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# THE EFFECTIVENESS OF CORN SILK EXTRACT AGAINST DENTAL CARIES-CAUSING BACTERIA AND ITS FORMULATION IN MOUTHWASH PREPARATION

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**ABSTRACT**
Indonesia is undergoing a dental caries emergency with a prevalence of 51.1% (Riskesdas, 2018). This may cause tooth decay due to dental plaque bacteria such as *Streptococcus mutans*. Mouthwash can be a solution because it has antibacterial properties and reaches interspaces on the teeth. Unfortunately, the active ingredient of mouthwash, chlorhexidine, can cause cancer-related mutations if used continuously. Therefore, it requires alternatives to natural ingredients, such as corn silk. The research aims to determine the corn silk phytochemical profile and minimum inhibitory concentration (MIC) against *Streptococcus mutans*. The research started by macerating corn silk simplicia using 70% ethanol. Afterwards, thin layer chromatography (TLC) was conducted to determine its phytochemical profile. Disk-diffusion and broth microdilution methods were conducted using various concentrations of corn silk extract to determine its antibacterial activity and minimum inhibitory concentration (MIC) against *Streptococcus mutans*. Then, the $MIC_{50}$ was used as the minimum dose of corn silk extract concentration in mouthwash formulation which qualities controlled by pH and organoleptic tests. The yield of corn silk extracted was 16.17%w/w. The phytochemical profile from TLC showed that flavonoids, tannins, and terpenoids were present. Corn silk extract has antibacterial activities against *Streptococcus mutans* with $MIC_{50}$ of 7.2%w/v. Corn silk extract, tween 80, sorbitol, sodium benzoate, sodium metabisulfite, oleum menthae piperitae, and distilled water were used in four mouthwash formulas (F1-F4). The pH of all formulas was 5 and the organoleptic test showed that from 30 panelists, the majority chose F2 as the best in terms of taste, color, and smell.

**Keywords:** Corn silk; Caries; *Streptococcus mutans*; Mouthwash

## 1. INTRODUCTION

Oral and dental diseases have become a serious issue in Indonesia. Data from the Basic Health Research (2018) indicates that the prevalence of oral and dental problems in the Indonesian population is 57.6%, with 88.8% of that being dental caries. This is primarily due to the lack of attention to oral health, leading to plaque formation on teeth. One of the primary bacteria responsible for plaque and tooth decay is *Streptococcus mutans*, which produces acid that demineralizes teeth, making them mineral-deficient and prone to damage (Ambarawati et al., 2020).

Mouthwash can offer a solution to the problem of dental plaque, as it can kill bacteria, eliminate bad breath, prevent plaque formation, and reach small interdental spaces (Yuniarsih, 2017). Unfortunately, many of the mouthwashes used today still contain synthetic ingredients such as chlorhexidine, which, if used continuously, can have mutagenic (cancer-causing) effects

in the oral cavity (Rahayu et al., 2022). Therefore, the need for safer alternatives, such as natural ingredients, is evident.

Indonesia is a significant corn producer, with a production of 29.02 million tons of corn in 2020 (Komalasari, 2021). Regrettably, a substantial amount of corn silk is often discarded (Rohmadianto et al., 2018). However, corn is known to contain compounds like flavonoids, tannins, saponins, and other phenolic compounds, which possess antibacterial and antioxidant properties (Nurani et al., 2022). This enhances the potential of corn silk as a candidate active ingredient for an antibacterial mouthwash.

Several methods are required to test the activity of corn silk extract as mouthwash. First, corn silk is extracted using a maceration method to obtain the active compounds present in corn silk. Subsequently, the chemical compound profile is analyzed using Thin-Layer Chromatography (TLC), and the antibacterial activity is confirmed using solid diffusion methods. Afterward, microdilution antibacterial tests are conducted to determine the Minimum Inhibitory Concentration (MIC) of corn silk extract in inhibiting *S. mutans*. This MIC value serves as a reference for determining the extract dosage in mouthwash formulation. No previous research has explored the potential of corn silk extract as a mouthwash formulation, which is why we are interested in further investigating this topic.

## 2. METHODS

### 2.1. The Location and Time of The Research

The research was conducted for 4 months (July-October 2023) at the Pharmacognosy and Phytochemistry Laboratory, the Cellular Biology and Microbiology Laboratory, the Formulation Technology Laboratory of Semi-Condensate and Liquid Preparation of Faculty of Pharmacy, Gadjah Mada University.

