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RESEARCH ARTICLE

Anticariogenic potential of selected medicinal plants from Dayak Benuaq tribe, Indonesia

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Abstract

Several selected medicinal plants from the indigenous Dayak Benuaq tribe of Indonesia were analyzed for their potential as anticariogenic. The plant parts of leaves, stems, roots and tubers were extracted using methanol. *Streptococcus sobrinus* and *Streptococcus mutans* are used to determine antibacterial and anticariogenic activities. Toxicity activity was evaluated using *Artemia salina* with the brine shrimp lethality assay (BSLT) method. This study also calculated the content of total tannins, total flavonoids and total phenols. The results of this study indicate that four selected medicinal plants, namely *Cratoxylum sumatranum* (Jack) Blume., *Areca catechu* L., *Syzygium aromaticum* (L.) Merr. & L.M. Perry. and *Lepisanthes amoena* (Hassk.) Leenh., have the potential to inhibit the growth of *S. sobrinus* bacteria with inhibition values of 89.75%, 80.17%, 71.37% and 74.69% in concentrations of 100 ppm. The highest content of total tannins, total phenols and total flavonoids was in *Uncaria gambir* (W. Hunter) Roxb, with respective values of 205.94 g/g, 478.52 mg GAE/g and 0.725 g CE/g. Anticariogenic activity showed that *U. gambir, Helminthostachys. zeylanica, S. aromaticum, L. amoena* and *Eurycoma longifolia* (Jack) had the potential to inhibit acid production in bacteria with a pH of 6.380, 6.563, 6.140, 5.987 and 5.933, respectively. Besides that, it can also inhibit the attachment of bacterial cells with values of 31%, 50%, 43%, 53% and 40%. The results of this study indicate the potential of several selected medicinal plants from the Dayak Benuaq tribe in Temula village as natural antibacterial, anticancer and anticariogenic agents.

Keywords

cariogenic; Dayak Benuaq; dental care; oral health; *Streptococcus sobrinus; Streptococcus mutans*

Introduction

Dental caries can be suffered by anyone, whether children, adults, or the elderly (1). In 2021, the Ministry of Health of the Republic of Indonesia reported that in Indonesia, a developing country, the population with oral health problems reached 57.6%. Most problems in Indonesia are caused by damaged teeth, cavities and toothaches (2). Meanwhile, the oral health problem that many people experience is swollen gums or ulcers (3).

Dental caries is an infection and a progressive demineralization process in the hard tissues of the crown and root surfaces of teeth. Caries is an infectious disease due to the interaction of cariogenic (caries-causing) bacteria, the host and high-carbohydrate foods (4). Efforts to prevent caries involve controlling the growth

of caries-causing bacteria, namely *Lactobacillus*, *Actinomyces* and *Streptococcus*. Streptococcal bacteria that are often found in teeth are *Streptococcus mutans* and *Streptococcus sobrinus* (5).

S. sobrinus is a non-motile, round and Gram-positive bacterium. *S. sobrinus* plays an important role in the pathogenesis of caries due to its ability to produce acid (acidogenic) and its ability to multiply in an acidic environment (aciduric). The same acid is also the main cause of demineralization of tooth enamel, resulting in carious lesions. *S. sobrinus* can also produce the enzyme glucosyltransferase (GTase). GTase plays a role as a catalyst for converting sucrose into glucan, which then plays an important role in the attachment and colonization of bacteria on the tooth surface, resulting in the formation of black plaque around the teeth (5).

There are many ways to clean teeth from caries and remove plaque; the most common is to use a brush and toothpaste. However, this method is not easy enough because not all tooth surfaces are reached by a toothbrush. Often, mouthwash made from synthetic chemicals and antiseptics is an alternative, but besides the high price, people are not used to using it (6).

One thousand four hundred years ago, many people used miswak wood to clean their teeth (7) and some people still use it today. It is known that miswak has high antibacterial activity, with an inhibition value of 70% at a concentration of 100 ppm (8). Traditional communities have also made extensive use of medicinal plants to cure various oral diseases. The local name for this is *Nginang* and the materials used include *Uncaria gambir* (W.Hunter) Roxb., *Areca catechu* L. and *Piper betle* L. These plants are used by the Javanese tribe to make teeth and gums stronger and prevent thrush (9).

