PHYSICOCHEMICAL EVALUATION AND ANTIBACTERIAL ACTIVITY OF FIBRAUREA TINCTORIA LOUR HERBAL TOOTHPASTE

Evaluasi Fisikokimia dan Aktivitas Antibakteri Pasta Gigi Herbal Fibraurea tinctoria Lour

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ABSTRAK

Penyakit mulut merupakan salah satu penyakit tidak menular (NCD) yang paling umum terjadi di seluruh dunia, dan mempengaruhi sekitar 3,5 miliar orang. Penyakit mulut utama ini mempengaruhi hampir 480 juta orang (43,7% dari populasi) pada tahun 2019. Karies gigi adalah NCD yang paling umum di seluruh dunia, dengan lebih dari sepertiga populasi global hidup dengan karies gigi yang tidak diobati. Penyebab utama adalah bakteri Streptococcus mutans. Penelitian ini bertujuan mengevaluasi aktivitas antibakteri ekstrak Fibraurea tinctoria Lour dalam pasta gigi herbal terhadap S. mutans dengan desain penelitian eksperimental laboratorium. Metode difusi cakram Kirby-Bauer digunakan untuk mengevaluasi aktivitas antibakteri, sementara sifat sensorik dan fisikokimia pasta gigi dievaluasi. Hasil menunjukkan bahwa pasta gigi dengan ekstrak etanol akar kuning pada konsentrasi 3,125% dan 6,250% memiliki aktivitas antibakteri yang signifikan, dengan zona hambat rata-rata 19,36 mm dan 11,04 mm, sedangkan konsentrasi 0,3125% tidak menunjukkan aktivitas hambatan. Penggunaan bahan pengikat CMC-Na pada konsentrasi 3% dan 5% memenuhi standar kualitas, sementara pada 1% tidak memenuhi uji viskositas. Hasil ini menunjukkan potensi pengembangan pasta gigi herbal sebagai alternatif yang efektif dalam mengatasi masalah karies gigi, dengan mengurangi ketergantungan pada produk berfluorida yang berpotensi menimbulkan efek samping.

***Kata kunci**: antibakteri, karies gigi, Fibraurea tinctoria Lour, Streptococcus mutans, pasta gigi*

ABSTRACT

Oral diseases are among the most common non-communicable (NCD) diseases worldwide, affecting an estimated 3.5 billion people. Major oral diseases affected almost 480 million people (43.7% of the population) in 2019. Dental caries is the most common NCD worldwide, with more than one-third of the global population living with untreated dental caries. This study aimed to evaluate the antibacterial activity of Fibraurea tinctoria Lour extract in herbal toothpaste against S. mutans by using a laboratory experimental design. The Kirby-Bauer disc diffusion method was used to evaluate antibacterial activity, and the sensory and physicochemical properties of the toothpaste were assessed. The results showed that toothpaste with ethanol extract of yellow root at concentrations of 3.125% and 6.250% had significant antibacterial activity, with average inhibition zones of 19.36 mm and 11.04 mm, respectively, while the 0.3125% concentration did not show inhibitory activity. The use of 3% and 5% CMC-Na binding agents met the quality standards, while at 1%, it did not pass the viscosity test. These results indicate the potential development of herbal toothpaste as an effective alternative for addressing dental caries problems and reducing dependence on fluoride-containing products that may have potential side effects.

Keywords: antibacterial, dental caries, Fibraurea tinctoria Lour, Streptococcus mutans, toothpaste