### 2.2. Materials and Tools

Erlenmeyer, gauze, filter paper, blender, water bath, shaker, Buchner funnel, UV lamp, Petri dish, measuring cylinder, tweezers, micropipette, yellow tip, blue tip, white tip, Vernier caliper, 96-well microplate, microplate reader, vortex, magnetic stirrer, stirrer bar, ultra turrax homogenizer, pH meter, centrifuge, analytical balance, viscometer, beaker glass, ethanol 96% (Brataco), citroborate reagent, silica gel 60 F254 (Merck), $FeCl_3$ reagent, Dragendorff reagent, anisaldehyde reagent, toluene, ethyl acetate, diethylamine acid, glacial acetic acid, formic acid, hexane, Luria Bertani (LB) media, agar, *Streptococcus mutans* ATCC 25175, tween 80, oleum menthae piperitae, sodium metabisulfite, sodium benzoate, sorbitol (Brataco), distilled water, and corn silk simplicia (age of 50 days) from Gemahan, Ringinharjo, Bantul, Special Region of Yogyakarta, Indonesia.

### 2.3. Data Collection Method

#### 2.3.1. Preparation and Maceration of Corn Silk Extract

A total of 301.55 grams of corn silk was dried in an oven at 50°C and ground into a powder. Then, the powder was macerated in 70% ethanol at room temperature for 3 days with a powder-to-solvent ratio of 1:5. It was then separated into supernatant and residue through filtration. The residue was macerated again with the same ratio for 2 days and separated from the supernatant through filtration. The filtrate was evaporated in a water bath to obtain a concentrated extract (Kurnia et al., 2021).

#### 2.3.2. Thin-Layer Chromatography (TLC) Test

The TLC plate, used as the stationary phase, was heated in an oven at 105°C for 5 minutes. Approximately 100 mg of corn silk extract was dissolved in 300 µL of ethanol and 200 µL of distilled water, and then spotted on the plate. Reference substances (rutin and quercetin for flavonoids, quinine for alkaloids, gallic acid for tannins, and thymol for terpenoids) were also spotted on the plate. The plate was then placed in the mobile phase. The mobile phase used for

testing the rutin group of flavonoids (Yadnya-Putra et al., 2019) was a mixture of ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:27), while testing quercetin flavonoids (Yanti et al., 2021) used a mixture of hexane: ethyl acetate: formic acid (6:4:0.2). The alkaloid test (Fahturroji and Riza, 2020) used a mixture of toluene: ethyl acetate: diethylamine (7:2:1). The tannin test (Rakasiwi and Soegiharjo, 2014) used a mixture of ethyl acetate: formic acid: toluene: water (6:1.5:2:0.5), and the terpenoid test (Fahturroji and Riza, 2020) used hexane: ethyl acetate (93:7). Subsequently, the plate was sprayed with various reagents to visualize its chemical content. Spray reagents used included sitroborate for flavonoids, $FeCl_3$ for tannins, Dragendorff for alkaloids, and anisaldehyde for terpenoids (Kurnia et al., 2021).

### 2.3.3. Solid Diffusion Antibacterial Test

Corn silk extract was dissolved in 1% DMSO. Positive control used 1% ampicillin, and the negative control was 1% DMSO. Next, 10 µL of the sample, positive control, and negative control were placed on paper disks. Each paper disk, with the liquid applied, was placed on LB (Luria-Bertani) media in a petri dish containing *S. mutans*. Incubation was carried out for 24 hours at 37°C, and then the inhibitory zones formed were observed (Fajrina et al., 2021).

### 2.3.4. Microdilution Antibacterial Test

*S. mutans* bacterial inoculum was prepared in liquid LB media with an OD600 value of 0.264-0.30. Then, in a 96-well microplate, 100 µL of sterile LB media, 50 µL of bacteria, and 50 µL of extract (2.5%, 5%, and 10% w/v) with 1% DMSO as a solvent were added. Each concentration was replicated three times. The negative control was filled with 200 µL of media, while the positive control contained 150 µL of media plus 50 µL of bacteria. The microplate containing negative control, positive control, and extracts was then incubated for 24 hours at 37°C. Subsequently, it was analyzed using a microplate reader at a wavelength of 600 nm to obtain the percentage of inhibition and determine the minimum inhibitory concentration (MIC) value (Septiani et al., 2017).

