glycosides, terpenoids, saponins, and polyphenols. *Candida albicans* also has a stronger and more complex structure with a thickness of 100–400 nm, making it difficult to decompose. *Candida albicans* has six layers (from the inside out), namely the plasma membrane, mannoprotein, B-glucan-chitin, B-glucan, mannoprotein, and fibrillar layers, so that the compounds contained in the ethanol extract of Intersection Blenyeh are not effective in destroying the cell wall of the *Candida albicans* fungus (Putri, 2013). Then the saponin compounds work by lowering the surface tension of the cell wall, thereby disrupting the permeability of the cell

Antimicrobial activity of temu blenyeh membrane, which results in damage to the cell membrane so that the bacteria experience leakage and cell death (Syahrani, 2021). According to Barile (2007), saponins are detergents that work to form complexes with sterols in the membrane, causing membrane damage (Barile, et al., 2007). Damage to the bacterial cell membrane causes the cell to lose cytoplasm, leakage occurs, the transport of substances is disrupted, and metabolism is inhibited, causing the bacterial cell to lyse (damage). Furthermore, terpenoid compounds have antibacterial activity, which works by reacting with porins (transmembrane proteins) that form strong polymer bonds on the outer membrane of the bacterial cell wall, resulting in damage to the porin, which can cause bacterial cells to lack nutrition so that bacterial growth is inhibited or dies (Rachmawati, Nuria, & Sumantri, 2011).

CONCLUSION

The results showed that the ethanol extract of intersection blenyeh (*Curcuma purpurascens* Blume) with various concentrations of 30; 40; 50; 60; and 70% had medium-category *Streptococcus mutans* antimicrobial activity with an inhibition zone of 6.75; 7.00; 7.08; 7.16; and 8.26 mm and a very weak category on *Candida albicans* with inhibition of 2.75; 3.16; 3.33; 3.60; and 3.67mm. The results showed that the ethanol extract of Intersection blenyeh (*Curcuma purpurascens* Blume) had greater antimicrobial activity against *Streptococcus*

mutans than *Candida albicans*. Further research is needed to determine the content of active compounds in the rhizome of Intersection blenyeh that act as antibacterial and antifungal agents, along with their inhibition mechanisms, and to test them against other bacteria.

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