INTRODUCTION

Oral diseases are among the most common non-communicable (NCD) diseases worldwide, affecting an estimated 3.5 billion people. The major oral disease affected almost 480 million people (43.7% of the population) in 2019. Dental caries is the most common NCD worldwide, with more than one-third of the global population living with untreated dental caries [1]. According to data from the Directorate of Basic Health Efforts, Ministry of Health of the Republic of Indonesia, in 2013, the national prevalence of dental and oral problems was 25.9%, which increased to 57.6% in 2018; the prevalence of caries experience was 72.3%, and the national prevalence of active caries was 53.2%. Thus, various efforts are still needed to improve Indonesian people's dental and oral health [2]. Dental caries are the most common dental and oral diseases. This disease damages tooth tissue and can negatively impact the mastication process and aesthetic appearance. Dental caries are formed because food residues that stick to the teeth undergo a fermentation process by bacteria in the mouth, which will form plaques on the teeth, which in turn will cause calcification of the teeth so that the teeth become porous, perforated, and even broken [3]. Many factors cause dental caries, including cariogenic microflora, fermentable carbohydrates, and plaque on teeth. The interaction of these variables, host susceptibility, and acidogenic bacteria frequently use sucrose as a substrate, resulting in dental caries. *Streptococcus mutans* is a cariogenic bacterium that causes dental caries. *Streptococcus mutans* is a gram-positive bacterium that converts carbohydrates to lactic acid. An increased concentration of lactic acid is responsible for the demineralization of enamel when saliva cannot prevent its dissolution, leading to caries [4].

One way to prevent caries is to brush the teeth with toothpaste. The addition of antibacterial ingredients to toothpaste can reduce the number of caries-causing bacteria. Most toothpastes recommended by the World Dental Federation (WDF) and American Dental Association (ADA) contain fluoride [5]. Evidence has shown that using fluoridated toothpaste twice daily is sufficient to provide the slow release of fluoride required to protect teeth from caries [6]. Although fluoride-containing toothpastes have benefits, the excessive use of these chemicals also has negative effects. Fluoride can be toxic when ingested excessively. Fluoride can cause intestinal and oral flora changes, dental staining, dental fluorosis, vomiting, and oral cancer. Fluoride toxicity has been reported to affect the endocrine system, thyroid-stimulating hormone and insulin levels, and the central nervous system when ingesting high amounts of fluoride exceeds the probable toxic dose of 5 mg F/kg [6]–[8].

Natural materials must be developed as alternative materials to prepare healthy toothpaste. Plant extracts and herbal toothpaste have been reported to effectively treat several diseases, including gingivitis, gum bleeding, bad breath, and dental cavities. Several studies have examined the effects of plant extracts on specific oral pathogens. Other studies have focused on inhibiting biofilm formation and reducing microbial adhesion, both of which are primarily responsible for dental plaque formation [5], [9]. One plant that can be used is yellow root (*Fibraurea tinctoria* Lour). Taxonomically, the *Fibraurea tinctoria* Lour plant belongs to the *Menispermaceae* family [10]. This plant contains protoberberine alkaloids such as berberine, palmatine, jatrorrhizine, columbamine, and several furanoditerpenoids [11], [12]. This plant also contains flavonoids, tannins, saponins, and steroids/triterpenoids [13]–[16]. Several studies have shown that the *Fibraurea tinctoria* Lour plant has antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae*, and *Bacillus cereus* [17]–[19].

This study presents a novel approach to the development of herbal oral care products. This study explored the potential of *Fibraurea tinctoria* Lour, an herbal ingredient, as an alternative to fluoride toothpaste for combating dental caries. This shift towards herbal alternatives responds to the increasing awareness of the side effects of fluoride toothpaste and the growing consumer interest in natural products [20]. This study evaluated the antibacterial activity of *Fibraurea tinctoria* Lour extract against *Streptococcus mutans*, a key bacterium involved in dental caries, which is crucial for the discovery of effective natural antibacterial agents against such pathogens [21]. Additionally, the toothpaste formulation was optimized by adjusting the yellow root ethanol extract and CMC-Na binder concentrations to enhance its antibacterial efficacy while ensuring compliance with the relevant standards [22]. Moreover, it evaluates the sensory and physicochemical properties of herbal toothpaste, which are vital for ensuring consumer acceptance and product quality [23]. Finally, research has shown that the formulated toothpaste meets the requirements of regulatory standards, such as the Indonesian National Standard 12-3524-1995, underscoring the importance of adhering to such standards in the development and commercialization of herbal oral care products [24]. Overall, this combination of exploring herbal alternatives, optimizing formulations, evaluating sensory and physicochemical properties, and meeting regulatory standards will contribute to the novelty and advancement of natural oral care products [20]. This study aimed to assess the antibacterial activity of *Fibraurea tinctoria* Lour extract in herbal toothpaste formulations against *Streptococcus mutans*, one of the main causes of dental caries. In addition, this study also aimed to determine the effect of varying concentrations of extracts and binders in toothpaste formulations on the antibacterial activity, sensory properties, and physicochemical properties of toothpaste.