### 2.3.5. Mouthwash Formulation

The preparation of the mouthwash formulation was made in a quantity of 100 mL with various concentrations of corn silk extract in the formula. The formulation started by mixing tween 80, sorbitol, sodium metabisulfite, sodium benzoate, and peppermint oil in a glass beaker. Then, the corn silk extract obtained from the maceration step was added according to its minimum inhibitory concentration. All ingredients were mixed using an ultra turrax homogenizer until homogenous, and distilled water was added to the specified calibration limit. The formulation was then transferred to plastic bottles, and an evaluation of the mouthwash formulation was conducted, including pH testing and organoleptic evaluation (Thomas et al., 2022).

## 2.4. Analytical Methods

### 2.4.1. Maceration of Corn Hair Ethanol Extract

The percentage yield of corn hair extract obtained is calculated using the formula,

$$\%Extract\ Yield = \frac{The\ weight\ of\ the\ evaporated\ extract}{The\ weight\ of\ the\ dried\ corn\ silk\ powder}\ x\ 100\%$$

### 2.4.2. Thin-Layer Chromatography Test

The data analysis of the thin-layer chromatography test results in a chemical content profile in the extract, which is represented by the color changes (chromatogram) on the thin-layer chromatography plate. The color changes indicate the presence or absence of the analyzed compounds.

### 2.4.3. Solid Diffusion Antibacterial Test

The diameter of the inhibition zones, represented as clear areas around the paper discs, indicates whether the corn hair extract possesses antibacterial properties or not. The measurements are taken with a ruler, and the data is graphed in a bar chart using Excel 2019 for comparison.

### 2.4.4. Microdilution Antibacterial Test

The absorbance data obtained from the ELISA reader is used to calculate the percentage of inhibition. The formula for calculating the percentage of inhibition is as follows:

$$\%Bacterial\ inhibition = \frac{Negative\ control - (treatment\ extract - control\ extract)}{Negative\ control} x\ 100\%$$

The percentage of inhibition from three replicates is averaged, and a linear regression is performed using Microsoft Excel 2019 to obtain the equation y = bx + A, where "y" represents the percentage of inhibition (%) and "x" represents the concentration of corn silk extract (% v/v). The minimum inhibitory concentration (MIC) can be calculated by inputting 50 for the "y" variable and obtaining the value of "x" as the MIC, which represents the concentration at which 50% inhibition is achieved.

### 2.4.5. Quality Control Testing of Dosage form

The pH measurement of mouthwash is carried out using pH paper, which changes color upon contact. The resulting color can be matched with the pH paper container's instructions. The pH data from three replicates is averaged and a bar graph is created using Microsoft Excel 2019, allowing for a comparison of pH values against each other to determine the quality criteria for mouthwash pH.

For organoleptic evaluation, responses from the public are collected through a Google Form filled out by a panel of individuals after tasting, smelling, and visually examining the formulated mouthwash. Evaluation is based on a scale of 1-5, with each person allowed to provide a single rating. The total scores are then compared to determine which formulation receives the best response from the public.

## 3. RESULTS AND DISCUSSION

### 3.1. Sample Preparation

Samples were obtained with an age of 50 days from Gemahan, Ringinharjo, Bantul, Special Region of Yogyakarta, Indonesia. The Laboratory of Microbiology and Cell Biology of the Faculty of Pharmacy UGM determined that the sample is a species of Zea mays L. of the Poaceae family. Subsequently, the samples were dried at 50°C for 2 days to prevent fungal growth before the extraction process to longer storage time. The dried samples were powdered, and 285.49 grams powder was obtained.

### 3.2. Extraction

Simplicia powder was soaked with ethanol 70% for 72 hours with a powder-ethanol ratio of 1:5. Subsequently, the result was filtered using the Buchner funnel and re-macerated with the same ratio for 24 hours and then re-filtered. The resulting filter applied onto the water bath, then a condensed extract of 46.19 grams was obtained with 16.17%b/b yield.

Extraction was done by maceration method. Maceration was used because it is easy, simple, and does not damage the active compounds during extraction. The cell walls and cell membranes will break down due to the pressure difference between the outside and the inside of the cell so that the secondary metabolites present in the cytoplasm will break and dissolve in the organic solvent used (Novitasari & Putri, 2016). Therefore, a suitable solvent is required to dissolve the active compound of simplicia.

The solvent used is 70% ethanol. Ethanol has a low boiling point of 79°C, which requires less heat for evaporation. Ethanol is also one of the solvents that is safe or non-toxic when consumed due to its low level of toxicity. Another reason for choosing ethanol 70% solvent is because the flavonoid compound that is the majority of compounds in corn silk is generally in the form of a polar glycoside, so it has to be dissolved with a polar solvent.

