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ANTIBACTERIAL ACTIVITY OF THE COMBINATION OF ETHANOL EXTRACTS OF KEPOK BANANA PEEL (*Musa paradisiaca* L.) AND BETEL LEAVES (*Piper betle* L.) AGAINST *Streptococcus mutans* CAUSING DENTAL CARIES

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ABSTRAK

Karies gigi adalah masalah kesehatan mulut yang umum disebabkan oleh *Streptococcus mutans*. Agen antibakteri alami semakin dieksplorasi sebagai alternatif bahan kimia sintetis. Salah satu kombinasi bahan herbal yang diyakini memiliki potensi untuk terapi karies gigi adalah kombinasi antara kulit pisang kepok (*Musa paradisiaca* L.) dan daun sirih (*Piper betle* L.). Penelitian ini bertujuan untuk mengetahui aktivitas antibakteri dari ekstrak etanol kulit pisang kepok dan daun sirih, baik secara tunggal maupun kombinasi terhadap *S. mutans* (ATCC 35668) menggunakan metode mikrodilusi. Metode mikrodilusi digunakan untuk menentukan KHM dan KBM dari ekstrak dan metode mikrodilusi *checkerboard* digunakan untuk menentukan Indeks FKI dari kombinasi kedua ekstrak tersebut. Skrining fitokimia menunjukkan adanya flavonoid, alkaloid, triterpenoid, steroid, tanin, dan polifenol pada kedua ekstrak. Hanya ekstrak daun sirih yang menunjukan adanya senyawa saponin. Nilai KHM untuk ekstrak kulit pisang kepok dan daun sirih adalah 16 dan 4 mg/mL. Nilai KBM yang diperoleh untuk ekstrak kulit pisang kepok sebesar >32 mg/mL dan untuk ekstrak daun sirih sebesar 4 mg/mL. Kombinasi ekstrak menunjukkan efek aditif (Indeks FKI = 0,515). Hal ini menunjukan bahwa aktivitas antibakteri kombinasi kedua ekstrak terhadap *S. mutans* meningkat dibandingkan saat pengujian ekstrak secara tunggal. Kombinasi ekstrak etanol kulit pisang kepok dan daun sirih memiliki potensi untuk dikembangkan sebagai bahan baku produk perawatan gigi untuk mencegah karies gigi berdasarkan efeknya terhadap *S. mutans*.

Kata kunci: Antibakteri, Mikrodilusi, Kulit pisang kepok, Daun sirih, *Streptococcus mutans*

ABSTRACT

Dental caries is a prevalent oral health issue caused by *Streptococcus mutans*. Natural antibacterial agents are increasingly explored as alternatives to synthetic chemicals. One of the combinations of herbal ingredients believed to have the potential for dental caries therapy is the combination of kepok banana peel (*Musa paradisiaca* L.) and betel leaves (*Piper betle* L.). This study aimed to determine the antibacterial activity of ethanol extracts of kepok banana peel and betel leaves, individually and in combination, against *S. mutans* (ATCC 35668) using the microdilution method. The microdilution method was used to determine the MIC and MBC of the extracts, and a microdilution checkerboard was applied to determine the FICI of the combination of the two extracts. Based on the results of phytochemical screening, flavonoids, alkaloids, triterpenoids, steroids, tannins, and polyphenols were detected in both extracts, but only the betel leaves extract showed the presence of saponin compounds. Banana peel extract exhibits antibacterial activity against *S. mutans* with MIC of 16 mg/mL and an MBC > 32 mg/mL. Meanwhile, betel leaf extract had higher antibacterial activity than kepok banana peel against *S. mutans*, with MIC and MBC values of 4 mg/mL. The combined effect of the two extracts increased the antibacterial activity, resulting in an additive effect (FICI = 0.515) compared to the antibacterial activity individually. The combination of ethanol extracts from kepok banana peel and betel leaves has the potential to be developed as a natural-based material for dental care products to prevent dental caries, based on its effects on *S. mutans*.