The Dayak Benuaq tribe from Temula village is in Nyuatan sub-district, West Kutai district, East Kalimantan, Indonesia. As an indigenous community, the Dayak Benuaq tribe still uses medicinal plants as the main choice in treating various diseases, with the inheritance of knowledge from their ancestors. Dayak community in Mandomai village, central Kalimantan, is known to have used as many as 55 species of medicinal plants, with 48 species already known and 7 species unknown; most use natural leaves (10). There is still little evidence of the benefits of medicinal plants from the Dayak Benuaq tribe; we selected several types of plants used by the Dayak Benuaq tribe to explore their potential as a source of caries and plaque prevention alternative medicine components. Here, we present the data and inhibitory mechanisms of ten selected plants from the Dayak Benuaq tribe, Temula village, Indonesia.

Materials and Methods

Plants and materials

The raw materials used in this study were medicinal plants that grow in Temula Village (Fig. 1), East kutai is a part of east kalimantan province. The parts of the plants used are the leaves, tubers and bark. The selected plants were *Cratoxylum sumatranum* (Jack) Blume., *Merremia peltata* (L.) Merr., *Dioscorea villosa* L., *Eurycoma longifolia* Jack., *Areca catechu* L., *Helminthostachys zeylanica* (L.) Hook., *Uncaria gambir* (W.Hunter) Roxb., *Carica papaya.* L., *Syzygium aromaticum* (L.) Merr. & L.M. Perry. and *Lepisanthes amoena* (Hassk.) Leenh. (Table 1). The plants were identified by the Dendrologies of Forestry Faculty at Mulawarman University and their identification had been verified by references. In the Laboratory of Forest Products Chemistry, Faculty of Forestry, Mulawarman