### 3.3. Thin-Layer Chromatography (TLC)

Thin-layer chromatography (TLC) was observed through the color changes that occurred after the addition of visualization reagents. Flavonoids appeared yellow after the addition of sitroborate due to the reaction between sitroborate and the ortho-hydroxy groups in flavonoids. Tannins changed to a brown color when sprayed with $FeCl_3$, resulting from the formation of a colored complex compound, trisianoferitric potassium ferric (III). Terpenoids turned purplish Panelistse after the addition of anisaldehyde and sulfuric acid due to the ability of terpenoids to form color in the presence of $H_2SO_4$ in the solvent. Additionally, alkaloids could undergo a color change to red when Dragendorff reagent was added, leading to the formation of reddish-colored $[BiI_4]^-$ (Fahrurroji & Riza, 2020).

**Table 1.** Results of Thin-Layer Chromatography

| Chemical Compund | Reagent | Before | After | Result |
|---|---|---|---|---|
| Alkaloid | Dragendorff | Colorless | Colorless | negative |
| Flavonoid | Sitroborate | Colorless | Yellow | positive |
| Tannin | $FeCl_3$ | Colorles | Brown | positive |
| Terpenoid | Anisaldehyde-sulfuric acid | Colorless | purplish Panelistse | positive |

Based on Table 1, it is evident that the corn silk extract contains compounds from the flavonoid, tannin, and terpenoid groups, while alkaloids were not detected. This conclusion is drawn based on the color changes that occurred after applying the visualization reagents, as depicted in the supplementary figures. In the flavonoid test, both the sample and control (either rutin or quercetin) turned from colorless to bright yellow upon spraying with sitroborate, and this change was observable under 366 nm light. Similarly, the tannin test showed color changes in both the sample and control (gallic acid) after reacting with $FeCl_3$, transitioning from a vague or unclear color to a visible brown hue under visible light. Furthermore, the terpenoid test resulted in color changes in both the sample and control (thymol) after adding anisaldehyde, transforming from colorless to purplish Panelistse. However, in the alkaloid test, only the control (quinine) exhibited a color change from colorless to reddish Panelistse after the addition of Dragendorff reagent, while the sample showed no color change.

### 3.4. Agar Diffusion Antibacterial Test

The solid diffusion antibacterial test is based on the ability of extract compounds to diffuse, with the sample placed on paper discs that diffuse into a medium containing *Streptococcus mutans* at an OD600 (optical density or turbidity level indicating the bacterial population) of 0.25. This OD600 value is chosen because it corresponds to a phase of rapid bacterial growth, making it suitable for the test. Compounds that diffuse create an area, and if these compounds have antibacterial properties, bacteria cannot grow in that area, resulting in a visible difference in color or what is commonly referred to as an inhibition zone (Balouiri et al., 2015).

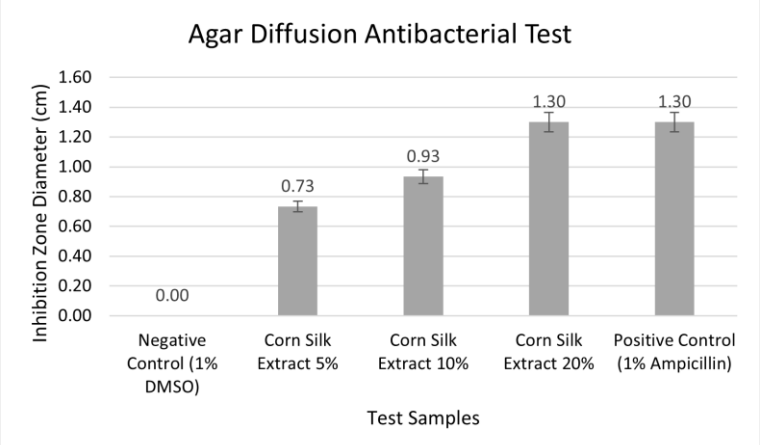


**Figure 1.** Graph of Solid Diffusion Antibacterial Inhibition Zones

As shown in Figure 1, it is evident that corn silk extract possesses antibacterial properties. The diameter of the inhibition zone increases with the rising concentration of the extract. The respective lengths of the inhibition zone diameters for the 5%, 10%, and 20% extracts are 0.73, 0.93, and 1.3 cm. Furthermore, in the positive control (1% ampicillin), the diameter of the inhibition zone is 1.3 cm, while there is no inhibition zone in the negative control. The positive control serves as an indicator of bacterial growth inhibition, which is visually observable through the formation of inhibition zones. Additionally, the negative control demonstrates that the solvent used does not possess antibacterial properties. Thus, it is highly likely that the inhibition zones observed in both the extract and the positive control are indeed due to the compounds themselves, rather than the solvent.