Keywords: Antibacterial, Microdilution, Kepok banana peel, Betel leaves, Streptococcus mutans

INTRODUCTION

Dental caries is a widespread dental issue globally impacting individuals of all ages (Wongsariya *et al*., 2024). This condition arises when dental tissues become damaged, starting from tooth surfaces such as pits, fissures, and interproximal areas, eventually reaching the pulp. One of the primary culprits behind dental caries is the bacterium *Streptococcus mutans*, which produces large amounts of glucan and acid, surpassing saliva's buffering capacity. This enables the bacteria to adhere firmly to teeth and thrive in acidic environments (Almoudi *et al*., 2018).

Antimicrobial agents are commonly used in oral care product to prevent dental caries by maintaining oral health and reducing disease recurrence. However, the growing issue of antimicrobial resistance has prompted interest in alternative treatments, including herbal extracts and natural products. These alternatives are also supported by antimicrobial stewardship efforts to address resistance challenges (Wongsariya *et al*., 2024). One such natural source is the kepok banana peel (*Musa paradisiaca* L.), which contains active antibacterial compounds. Research by Sari *et al*. (2023) demonstrated that a 50% concentration of kepok banana peel extract was particularly effective against *S. mutans* (Sari *et al*., 2023). Furthermore, Wahyuni *et al*. (2019) reported that yellow kepok banana peel could inhibit *S. aureus* and *E. coli*, achieving inhibition diameters of 14.75 mm and 14 mm, respectively. Despite these findings, limited studies focus on the peel's efficacy against *S. mutans* (Wahyuni *et al*., 2019).

Combining kepok banana peel with betel leaves (*Piper betle* L.) is proposed to enhance the antibacterial properties. Betel leaves, known for their antibacterial properties, can be used in their raw extract form, which offers higher antibacterial activity than essential oil alternatives (Wahyuni *et al*., 2019). Nayaka *et al*. (2021) highlighted the efficacy of betel leaves against Gram-positive and Gram-negative bacteria (Nayaka *et al*., 2021). Betel leaves contain numerous active compounds, including alkaloids, terpenes, steroids, and propenylphenols such as hydroxychavicol and eugenol, which are believed to contribute to their antibacterial effects (Singtongratana *et al*., 2013). Owu *et al*. (2020) also found that ethanol extracts of betel leaves, which contain tannins, effectively inhibited *S. mutans* with a minimum inhibitory concentration (MIC) of 15% (Owu *et al*., 2020).

Several tests have been used to evaluate the inhibitory effects on bacterial growth, such as the dilution method and the diffusion test. Most published research uses the diffusion method to focus on antibacterial effects against bacterial strains. There is no research yet on the antibacterial effects of the combination of kepok banana peel and betel leaves extracts using the microdilution checkerboard method. The present study aimed to determine the antibacterial effects of the combination of kepok banana peel extract and betel leaves using a microdilution checkerboard method. This study will focus on determining the Fractional Inhibitory Concentration Index (FICI) of the combination of kepok banana peel extract and betel leaves extract. Therefore, single Minimum Inhibitory Concentration (MIC) data for each extract is needed to perform this combination test. Hopefully, this combination will provide an optimal antibacterial effect against dental caries caused by *S. mutans*. These materials can potentially be combined into a pharmaceutical preparation for treating dental caries.

MATERIALS AND METHODS

Plant Material

Kepok banana peel (*Musa paradisiaca* L.) was purchased from Anyar Sari Market, Denpasar, Bali, in April 2024. The plant material for betel leaves (*Piper betle* L.) was obtained from the Traditional Health Service Unit of Tawangmangu, Dr. Sardjito General Hospital, in May 2024. Authentication of plant materials was done in Materia Medica, East Java.