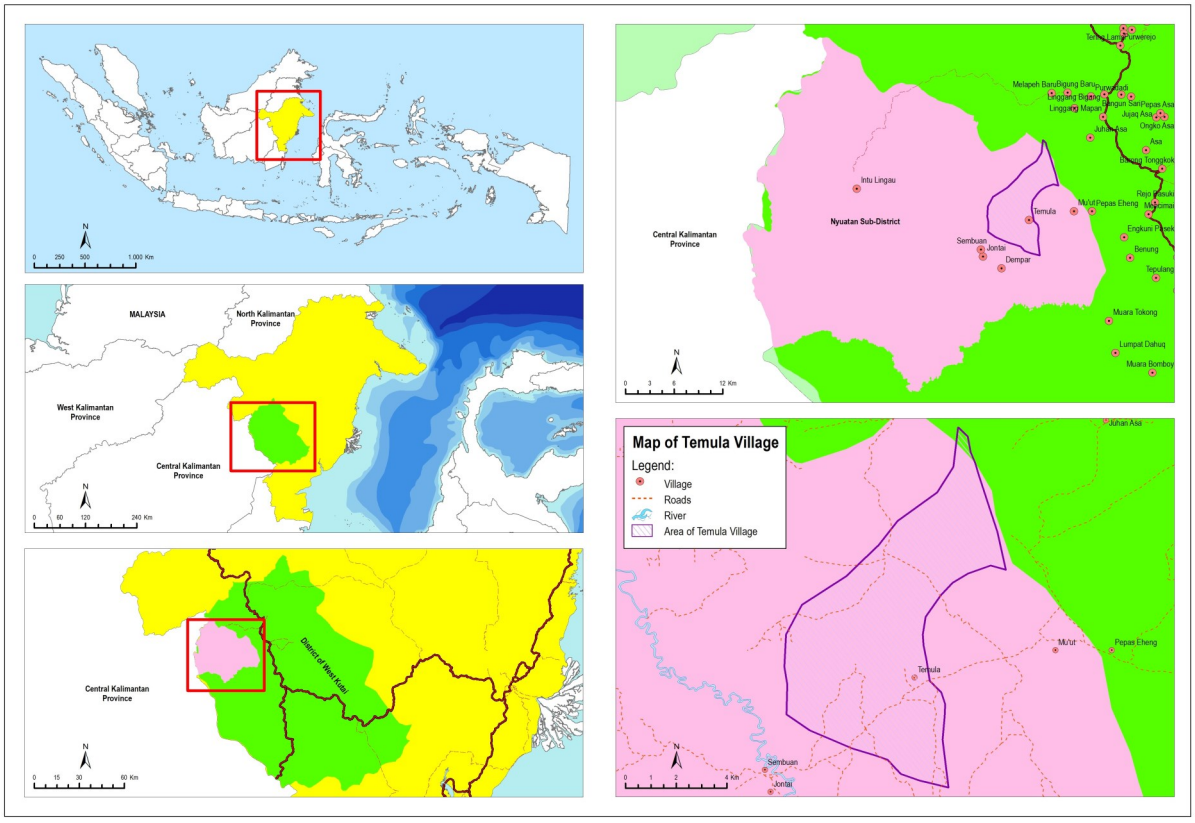

Fig. 1. Dayak Benuaq Tribe, Temula Village, West Kutai, East Kalimantan, Indonesia -0.2806496854881106 °N, 115.52422798651043 ˚E.

University, voucher specimens were deposited. Other materials such as distilled water, ethanol, methanol, acetone, dimethylsulfoxide (DMSO) were purchased from Merck (Darmstadt, Germany). DPPH solution (1,1-diphenyl-2-picrylhydrazyl), ascorbic acid, bismuth (III) nitrate, potassium idoide, acetic acid, sulfuric acid, magnesium, hydrochloric acid and 1-Naphtol were obtained from Sigma (St. Louis, MO, USA). The solvents used are HPLC-grade standards.

Extraction

The extract preparation begins with drying the small pieces at room temperature using a blender. The methanol was consecutively extracted from ground plant samples (45-100 g) at room temperature while continuously shaken on a shaker (7400 Tübingen; Edmun Buchler, Germany) for 48 h. Then, this procedure was repeated. After the solution was filtered through Whatman filter paper No. 2 (Maidstone, UK), the crude alcohol extracts were roto-evaporated at 40 °C and placed in a vacuum oven to almost dry to produce the plant extract shown in Table 1.

Brine Shrimp Lethality Assay

The toxicity activity of the plant extracts was evaluated by Brine Shrimp Lethality Assay using *Artemia salina. A. salina* has widely been used to test the cell viability or anticancer potential of various types of samples. Samples were prepared with a homogeneous 16 mg dose of 8 mL ethanol, so the initial concentration was 10000 ppm. Then 1000 µl, 100 µl and 10 µl of the initial concentration were taken, put in vials test and evaporated. The test was replicated thrice and followed with slight modifications (11). A specified number of *A. salina* eggs were put in and set under a light. *A. salina* eggs hatch into nauplii in 24 to 48 hh, suitable for investigation. 3 mL of seawater was put into each vial, which contained a sample solution and was homogenized until dissolved. Using a pipette, ten shrimps of *A. salina* larvae were put into each vial; water was added to make 5 mL. The control solution consisted of 5 mL of seawater and a solvent containing 10 shrimp larvae. After 24 h, the number of dead shrimp larvae was counted.

Antibacterial assay

Bacteria were grown on nutrient broth and glucose media with slight modifications (12). All bacterial cultures were treated at 37 ˚C for 18-24 h. Suspension Bacterial colonies were in 5 mL of 0.9% salt water to make standard McFarland (10^8 CFU/mL). Standard Manufacturing McFarland 0.5 Weigh as much as 0.1175 grams of $BaCl_2$. $2H_2O$ and put it in a measuring cup, then add distilled water to a volume of 10 mL as solution A. Prepare 1 mL of H_2SO_4 solution to put in a tube, then add distilled water to a volume of 100 mL as solution B. added 0.5 mL of solution B and add 0.5 mL of solution A; and measured with a spectrophotometer with 625 nm (the ideal absorbance value is between 0.008 and 0.13 nm). Antibacterial activity was weighed with as much as 8 g of nutrient broth and 10 grams of glucose, then added with distilled water until it reached a volume of 1000 mL. Sterilized in the autoclave at 121 ˚C for ± 15 min with a pressure of 2 atm. 5 mL of the Nutrient Broth solution was put in 12 test tubes (3 positive control tubes, 3 negative control tubes, 3 test tubes (media + sample extract)), then added 400 µl of bacterial suspension and 500 µl of sample extract with a concentration of 100 ppm in 5 mL of test medium. Incubated for 18-24 h, the absorbance was read at 550 nm.