METHODS

Research Design

The design of this study was experimental, and the method used was based on a laboratory experiment. The time and place of research were February - August 2023. The plant samples used in this research were yellow root plants of the species *Fibraurea tinctoria* Lour.

Plant Material

The plants used in this study were collected from Menua Sadap Village in Kapuas Hulu District, West Kalimantan, and identified at the Biology Laboratory, Tanjungpura University, Pontianak, Indonesia.

Microorganisms

Pathogenic bacterial isolates of *Streptococcus mutans* were obtained from Tanjungpura University, Pontianak, Indonesia. *Streptococcus mutans* was cultured in brain heart infusion broth (BHI) at 37°C for 48 h.

Extraction of Plant Materials

The stems of the yellow root (1kg) were washed and dried for two weeks in an aerated manner, then crushed using a wood hammer mill and sifted with a particle size of 40 mesh. The extraction was carried out by maceration for four days using ethanol, which was replaced daily. The filtrate was evaporated at 50°C until a thick extract was formed.

Antibacterial Activity Determination against Streptococcus mutans

Antibacterial activity testing in this study used the Kirby-Bauer method, namely, the diffusion method with paper discs. Paper discs soaked with various yellow root extract toothpaste concentrations were placed on media planted with bacteria and incubated at 37 °C for 48 h. A clear zone formed around the disc was measured for each sample [25].

The Toothpaste Formulation

The toothpaste formulas are shown in Tables 2 and 3. Na-CMC was sprinkled in hot water as much as 20 times the weight of Na-CMC, let stand for 30 minutes, and grind until homogeneous (Mass I). Calcium, glycerin, and sorbitol were crushed in different mortars (Mass II). Mass I and Mass II combined to form Mass III. Furthermore, Mass III was gradually added to the extract until it became homogeneous (Mass IV). Methyl Paraben was dissolved in 4 mL of hot water, added to Mass IV, and ground until homogeneous. Finally, add Na. Lauryl sulfate until homogeneous [25]. Toothpaste formulation was done; then, it was evaluated for organoleptic, viscosity, pH, and foam height.

Physical evaluation of toothpaste

Viscosity test

The viscosity was measured using a Brookfield viscometer. The spindle was then inserted into the sample to a certain depth. Rotate the spindle using an electric current until the viscometer needle has a certain number of arrows. Four types of metal spindles were used based on the thickness of the material to be measured. Spindle no.7 was used at a speed of 20 rpm. The result of the viscosity measurement was then obtained as a number displayed on the viscometer monitor. The viscosity measurements were performed at room temperature [25].

pH Test

The sample was prepared at a concentration of 1%; 1 g of the preparation was weighed and dissolved in 100 ml of distilled water. The electrode was then dipped into the electrode. The pH was recorded using a monitor pH meter [25].

Foam Height Test

The toothpaste foam formation test was carried out using 2 g of toothpaste, placed in a 25 mL measuring cup, and dissolved in 15 mL of distilled water. The measuring cup was closed and shaken five times, and the height of the foam formed was recorded [26].

Organoleptic Test

The toothpaste organoleptic observations included shape, color, and odor. The physical form was observed by observing toothpaste preparation [27][28].

RESULT

The Extraction Results

Table 1 shows the extraction results obtained with an extract yield of 2.63% (w/w).