Chemicals

A technical grade of 96% ethanol was used for extraction. The solvent was distilled prior to use. A HCl (Merck®, Germany), boric acid, acetone, oxalic acid, ether, $FeCl_3$, Dragendorff's reagent (consisting of bismuth (III) nitrate, HNO_3, potassium iodide, and distilled water), Mayer's reagent (containing potassium iodide, $HgCl_2$, and distilled water), Bouchardat's

reagent (containing potassium iodide, iodine, and distilled water), and Liebermann-Burchard reagent (containing anhydrous acetic acid, sulfuric acid, ethanol, and distilled water) were used for phytochemical screening. All reagents were prepared according to previously published procedures by Jones and Kinghorn (2012). A Mueller Hinton Agar (Himedia®, India), Mueller Hinton Broth (Himedia®, India), DMSO (Merck®, Germany), and Chloramphenicol (Nalgane®, USA) were used for the antibacterial test.

Equipment

This study used the following equipment including a rotary evaporator (Heidolph®, Germany), incubator (Binder®, Germany), TLC-Scanner 4 (CAMAG®, Switzerland), autoclave (Biobase®, China), UV-Vis spectrometer (Thermo Scientific®, USA), *microplate-96* (Iwaki®, Japan), oven (Binder®, Germany), micropipette (JoanLab®, USA), and laminar air flow (JLabTech®, South Korea) were used in this study.

Methods

Preparation of Plant Material and Extraction

The plant materials were air dried at room temperature. The dried plant materials were powdered and sieved through a 60-mesh sieve. Powdered herbal material (1 kg) was extracted with 96% ethanol (5 L) and allowed at room temperature for 24 h. After filtration, the residue was extracted with 96% ethanol (3 L). This process was repeated twice, followed by solvent removal from the resulting filtrate to obtain the crude ethanol extracts of kepok banana peel and betel leaves, respectively (Kemenkes, 2017).

Loss on Drying

Each dried plant material or simplicia (1 g) was placed into a weighing bottle, followed by drying using oven at 105°C for 30 min. Additional drying was done to reach the constant mass of each plant material. Constant mass is defined as a state where the difference of mass of plant material after 2 subsequent drying at a designated time interval is less than 0.25%. This experiment was done in triplicate and the percentage of loss on drying (%w/w) was calculated (Kemenkes, 2017).

Phytochemical Screening

Phytochemical screening was carried out to detect the presence of secondary metabolites such as: flavonoids, alkaloids, saponins, polyphenols, tannins, steroids, and triterpenoids in ethanol extract of kepok banana peel and betel leaves.

a. Flavonoids

The test solution (1 mL) was evaporated, the residue left behind was then added with acetone P (1 mL). Boric acid powder and oxalic acid were then added in small amounts, and the mixture was heated. Afterward, the residue was combined with ether (1 mL). The sample was examined under UV light at 366 nm, where a bright yellow fluorescence confirmed the presence of flavonoids (Ariantari *et al*., 2024).

b. Alkaloids

The test solution (4 mL) was mixed with 2N HCl (4 mL). The resulting solution was transferred into a tube. The solution was then divided into four reaction tubes: the first tube served as a blank, the second tube had three drops of Bouchardat's reagent added, the third tube had three drops of Dragendorff's reagent added, and the fourth tube had three drops of Mayer's reagent added. The presence of alkaloids was indicated by the formation of a brown to blackish precipitate in the second tube, an orange precipitate in the third tube, and a yellowish precipitate in the fourth tube (Ariantari *et al*., 2024).

c. Saponins

The test solution (1 mL) was added to hot water (4 mL) and shaken for 10 seconds until foam forms. Then, a drop of 2N HCl wass added to the solution. The formation of stable foam that lasts for more than 10 minutes with a height of more than 1 cm indicated the presence of saponin (Jones & Kinghorn, 2012).

d. Polyphenols

To the test solution (1 mL), 10% $FeCl_3$ (3-4 drops) was added. A color change of the test solution to dark blue, blackish blue, or greenish black indicates polyphenols (Ariantari *et al*., 2024).