The antibacterial properties of the corn silk extract are likely attributed to the presence of flavonoids, tannins, and terpenoids. Flavonoids can inhibit nucleic acid synthesis, disrupt cell membrane function, and interfere with energy metabolism. Tannins can form hydrophobic complexes with bacterial proteins, inactivate enzymes and transport proteins in the cell wall, and cause the cell wall to contract, disrupting its permeability. Additionally, terpenoids can damage porins (transmembrane proteins in bacteria), disrupting their permeability and causing bacterial nutrient deprivation (Xie et al., 2015).

### 3.5. Microdilution Antibacterial Test

The microdilution antibacterial test relies on the turbidity level of bacteria. The more turbid, the more bacteria have grown. In a 96-well microplate, compounds are mixed with bacteria, and their turbidity is assessed. If a compound exhibits antibacterial properties, its turbidity will not differ significantly from the control without bacteria. Concentration series of the test compound are used for the microdilution test, allowing the creation of a linear regression equation to calculate the minimum inhibitory concentration at 50% ($MIC_{50}$), indicating the concentration of the test compound required to inhibit 50% of the bacteria (Balouiri et al., 2015).

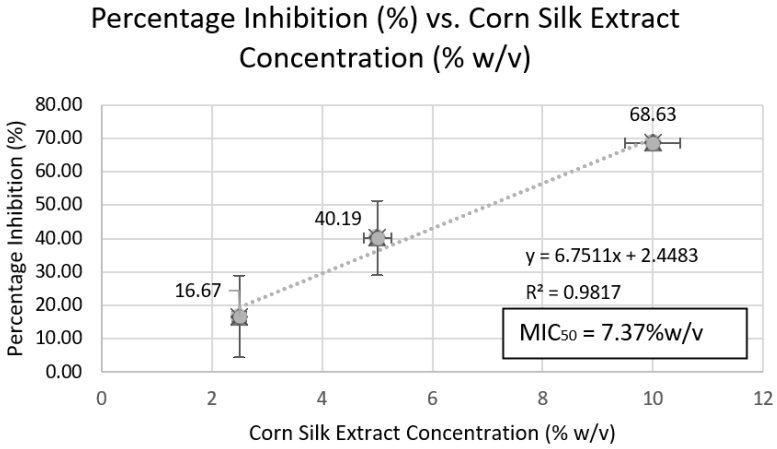


**Figure 2.** Graph of Percentage Inhibition of *S. mutans* vs. Corn Hair Extract Concentration

Based on Figure 2, it is evident that the percentage inhibition or growth inhibition of *S. mutans* bacteria increases with the rising concentration of the extract. The percentage inhibition of *S. mutans* from concentrations of 2.5%, 5%, and 10% is 14.73%, 38.17%, and 68.09%, respectively. This data is plotted in the form of a graph, and a linear regression equation is derived, y = 6.9531x - 0.23, where "y" represents the percentage inhibition of *S. mutans*, and "x" represents the concentration of corn silk extract (% w/v). To find the $MIC_{50}$, the value of 50 is input into the variable "y," resulting in a value of "x" of 7.22. Therefore, the $MIC_{50}$ of corn silk extract against *S. mutans* is 7.37%, and this concentration serves as a reference for calculating the dosage in the formulation of corn silk extract mouthwash.

### 3.7. Mouthwash Formulation

**Table 2.** Mouthwash Formula

| Ingredients | F1 (%w/w) | F2 (%w/w) | F3 (%w/w) | F4 (%w/w) |
|---|---|---|---|---|
| Corn silk extract | 8 | 8 | 8 | 8 |
| *Tween* 80 | 1 | 1 | 1 | 1 |
| Sorbitol | 10 | 12 | 10 | 12 |
| Sodium metabisulfite | 0.1 | 0.1 | 0.1 | 0.1 |
| Sodium Benzoate | 0.1 | 0.1 | 0.1 | 0.1 |
| *Oleum menthae pip.* | 0.2 | 0.18 | 0.2 | 0.18 |
| water | Ad 100 | Ad 100 | Ad 100 | Ad 100 |