Table 1. The Extraction Results

Sample mass (gram)	Extract mass (gram)	Yield (%)
1000	26.3	2.63

The Determination of The Concentration of Yellow Root Extract in Toothpaste

The toothpaste formulations with various concentrations of yellow root ethanol extract are presented in Table 2.

Table 2. The Toothpaste Formulations with Various Concentrations of Yellow Root Ethanol Extract

	F1 (%)	F2 (%)	F3 (%)
Extract	6.250	3.125	0.3125
Na-CMC	3	3	3
Calcium	20	20	20
Glycerin	5	5	5
Sorbitol	10	10	10
Methyl Paraben	0.5	0.5	0.5
Na. Lauryl Sulfate	1	1	1

Table 2 shows three variations of the extract concentrations used in the toothpaste formula. This variation was based on the MIC value of the yellow root ethanol extract of *Streptococcus mutans*, which was 3125 ppm (0.3125%). The concentration variations were 6.250% (20 times the MIC value), 3.125% (10 times the MIC value), and 0.3125% (the same as the MIC value). These three formulations were tested for antibacterial activity against *Streptococcus mutans*.

Antibacterial Activity Determination against Streptococcus mutans

The results of the antibacterial activity test of the toothpaste formula with various concentrations of the yellow root ethanol extract against *Streptococcus mutans* are presented in Table 3.

Table 3. The Results of The Antibacterial Activity

Replication	F1 (mm)	F2 (mm)	F3 (mm)
1	22.25	7.30	-
2	16.93	12.75	-
3	18.90	13.08	-
Average	19.36	11.04	-

The results of the antibacterial activity test showed that formula 1, with an extract concentration of 6.250%, and formula 2, with an extract concentration of 3.125%, showed strong antibacterial activity against *Streptococcus mutans* because it has a diameter of inhibition between 10-20 mm. In contrast, formula 3, with an extract concentration of 0.3125%, did not exhibit antibacterial activity against *Streptococcus mutans*. Among the three formulas, formula 2 was chosen for further formulation of the toothpaste with various CMC-Na ingredients. Formula 2 was selected because, at this concentration, it could provide strong antibacterial activity against *Streptococcus mutans*.

The Yellow Root Extract Toothpaste Formulation with Variations of CMC-Na

The material used in this study was CMC-Na, which functions as a binding agent. The formula for the yellow root ethanol extract toothpaste with variations in CMC-Na is presented in Table 3.

Table 3. The Formula for The Yellow Root Ethanol Extract Toothpaste with Variations in CMC-Na

	F1 (%)	F2 (%)	F3 (%)
Extract	3.125	3.125	3.125
Na-CMC	1	3	5
Calcium	20	20	20
Glycerin	5	5	5
Sorbitol	10	10	10
Methyl Paraben	0.5	0.5	0.5
Na. Lauryl Sulfate	1	1	1

Physical Evaluation of Toothpaste

Viscosity test

The viscosity test aims to determine the thickness of the resulting toothpaste, where viscosity indicates the strength of the liquid to flow. According to the SNI (12-3524-1995), the viscosity value of toothpaste ranges from 20,000 to 50,000 (Cps) [29]. The viscosity test results for the yellow root ethanol extract toothpaste with variations in CMC-Na are presented in Table 4.

Table 4. The Viscosity Test Results

Replication	F1 (Cps)	F2 (Cps)	F3 (Cps)
1	11,400	26,000	32,200
2	11,400	28,000	32,000
3	10,200	28,000	42,000
Average	11,000	27,333	35,400

Table 4 shows among the three toothpaste formulas, only formulas 2 and 3 met the viscosity requirements specified by the SNI. By contrast, formula 1 does not comply because it has a viscosity value of less than 20,000 Cps.

pH Test

The pH test aims to test the suitability of the degree of acidity of the preparation for the oral mucosa. A good toothpaste pH value falls within the pH range that can be accepted by the oral mucosa and the range set by SNI No. 12-3524-1995, which ranges from 4.5 to 10.5 [30]. The pH test results for the yellow root ethanol extract toothpaste with CMC-Na variations are presented in Table 5.