e. Tannins

The test solution (1 mL) was added with 1% $FeCl_3$ (3 drops). A dark blue or greenish-black color of the test solution indicates the presence of tannins (Jones & Kinghorn, 2012).

f. Steroids and Triterpenoids

A test solution (2 mL) was evaporated using an evaporating dish over a water bath until a residue was obtained. After dissolving the residue in chloroform (0.5 mL), the solution was transferred to a reaction tube. To the reaction tube, anhydrous acetic acid (0.5 mL) and concentrated sulfuric acid (2 mL) were

added slowly through the tube wall. A brown or violet ring at the solution interface indicates the presence of triterpenoids. A bluish-green ring indicates the presence of steroids (Ariantari *et al*., 2024).

Antibacterial Assay

Antibacterial assay of each tested extract and its combination against *S. mutans* ATCC 35668 was conducted using the microdilution method in a 96-well microplate, according to the protocol from CLSI (2024). Mueller Hinton Broth (MHB) and Mueller Hinton Agar (MHA) media were prepared. Stock solutions of kepok banana peel concentrated extract at 64 mg/mL and betel leaves concentrated extract at 16 mg/mL were prepared. A stock solution of chloramphenicol at 0.512 mg/mL was also prepared. The tested bacterium *S. mutans* ATCC 35668 of 5 x 10^6 CFU/mL in MHB was prepared for the assay.

All microwells in the 96 microwells plate were filled with 100 µL of MHB. Then, 100 µL of the test sample was added to the wells in column 12^{th}. A twofold serial dilution was performed from column 12^{th} to column 3^{rd}. Afterward, 10 µL of the bacterial test suspension was added from column 2^{nd} to column 12^{th}. The wells in the first column served as the media control, and the wells in the second column served as the bacterial control. The microdilution plate was incubated for 18-24 h at 37°C. The concentration of extract that inhibits bacterial growth, as shown by the clear zone in the microwell, was determined as the Minimum inhibition concentration (MIC) (CLSI, 2015).

Minimum Bactericidal Concentration (MBC) was determined by inoculating treated *S. mutans* in each well of the microplate, which showed inhibition of bacterial growth in Mueller Hinton Agar (MHA) media. The inoculated MHA media was then incubated at 37°C for 18-24 h. The MBC value was determined from the concentration of tested extract where no bacterial colonies were observed (CLSI, 2015).

Determination of the Fractional Inhibitory Concentration Index (FICI) using the Checkerboard Microdilution Method

The tested solutions were prepared starting from the highest concentration (2× MIC), followed by two-fold serial dilutions. In the checkerboard microdilution setup, betel leaf extract was diluted across the columns 12th to 2nd (right to left) in a horizontal direction, while kepok banana peel extract was diluted in vertical direction from top to bottom of microwell plate, starting from rows A to H. This design enabling combinations between the two extracts at varying concentrations in each well. Bacterial suspension (10 µL) was then added to each well. The assay was carried out in triplicates. The microwell plate was then incubated at 37°C for 18-24 hours. The procedure for preparing the tested bacterial suspension was the same as that of the antibacterial assay described before. The assay was carried out in triplicates. The microplate was then incubated at 37°C for 18-24 hours (Leliqia *et al*., 2021). The FICI values were calculated for all wells showing a color change from turbid to clear using the formula:

$$FICI = \frac{MIC_{KBPE}\ combination}{MIC_{KBPE}\ single} + \frac{MIC_{BLE} combination}{MIC_{BLE}\ single}$$

FICI : Fractional Inhibitory Concentration Index
MIC_{KBPE} : Minimum Inhibitory Concentration of Kepok Banana Peel Extract
MIC_{BLE} : Minimum Inhibitory Concentration of Betel Leaf Extract