Total phenol content

The standard calibration curve was prepared using gallic acid (13). The total phenol content was determined by the Folin-Ciocalteu method. 0.25 mL of gallic acid with various concentrations (0-100 ppm)/extract sample was added to 0.75 mL of Folin-Ciocalteu solution and then stirred until homogeneous. After 5 min, 0.75 mL of 4% Na_2CO_3 (sodium carbonate) was added and then stored at room temperature for 120 min. Absorbance was read with a spectrophotometer at a wavelength of 740 nm. The total phenol concentration was calculated using the gallic acid standard calibration curve (0 ppm -100 ppm) expressed in mg/g gallic acid equivalents (GAE).

Total flavonoid content

The standard was prepared by dissolving 0.1 mg of catechin in 10 mL distilled water (14). A total of 0.25 mL with various concentrations of catechins (0-100 ppm) and 0.25 mL of extract with a concentration of 100 ppm were used. Each catechin and extract sample was mixed with 0.5 mL of water, then mixed with 100 µl of $NaNO_2$ (5%) and then, after 5 min, mixed again with 100 µl of $AlCl_3$ (10%). After 6 min, added 0.5 mL of 1M NaOH and centrifuged at 1000 rpm for 1 min. The supernatant was measured using a spectrophotometer at a wavelength of 420 nm. The percentage of flavonoids was calculated in mg Catechin equivalents (CE)/g.

Anticariogenic assay

This anticariogenic assay uses *S. mutans* and *S. sobrinus* 6715 bacteria and the method was followed with slight modifications (15). Bacteria were grown in nutrient broth media 0.8 g of nutrient broth and 2 g of glucose dissolved in 100 mL of distilled water. All bacterial cultures were incubated at 37 ˚C for 18 h. The bacteria to be used were adapted to Mc. Farland 0.5 by adding 99.5 mL of H_2SO_4 (1%) with 50 µl of $BaCl_2$ (1%).

Production of acid

S. mutans and *S. sobrinus* bacteria were cultured in the media. 100 µl in 25 mL of red phenol broth containing 1% glucose and 100 µl of extract with a concentration of 100 ppm were used. Then incubated at 370 °C for 16–20 h. A sample of 5 mL of suspension was taken from the culture periodically, every hour, to record the pH level. The test was carried out in three replicates.

Cell adhesion

S. mutans and *S. sobrinus* bacteria, that had been cultured in nutrient broth containing 2% glucose, were dissolved in 0.9% sterile NaCl and the suspension was adjusted to 0.5 McFarland scale. 100 µl of the suspension was added to nutrient broth containing 2% glucose and mixed thoroughly. Then 2.8 mL of the mixture was taken and transferred to a test tube, where 100 µl of extract with a concentration of 100 ppm was added and then incubated at 37 ˚C for 20 h. After incubation, the cells attached to the glass surface were washed again with 0.9% NaCl and the cells were observed spectrophotometrically at a wavelength of 550 nm using the procedure (15). Repetition was carried out three times.