Table 5. pH Test Results

Replication	F1	F2	F3
1	8.021	7.704	7.556
2	7.946	7.642	7.772
3	7.974	7.634	7.733
Average	7.980	7.660	7.687

Table 5 showed that all formulas met the pH value requirements determined by SNI; therefore, it can be concluded that the three toothpaste formulas have a pH acceptable to the oral mucosa.

Foam Height Test

The foam height test measures the foam produced when brushing teeth to remove debris [31]. The foam height test results for the yellow root ethanol extract toothpaste with variations in CMC-Na are presented in Table 6.

Table 6. The Foam Height Test Results

Replication	F1 (cm)	F2 (cm)	F3 (cm)
1	1.2	1.0	1.3
2	1.2	1.0	1.2
3	1.5	1.0	1.1
Average	1.3	1.0	1.2

Organoleptic Test

The organoleptic test aimed to determine the physical appearance of toothpaste preparations. The organoleptic test observed toothpaste preparations' form, color, and odor [32]. The organoleptic test results for the yellow root ethanol extract toothpaste with CMC-Na variation are presented in Table 7.

Table 7. The Organoleptic Test Results

Parameters	F1	F2	F3
Form	Semi-solid paste	Semi-solid paste	Semi-solid paste
Color	Thick yellow	Thick yellow	Thick yellow
Odor	The characteristic odor of the extract	The characteristic odor of the extract	The characteristic odor of the extract

The results of the organoleptic test showed that the three toothpaste formulations were in the form of a semi-solid, thick yellow color and had a characteristic odor of the extract.

DISCUSSION

Dental caries is a multifactorial disease characterized by high levels of cariogenic bacteria. *Streptococcus mutans* is the most frequently isolated bacterium in human dental plaques. It was chosen for this investigation because it is thought to be the main causative microorganism for tooth caries. Because of its capacity to create acids that can decrease the pH of the oral cavity and result in tooth demineralization, *Streptococcus mutans* is the bacterium that most significantly contributes to the prevalence of dental caries [33].

An antibacterial activity test was performed to determine the ability of a material to inhibit bacterial growth. Antibacterial activity testing was conducted in vitro against Streptococcus mutans using the disk diffusion method. In this method, the sample to be tested is absorbed onto paper discs and attached to agar media, which is homogenized with bacteria and then incubated. Antibacterial activity is positive if a clear zone is formed around the disc paper, where the part that is counted is the diameter of the inhibition zone. The diameter of the inhibition zone also describes the drag force classification. The antibacterial strength was divided into four categories: weak inhibition (<5 mm), moderate (5-10 mm), strong (10-20 mm), and very strong (>20 mm) [34].

In this study, three variations of the extract concentrations were used in the toothpaste formula. This variation was based on the MIC value of the yellow root ethanol extract of *Streptococcus mutans*, which was 3125 ppm (0.3125%), consistent with the results of several previous studies [17]–[19], [35]. The concentration variations were 6.250% (20 times the MIC value), 3.125% (10 times the MIC value), and 0.3125% (the same as the MIC value). Replication was performed thrice for each formulation. The antibacterial activity test showed strong activity against *Streptococcus mutans* at concentrations of 3.125% and 6.250%, with an average inhibition zone value of 19.36 mm and 11.04 mm, respectively. By contrast, 0.3125% did not show inhibitory activity.

The research results showed that although the MIC value of yellow root ethanol extract against *Streptococcus mutans* was 3125 ppm (0.3125%), when the toothpaste formulation was carried out, the concentration of the extract that could produce antibacterial activity was at a concentration of 3.125% (10 times the MIC value) and 6.250% (20 times the MIC value). In addition, the results also showed that the higher the extract concentration in toothpaste, the greater the diameter of the inhibition zone against *Streptococcus mutans*. This is because the higher the concentration of the extract, the greater the amount of active substance contained in the extract and the greater the ability of the extract to inhibit the growth of bacteria.