RESULTS AND DISCUSSION

Loss on Drying

The measurement of loss on drying of dried plant materials (simplicia) used in this study was done to determine the amount of compounds lost during the drying process. Loss on drying is a parameter synonymous with the moisture content in the simplicia. High moisture content can cause simplicia to be easily contaminated by microbes and fungi, thereby reducing the stability and pharmacological activity of the simplicial (Andini & Putri, 2021). According to the Kemenkes RI (2017), the requirement for loss on drying of kepok banana peel and betel leaves simplicia is below 10%. Our data showed the loss on drying of kepok banana peel and betel leaves simplicia was 2,84±0,04 % and 5,19±0,02 %, respectively, indicating that both simplicia have met the acceptable criteria for

loss on drying as suggested in The Herbal Pharmacopoeia of Indonesia (2017) (Kemenkes, 2017).

Extraction

Maceration was used to extract secondary metabolites of kaepok banana peel and betel leaves using 96% ethanol as the solvent. Maceration is an extraction method that is easy to apply and relatively low-cost. This method also prevents damage of heat-sensitive compounds during the extraction process. The process involves soaking the material in an appropriate solvent, allowing the solvent to extract the active compounds (Chairunnisa *et al*., 2019). According to Farabi *et al*. (2023), 96% ethanol was chosen as the solvent due to its universal nature, easy availability, selectivity, non-toxicity, good absorption, and high extraction capability. It can extract non-polar, semi-polar, and polar compounds. The 96% ethanol solvent penetrates the sample's cell walls more quickly than lower concentration ethanol solvents, thus enabling maximum maceration of the simplicial (Farabi *et al*., 2023). The maceration process is carried out for 3x24 hours with two rounds of maceration using a fresh solvent. According to Woran et al. (2021), maceration ensures that all remaining active compounds are optimally extracted, leaving no essential components wasted (Woran *et al*., 2021). The obtained filtrate is then evaporated using a rotary evaporator to ensure the solvent used during extraction is completely removed, leaving only the concentrated active compounds and yielding a thick extract. The evaporation resulted in ethanol extract yields 6.50%w/v for kepok banana peel and 14.05%w/v for betel leaves. The results met the requirements of the Kemenkes RI. (2017), which states that the yield should not be less than 5.0%w/v (Kemenkes, 2017).

Phytochemical Screening of Extracts

Phytochemical screening is the process of testing to identify secondary metabolites present in the ethanol extract of kepok banana peel and betel leaves. According to Ariantari *et al*. (2024), this method uses specific coloring reagents to detect particular chemical compounds. Changes observed in the extract solution after adding reagents indicate the particular class of compounds contained in the extract (Ariantari *et al*., 2024).

The data in Table 1 indicates that the ethanol extract of kepok banana peel and betel leaves contained flavonoids, alkaloids, triterpenoids, steroids, and polyphenols, while saponins were not detected in this extract. Flavonoids are a group of phenolic compounds widely distributed in the plant kingdom and are known for their diverse biological activities, including antibacterial, antioxidant, and anti-inflammatory effects (Górniak *et al*., 2019). Research by Cushnie and Lamb (2011), demonstrated that flavonoids can damage bacterial cell membranes and inhibit key bacterial enzymes (Cushnie & Lamb, 2011). Alkaloids are heterocyclic nitrogen compounds commonly found in plants and are known to have potent pharmacological effects (Latti *et al*., 2021). Another study showed alkaloids have significant potential as antimicrobial agents against oral pathogens, with mechanisms of action that include damaging bacterial cell walls (Yan *et al*., 2021).