Results and Discussion

Ethnobotanical studies on the Dayak Benuaq tribe of Borneo reveal a rich knowledge of plant-based remedies deeply embedded in their cultural practices. The tribe utilizes a wide array of forest plants to treat various ailments, such as fever, wounds, respiratory issues and digestive disorders. Notable medicinal plants include *Curcuma longa* (turmeric), *Eurycoma longifolia* (tongkat ali) and *Centella asiatica* (gotu kola), valued for their anti-inflammatory, analgesic and antimicrobial properties. The Dayak Benuaq's traditional plant knowledge, often transmitted orally across generations, is intricately linked to their spiritual beliefs and environmental stewardship. Ten traditional medicinal plants from Dayak Benuaq ethnic group, from the Temula village community were determined for total phenol, antibacterial, toxicity and anticariogenic activity. This plant was chosen based on information from indigenous people who use it as a medicinal plant and have used it for generations.

Plant Extract

Leaves, tubers, fruit, roots and wood of ten medicinal plants were macerated using methanol at room temperature (Table 1). Yields with a value of 3.84-18.13% were obtained from samples under dry conditions. *D. villosa* showed the lowest yield, while *S. aromaticum* showed the highest yield.

Table 1 shows that the yield obtained from the methanol extraction process was grouped into 5 parts, namely fruit, leaves, stems, tuber and roots. The yield of leaves was higher than the three parts of fruit: stem, roots and tuber. This is because the leaves are easier to extract than the fruit, stem and roots as physically, the leaves are softer than the harder parts of the fruit, stems and roots (sweet potatoes) and besides that, the leaves contain a lot of leaf green substances or chlorophyll. Methanol is the most widely used solvent in isolating natural organic compounds because it can dissolve all secondary metabolites. The ideal solvent for the extraction process must have certain conditions, namely, that it can dissolve extractive substances, have a uniform boiling point, be inert (does not react with the substance to be extracted), have a low enough boiling point to be easily evaporated without high temperatures (16).

Toxicity activity

Toxicity activity is used to determine the level of toxicity of a traditional medicinal plant. The Brine shrimp lethality test (BSLT) method is often used as an initial test in selecting samples that have toxic compounds. This test considers the mortality rate of *A. salina* shrimp larvae that have been incubated for 24 h at sample concentrations of 1000, 100 and 10 ppm. Table 2 shows the percentage of death of *A. salina* larvae from 0 to 100%. The lowest mortality percentage was found in *U. gambir* and *L. amoena* plants with the same value of 10%, but there were 3 other samples that had low mortality rates, namely *M. peltate, C. sumatranum* and *S. aromaticum* with values of 13.33, 20 and 25%, respectively. There were 5 plants with high mortality rates, *namely E. longifolia, D. villosa, H. zeylanica* and *A. catechu*, with values of 100, 93.33, 86.67 and 70%, respectively. Plants are said to be toxic in BSLT if the extract can cause 50% mortality (LC50) in test animals at a concentration of 1000 ppm. The LC50 values of *E. longifolia, D. villosa, H. zeylanica* and *A. catechu* extracts were 110.89 ppm, 112.40 ppm, 220.80 ppm and 373.07 ppm, respectively.

The high potential for toxicity in plants can come from secondary metabolite compounds used by plants for self-defence (17). Groups of chemical compounds in plants related to toxicity are also closely related to anticancer activity, including alkaloids, terpenoids, polyphenols, flavonoids and resin (18). Extract samples show toxicity activity in BST if the extract can cause 50% mortality in bioassay at concentrations less than 1000 ppm. The extraction results of several traditional medicinal plants usually contain monosaccharides glucose cellulose (70% dry weight), galactose, 3,6-anhydrogalactose, mannose, xylose and carrageenan. The types of carrageenan contained in medicinal plants are κ-carrageenan type and a little ι-carrageenan (19). Other sources explain that macroalgae natural products that have been tested for anticancer activity are algal polysaccharides, including polysaccharide sulphate, sodium alginate fractions G and M, carrageenan iota, carrageenan kappa, carrageenan lambda and porphyrin (20).