The mouthwash formulation is prepared with a final weight of 100 grams using two concentrations of corn silk extract, namely 8% and 10%, in several formulations with ingredient ratios as shown in Table 2. The dosage used should be equal to or greater than the $MIC_{50}$ value to ensure optimal efficacy of the mouthwash. Tween 80 is used as a surface tension reducer and surfactant, allowing the mouthwash to penetrate small crevices in the teeth. Sorbitol serves as a sweetener with low-calorie content, making it less likely for any remaining bacteria on the teeth to break down sorbitol into smaller sugar molecules compared to other sweeteners like aspartame and saccharin. Sodium metabisulfite is utilized as an antioxidant, while sodium benzoate acts as a preservative. Water is employed as the solvent. To maintain consistency and ensure that differences in antimicrobial activity between formulations are attributed solely to other variables, all formulations in this study contained the same concentrations of sodium metabisulfite and sodium benzoate. This uniform level of preservatives was selected to effectively prevent microbial contamination and ensure product stability, without affecting the antimicrobial activity of the corn silk extract or other components. The corn silk extract and these additional ingredients are varied in four different formulations (F1, F2, F3, and F4) to determine which one receives the best response from the public.

### 3.8. Quality Control Test

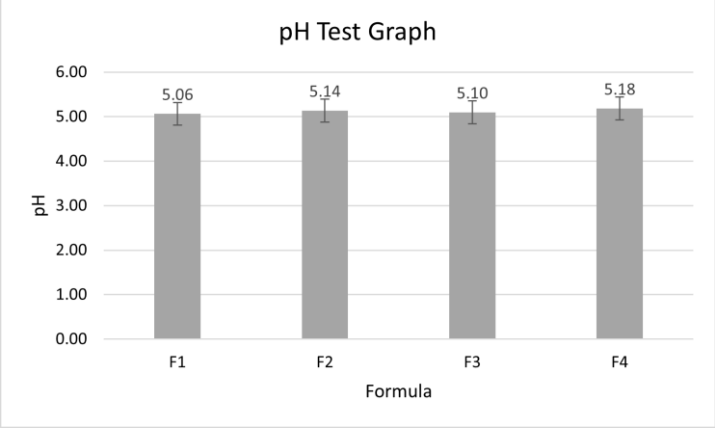


**Figure 3.** pH Test Graph

Based on Figure 3, all four formulations have a pH of 5 from three replicates, indicating that these formulations meet the criteria for a good mouthwash, with a pH range of 5-6. This is because bacteria typically thrive at a pH range of 6-7, so having a different pH can make it challenging for bacteria to survive (Hidayanto et al., 2017).

**Table 3.** Public Response Scores

| Formula | Score 5 | Score 4 | Score 3 | Score 2 | Score 1 | Total Score |
|---|---|---|---|---|---|---|
| F1 | 2 Panelists | 24 Panelists | 4 Panelists | 0 Panelists | 0 Panelists | 118 |
| F2 | 22 Panelists | 8 Panelists | 0 Panelists | 0 Panelists | 0 Panelists | 142 |
| F3 | 4 Panelists | 22 Panelists | 4 Panelists | 0 Panelists | 0 Panelists | 120 |
| F4 | 5 Panelists | 20 Panelists | 5 Panelists | 0 Panelists | 0 Panelists | 120 |

Table 3 indicates that Formula F2 has the best public response with a total score of 142. Formula F3 and F4 have the second-best response with equal scores, both totaling 120. Formula F1 ranks last with a total score of 118.

Formula F2 has a slightly sweet taste, a reddish-brown color, and a sweet and fresh minty aroma. Formula F1 has a darker color than F2 with the same taste but a stronger aroma. Formula F3 has a darker color than F1 with a slightly more bitter taste. Formula F4 is not significantly different from F2 in terms of taste but has a brighter color than F1 and F4, with an aroma similar to F2.

## 4. CONCLUSION

Our research findings indicate that corn silk extract contains flavonoids, tannins, and terpenoids, while alkaloids are not present. The minimum inhibitory concentration ($MIC_{50}$) of corn silk extract against *Streptococcus mutans* is determined to be 7.37% w/v. Among the various formulations, Formula F2 garnered the most favorable response from the public, characterized by a reddish-brown color, a minty aroma, a slightly sweet taste, and consisting of 8% corn silk extract, 1% Tween 80, 10% sorbitol, 0.1% sodium metabisulfite, 0.1% sodium benzoate, 0.18% peppermint oil, and water to reach 100% w/w.

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## 6. CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

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