Table 1. Phytochemical Screening of Ethanol Extracts from Kepok Banana Peel and Betel Leaves

Phytochemicals	Reagents	Results	
		Kepok Banana Peel	Betel Leaves
Flavonoids	Wilson Taubock	(+)	(+)
Alkaloids	Dragendorff	(+)	(+)
	Mayer	(+)	(+)
	Bouchardat	(+)	(+)
Triterpenoids	Lieberman-Burchard	(+)	(+)
Steroids	Lieberman-Burchard	(+)	(+)
Saponins	Forth	(-)	(+)
Tannins	1% $FeCl_3$	(+)	(+)
Polyphenols	10% $FeCl_3$	(+)	(+)

(+) indicates a positive result/the corresponding chemical content was detected; (-) indicates a negative result/the corresponding chemical content was not detected

Antibacterial Assay

The medium used to determine the MIC was MHB, while MHA was used to determine the MBC. These two media are recommended for MIC and MBC testing because they meet the performance standards set by CLSI and show good reproducibility in each repeat without affecting the results of antibacterial testing (CLSI, 2024). The results of the single antibacterial activity tests of the ethanol extracts of kepok banana peel, betel leaves, and chloramphenicol against *S. mutans* are shown in Table 2.

Chloramphenicol was used as a reference antibiotic in this study. Chloramphenicol is a broad-spectrum antibiotic active against aerobic and anaerobic Gram-positive and Gram-negative organisms (Alghifari *et al*., 2017). Chloramphenicol exhibits antibacterial activity by acting as a bacteriostatic agent but can be bactericidal at high concentrations or against some highly susceptible microorganisms (Eliakim-Raz *et al*., 2014). The range of chloramphenicol concentrations used in the in vitro antibacterial testing using the microdilution method was 0.0005-0.256 mg/mL, where bacteria are considered susceptible to chloramphenicol if they are inhibited at a concentration of ≤0.004 mg/mL, while they are considered resistant when the MIC produced is >0.016 mg/mL (CLSI, 2024). The test results showed that the MIC of chloramphenicol was 0.008 mg/mL, which falls into the intermediate category against *S. mutans*.

Based on the data in Table 2, the antibacterial activity of the ethanol extracts of kepok banana peel and betel leaves against *S. mutans* is considered as weak activity (MIC values >1.5 mg/mL). However, the ethanol extract of betel leaves had a smaller MIC value than that of ethanol extract of kepok banana peel, indicating stronger growth inhibition of the betel leaf extract against *S. mutans*. The difference in activity between the ethanol extracts of kepok banana peel and betel leaves against the bacteria is suspected to be influenced by the varying sensitivity of the bacteria to the active compounds in each extract, as well as the difference mechanisms of these active compounds in the ethanol extracts of kepok banana peel and betel leaves when reacting as antibacterial agents (Khameneh *et al*., 2019). The phytochemical compounds in the ethanol extracts of kepok banana peel and betel leaves that are suspected to influence their antibacterial activity are flavonoids, saponins, polyphenols, and steroids/triterpenoids. Each of these phytochemical groups has different mechanisms as antibacterial agents.

Flavonoid compounds have three mechanisms of their antibacterial activity, they can inhibit nucleic acid synthesis, energy metabolism, and the function of the cell membrane, leading to bacterial cell lysis (Prestianti *et al*., 2018). The phenol content in polyphenol compounds has an antibacterial mechanism similar to that of flavonoids, as it can damage the cell membrane by causing protein denaturation in bacterial cells. Hydrogen bonds form between phenol and bacterial cell proteins, leading to damage to the cytoplasmic membrane and the protein structure of the cell wall, causing disruption of the cytoplasmic membrane and cell wall permeability, and imbalance of macromolecules and ions within the cell, resulting in cell lysis (Pelezar & Chan, 2008). The saponin content also has an antibacterial mechanism that causes damage to the bacterial cell membrane. Saponins, with their surfactant properties (consisting of hydrophilic and lipophilic parts), reduce the surface tension of the bacterial cell wall and damage the cell membrane permeability, leading to leakage of the cell cytoplasm. Triterpenoid content has an antibacterial mechanism by damaging the cell membrane. The bacterial cell phospholipid membrane, which is permeable to lipophilic compounds, binds with triterpenoids, which also have lipophilic properties, causing changes in membrane morphology, liposome leakage, cell fragility, and eventually cell lysis (Cowan, 1999).