Table 2. Toxicity activity

No	Scientific name	Toxicities (%)		
		1000 ppm	100 ppm	10 ppm
1	*Cratoxylum sumatranum* (Jack)	20.00	0.00	0.00
2	*Merremia peltata* (L.) Merr.,	13.33	13.33	5.00
3	*Dioscorea villosa* L.,	93.33	36.67	13.33
4	*Eurycoma longifolia* Jack.,	100.00	33.33	10.00
5	*Areca catechu* L.,	70.00	3.33	0.00
6	*Helminthostachys zeylanica* (L.) Hook.,	86.67	10.00	0.00
7	*Uncaria gambir* (W.Hunter) Roxb.,	10.00	6.67	3.33
8	*Carica papaya*. L.,	50.00	33.33	16.67
9	*Syzygium aromaticum* (L.) Merr. &	25.00	5.00	0.00
10	*Lepisanthes amoena* (Hassk.) Leenh.	10.00	0.00	0.00

Table 1. Selected plants from Temula village used for oral health

No	Scientific name	Local name	Family	Part	Rend. (%)
1	*Cratoxylum sumatranum* (Jack) Blume.,	Bentalengk	Hypericaceae	Leave	4.56
2	*Merremia peltata* (L.) Merr.,	Blayant	Convovulaceae	Leave	5.96
3	*Dioscorea villosa* L.,	Gadung	Dioscareaceae	Tuber	3.84
4	*Eurycoma longifolia* Jack.,	Pasak bumi	Simaroubaceae	Leave	17.65
5	*Areca catechu* L.,	Pinang	Arecaceae	Fruit	8.85
6	*Helminthostachys zeylanica* (L.) Hook.,	Tunjuk langit	Ophioglossaceae	Root	6.01
7	*Uncaria gambir* (W.Hunter) Roxb.,	Gambir	Rubiaceae	Leave	15.89
8	*Carica papaya*. L.,	Pepaya	Caricaceae	Stem	11.04
9	*Syzygium aromaticum* (L.) Merr. & L.M. Perry.,	Cengkeh	Myrtaceae	Leave	18.13
10	*Lepisanthes amoena* (Hassk.) Leenh.	Selekop	Sapindaceae	Leave	13.94

Artemia mortality in medicinal plant extract solutions dissolved in methanol and chloroform proves the existence of secondary metabolism that is polar and nonpolar. Secondary metabolite compounds from algae that are polar are flavonoids and alkaloids, while compounds that are nonpolar are terpenoids and steroids (21). The presence of flavonoids in the cell environment causes the OH^-group on flavonoids to bind to integral proteins of the cell membrane. This causes the active transport of Na^+ and K^+ to be blocked. The stopped active transport causes uncontrolled entry of Na^+ ions into the cell, which causes rupture of the cell membrane. The rupture of the cell membrane is what causes cell death (22).

Antibacterial activity

Antibacterial activity test uses two bacteria, *S. sobrinus* and *S. mutans*, at a concentration of 100 ppm as shown in Table 3. The 10 plant species tested showed a percentage of inhibition against *S. sobrinus* bacteria with a value of 19.96-89%, while in *S. mutans* bacteria, the rate of inhibition was 16.46-83.92%. The lowest percentage of inhibition on both bacteria was found in *D. villosa* plants, with an inhibition value of 19.96% and 16.46%, respectively, on *S. sobrinus* and *S. mutans* bacteria. While the highest inhibition was with *C. Sumatranum,* with an inhibition value of 89.75% and 83.92% on *S. sobrinus* and *S. mutans* bacteria, respectively. In addition, there are 3 plants that can inhibit above 70%, namely *S. aromaticum, L. amoena* and *A. catechu*, each of which has an inhibition value of 71.37%, 74.69% and 80.17% on S. sobrinus bacteria and inhibition values of 70.74%, 72.77% and 77.86% on S. mutans bacteria.

The growth of dental plaque can be inhibited by eliminating or reducing bacteria in the mouth, for example, with mouthwash containing antiseptics. Areca nut contains bioactive compounds, namely flavonoids, including tannins, which can strengthen teeth (23). Areca nuts can be eaten with betel and lime, which effectively strengthen teeth. Areca nut decoction water is also used as a mouthwash and tooth strengthener (24). Likewise, in this study, the results of the percentage of inhibition of the two types of bacteria above 80% are almost the same as the positive control. Traditionally, people have recognized cloves or cengkeh as a plant that can be used to cure various diseases. Clove has been widely used by the community as a traditional medicine, among others, for toothache, bad breath, nausea, menstrual pain, cough, fever due to malaria, impotence, blackening eyebrows, colds and beriberi. Clove plants have many antimicrobial chemical contents, both in the stem, flowers and leaves. Clove leaves contain saponins, flavonoids, tannins and essential oils. The contents of clove leaves that can be antibacterial are tannins and flavonoids (25).