The antibacterial activity of the extract can be attributed to the presence of bioactive/chemical components, including phenolics, flavonoids, tannins, alkaloids, saponins, and steroids/triterpenoids. Alkaloids can act as antibacterial agents by interfering with the constituent components of peptidoglycan in bacterial cells; therefore, the cell wall layer is not formed intact and causes cell death. Flavonoids inhibit bacterial growth by denaturing bacterial cell proteins and forming complex compounds with extracellular proteins, which disrupt the integrity of the bacterial cell membrane.

Phenolics contain hydroxyl functional groups that form complexes with bacterial cells, thereby disrupting the permeability of the bacterial cell membrane. Saponins function as antibacterial agents by interfering with the stability of the bacterial cell membrane and causing bacterial cell lysis. Tannins exhibit antibacterial activity by damaging cell membranes. The mechanism of action of triterpenoids as antibacterial agents is to react with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall, forming strong polymer bonds, resulting in damage to porins and inhibition of bacterial growth [36]–[39].

The extraction method and solvent are important in obtaining compounds with pharmacological activity. The type of extraction solvent affects the number of active compounds contained in the extract, according to the like-dissolves-like concept, where polar compounds dissolve in polar solvents, and nonpolar compounds dissolve in nonpolar solvents [40]. Several previous studies have reported that both the extraction method and the type of solvent used affect the extract's amount, compound content, and quality. A study by Candra et al. compared several extraction methods, namely maceration, soxhletation, reflux, and sonication, on the total phenolic and flavonoid contents in the ethanol extract of chickpeas (*Phaseolus vulgaris* L.). The results showed that the soxhletation method produces higher phenolic and flavonoid contents than other methods [41]. Another study conducted by Fauziyah et al. compared the extraction methods of maceration, microwave-assisted extraction (MAE), and ultrasonic-assisted extraction (UAE) on the total yield of the ethanol extract of butterfly pea flowers (*Clitoria ternatea* L.). The UAE extraction method produced butterfly pea flower extract with the highest total yield [42]. Research related to the effect of extraction solvent type on sinensetin levels in *Orthosiphon stamineus* Benth leaf extract was carried out by Arifianti et al. and showed that ethanol 96% was the best eluent for the manufacture of herbal medicine from Orthosiphonis folium [43].

The type of bacteria being inhibited is another factor that influences antibacterial activity. In addition, differences in the structure of bacterial cell walls determine the activity, penetration, and binding of antibacterial compounds. *Streptococcus mutans* is a gram-positive bacterium with a peptidoglycan layer that forms a thick and rigid structure and teichoic acid-containing alcohol and phosphate [44]

The yellow root extract at a concentration of 3.125% was then prepared into a toothpaste. In this study, three toothpaste formulas were prepared by varying the concentration of the binder, namely CMC-Na (1 %, 3 %, and 5 %). CMC-Na is a hydrogel binder that can absorb water and prevent separation between powder and liquid ingredients to provide a suitable shape and thickness for toothpaste. A lack of binder can cause a decrease in viscosity and an overly runny consistency. Meanwhile, an excess binder can reduce the ability to spread active ingredients and increase the density of preparation consistency [30].

Besides CMC-Na, other additives, such as calcium, glycerin, sorbitol, methylparaben, and sodium lauryl sulfate, are also used to manufacture toothpaste formulas. Calcium cleans and polishes the tooth surface and is the most significant component of the toothpaste. Glycerin is a moisturizer that increases fluffiness and protects the pasta from becoming stiff or dry. Sodium lauryl sulfate is a detergent that helps the cleaning movement of the toothbrush. In addition, it provides foam work that is comfortable for use. Sorbitol is a sweetener that covers the bad taste of other less pleasant ingredients. Methylparaben is a preservative used in toothpaste. Preservatives are needed in toothpaste preparations, given the presence of water and extracts, which can cause microbial growth [45], [46].