Table 2. Antibacterial Activity of Ethanol Extracts of Kepok Banana Peel and Betel Leaves against *S. mutans* ATCC 35668

Sample	Antibacterial Activity	
	MIC (mg/mL)	MBC (mg/mL)
Kepok Banana Peel	16	>32
Betel Leaves	4	4
Chloramphenicol	0.008	0.016

Experiments were done in triplicate; Chloramphenicol was used as a reference drug;
MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration

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Antibacterial Activity of Combination Ethanol Extracts of Kepok Banana Peel and Betel Leaves Using the Checkerboard Microdilution Method

The antibacterial activity of the combination of ethanol extracts of kepok banana peel and betel leaves was tested using the checkerboard microdilution method. The checkerboard microdilution method is a technique used to evaluate interactions between two or more antibacterial agents in inhibiting microbial growth. This method involves testing various concentrations of combined antimicrobial agents in microtiter wells arranged in a checkerboard pattern on a microplate (Choirunnisa & Sutjiatmo, 2017). It aims to determine whether the combination of antimicrobial agents exhibits synergistic, additive, indifferent, and antagonistic effects. The results of the combination antibacterial activity test of ethanol extracts of Kepok banana peel and betel leaves against *S. mutans* are shown in Table 3.

Based on the data in Table 3, the combination only requires 1/64 of the MIC of the kepok banana peel ethanol extract and 1/2 of the MIC of the ethanol extract of betel leaves to inhibit *S. mutans*. The effect is an additive with FIC Index of 0.515. This result indicates that the ability of the combination of the ethanol extracts of kepok banana peel and betel leaves to inhibit the bacterial growth that causes dental caries is better than that of each sample when applied individually. It also shows that betel leaf ethanol extract seems to enhance the antibacterial activity of kepok banana peel ethanol extract. This difference in ability is likely due to the different sensitivity of the bacteria to the combination of the two substances and the various mechanisms of the active ingredients when combined. The chemical compounds suspected to be responsible for the antibacterial activity when used individually also play a role in the combination effect. When combined, the active ingredients from each substance might work individually or interact with each other to enhance their antibacterial activity (Vaou *et al*., 2022).

Table 1. Antibacterial Activity of The Combination of Ethanol Extract of Kepok Banana Peel and Betel Leaves against *S. mutans* ATCC 35668

Sample	MIC Single (mg/mL)	MIC Combination (mg/mL)	FICI
Kepok Banana Peel	16	0.25	0.515 (additive effect)
Betel Leaves	4	2	

Experiments were done in duplo; MIC: Minimum Inhibitory Concentration; FICI: Fractional Inhibitory Concentration Index is calculated from the MICs of individual extract divided by the MICs of the corresponding extract in combination.

The antibacterial mechanism of the combination of ethanol extracts of kepok banana peel and betel leaves that produce an additive effect cannot yet be determined. Therefore, further research is needed on each sample's active ingredients or other factors that could influence the antibacterial activity of the combined extracts. Information about the additive effect of the combination of ethanol extracts of kepok banana peel and betel leaves can serve as a reference for other studies in the formulation of phytotherapeutic agents, such as the development of mouthwash or further studies related to proving the site of action through Scanning Electron Microscope (SEM) observation and the mechanism of its pharmacological activity.

CONCLUSION

The research highlights the antibacterial potential of combining ethanol extracts of kepok banana peel and betel leaves against *S. mutans* ATCC 35668, a primary bacterium causing dental caries. When subjected to antibacterial test separately, both ethanol extracts of kepok banana peel and betel leaves exhibited weak antibacterial activity against *S. mutans*, with MIC values of 16 and 4 mg/mL. However, combining these extract enhanced both extracts' antibacterial activity, resulting in additive antibacterial effect with the FIC index of 0.515. This study revealed the potential of the combination of ethanol extracts of kepok banana peel and betel leaves as alternatives to conventional antibiotics, addressing the rising concern of antibiotic resistance and suggesting further research for clinical application in dental care products.

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