Tannins have the same characteristics, namely aromatic rings containing one or two hydroxyl groups. In low concentrations (0.1-2%), phenol can damage the cytoplasmic membrane, which causes leakage of essential metabolites, besides that, it activates a few bacterial enzymes when used in high concentrations (26). Phenol works by damaging the cytoplasmic membrane in total and precipitating proteins. Phenol attacks the total cell boundary layer and damages the semi-permeability of the cytoplasmic membrane, which consists of lipids and proteins arranged in layers. The actual mechanism of inhibition by all phenol compounds is by damaging the plasma membrane, causing enzyme inactivity and protein denaturation. The destruction of the cell wall in bacteria can automatically affect the cytoplasmic membrane, mainly composed of proteins and phospholipids (27). Plant phenol compounds quickly form complexes with proteins, resulting in the work of enzymes being inhibited. Proteins in the cell membrane will undergo coagulation and denaturation. In such circumstances, the protein becomes dysfunctional again, resulting in the loss of permeability of the cytoplasmic cell membrane so that the transport of substances into and out of the cell is disrupted. When this happens, it will inhibit growth and even cause cell death (28).

Total tannin content

The total tannin was measured in two stages, namely by measuring total polyphenols and unabsorbed polyphenols (NAP). Analysis of polyphenol content using Folin-ciocalteu and tannic acid as standards was done to get a calibration curve y = 0.1352x-0.027 (R^2 = 0.9999). Table 4 shows that the measurement of total polyphenols from ten species of medicinal plants is very varied, ranging from 5.40 to 210 µg/g. The content of non-absorbed polyphenols varies between 0.12 and 7.26 µg/g. And the total tannin content varies from 5.28 to 205.94 µg/g. The three plants that contain the highest tannins, namely *C. sumatranum, A. catechu* and *S. aromaticum*, also have high inhibitory power against the two types of bacteria, so most likely because the compounds contain high tannins, the inhibitory effect against bacteria is also high. Tannins have several properties, such as astringents, antidiarrheals, antibacterial and antioxidants (29).

Total phenol and flavonoid content

This study determined the levels of phenols using folin-ciocalteu and gallic acid standards with concentrations of 0, 12.5, 25, 50 and 100 ppm measured at 740 nm. The gallic acid calibration curve obtained y = 0.018x + 0.059 (R^2 = 0.998). Fig. 2 shows that the total phenol content varied among plants from high phenol content in some plants to plants with no phenol content.

Table 3. Antibacterial activity

Scientific name	*S. sobrinus* (%)		*S. mutans* (%)	
	Samples	Antibiotic	Samples	Antibiotic
Cratoxylum sumatranum (Jack) Blume.,	89.75	91.46	83.92	87.29
Merremia peltata (L.) Merr.,	50.24	84.5	49.37	84.05
Dioscorea villosa L.,	19.96	84.89	16.46	83.97
Eurycoma longifolia Jack.,	43.36	84.11	37.17	84.05
Areca catechu L.,	80.17	95.35	77.86	87.29
Helminthostachys zeylanica (L.) Hook.,	31.47	84.5	29.68	84.05
Uncaria gambir (W.Hunter) Roxb.,	26.78	95.35	38.11	87.29
Carica papaya. L.,	32.87	84.89	30.8	89.38
Syzygium aromaticum (L.) Merr. & L.M. Perry.,	71.37	95.35	70.74	87.29
Lepisanthes amoena (Hassk.) Leenh.	74.69	91.46	72.77	83.97