The physical examination test of the preparation used in determining the best toothpaste formula and fulfilling the requirements included viscosity, pH, high foam, and organoleptic tests. Viscosity test results showed differences in the viscosity values of the three toothpaste formulations. This viscosity difference was due to the variations in the concentration of CMC-Na. The higher the CMC-Na concentration, the greater the viscosity. When CMC-Na is dispersed in water, the hydrophilic CMC-Na grains absorb water, resulting in swelling. Water that previously existed outside the granules and was free to move could not move freely anymore; therefore, the solution state was more stable, and there was an increase in viscosity [47]. The pH test showed that the three toothpaste formulas had a pH range of 7.6 – 8.0, so it could be concluded that they had a pH acceptable to the oral mucosa. CMC-Na was alkaline, with a range of 6.5-8.5 [48]. Toothpaste with a very low pH irritates and facilitates the growth of acidogenic bacteria that live in an acidic environment, such as *Streptococcus mutans* and *Lactobacillus*, at a pH of 4.5-5.5. In addition, a pH below 5.5 can potentially cause tooth demineralization and damage to tooth enamel, causing dental caries [30].

The foam height measurement results indicate the ability of the detergent to produce foam. The amount of foam produced was influenced by the concentration of detergent used. There are no requirements for foam height in toothpaste products. This is related to the aesthetic value of consumers [49]. This study used Na. Lauryl sulfate is used as a detergent in the manufacture of toothpaste. Na. Lauryl sulfate is an anionic surfactant with strong detergency [31]. The results showed that the three toothpaste formulas foamed well, with foam heights that did not differ significantly among the formulas. This is attributed to the amount of Na. lauryl sulfate used was the same (1 %). The organoleptic test results showed that variations in the CMC-Na concentration did not affect the physical composition of the toothpaste produced. It is indicated by the shape, color, and smell of the three toothpaste formulas, which are semi-solid, thick yellow, and have a characteristic odor.

The results showed that yellow root extract has the potential to act as an antibacterial agent against the oral pathogenic bacteria *Streptococcus mutans*. In addition, currently available toothpaste active ingredients, such as sodium fluoride and triclosan, are reported to have side effects. Excessive consumption of sodium fluoride is associated with dehydration and possible dental and skeletal fluorosis [45]. In addition to sodium fluoride, triclosan is one of the most widely used antimicrobial ingredients in several pharmaceutical and personal care products (PPCP), such as toothpaste, facial cleansers, deodorants, bar soap, and medical devices. Toothpaste containing TCS has been shown to reduce plaque formation and gingivitis. Although research on TCS has been conducted for many years, it remains an antibacterial agent that is controversial regarding its toxicity. Several reports have mentioned the toxicity of this compound, which belongs to the endocrine-disrupting chemicals (EDCs) class. TCS is often thought to disrupt the thyroid glands. In addition, TCS causes increased bacterial resistance, has a high impact on the gut microbiota and immune system, and has many other negative impacts on human health. In September 2016, TCS in soap products was banned by the U.S. Food and Drug Administration (FDA) and the European Union. Despite these problems, its application in personal care products within certain limits is still permitted [50]. Owing to concerns about the side effects of the currently available active ingredients in toothpaste, such as sodium fluoride and triclosan, yellow root extract can be an alternative replacement or can be administered in combination with other active ingredients in toothpaste formulas. Therefore, it is hoped that it can minimize the side effects of currently available toothpaste.

CONCLUSION

Based on the results obtained, it can be concluded that the yellow root ethanol extract toothpaste with concentrations of 3.125% and 6.250% had strong antibacterial activity in inhibiting the growth of *Streptococcus mutans,* with average inhibitory zone values of 19.36 mm and 11.04 mm, respectively. By contrast, 0.3125% did not show any inhibition. The yellow root ethanol extract toothpaste with 3% and 5% CMC-Na concentrations met the SNI 12-3524-1995 requirements for viscosity, pH, foam height, and organoleptic properties. In contrast, the 1% CMC-Na concentration did not meet one viscosity parameter. Further research needs to be done on other oral pathogenic bacteria, such as *Streptococcus sanguinis*, which plays an important role in forming biofilms on the teeth' surface and causes dental plaque formation.

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