Table 4. Total tannin content from ten medicinal plants

Scientific name	Total polyphenols (µg/g)	polyphenols Nonabsorban (NAP) (µg/g)	Total Tanin (µg/g)
Cratoxylum sumatranum (Jack) Blume.,	155.4	3.42	151.98
Merremia peltata (L.) Merr.,	158.4	1.01	157.39
Dioscorea villosa L.,	22	0.28	21.72
Eurycoma longifolia Jack.,	78	1.86	76.14
Areca catechu L.,	132.8	0.94	131.86
Helminthostachys zeylanica (L.) Hook.,	5.4	0.12	5.28
Uncaria gambir (W.Hunter) Roxb.,	210	4.06	205.94
Carica papaya. L.,	28.8	0.51	28.29
Syzygium aromaticum (L.) Merr. & L.M. Perry.,	197.6	7.26	190.34
Lepisanthes amoena (Hassk.) Leenh.	74	1.2	72.8

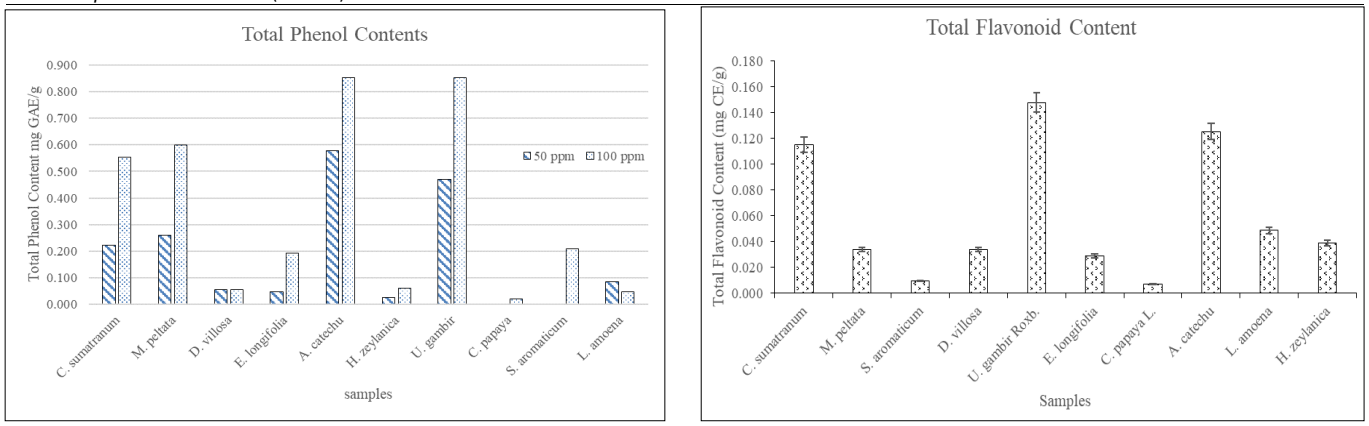

Fig. 2. Total phenol content and total flavonoid content.

Determination of flavonoid content was done using catechin standard solution with concentrations of 0, 12.5, 25, 50 and 100 ppm measured at 420 nm. The catechin calibration curve obtained y = 0.002x + 0.004 (R^2 = 0.999). Fig. 2 shows the total flavonoid content varied from plants with high levels to plants with no flavonoid content.

From the results of this study, *U. gambir* showed the highest value of phenol content; dry leaf extract of *U. gambir* contained 38.49–50.33% total phenol, total phenol content at a concentration of 200 ppm. *H. zeylanica* has the lowest value of total phenol content. While *L. amoena, C. papaya* and *D. villosa* did not show any phenol content, this is probably because *L. amoena, C. papaya and D. villosa* did not detect phenol content equivalent to gallic acid at a concentration of 100 ppm. Another possibility is that the three plant parts do not contain phenol.

U. gambir has prominent levels of total flavonoids, with values of 24.79 to 49.78% catechin. *U. gambir* is one of the medicinal plants commonly found in Indonesia. The active substances contained in *U. gambir* include catechin (30). *E. longifolia* has the lowest total flavonoid content, while papaya stems have no total flavonoid content.

Anticariogenic activity

Acid production in the mouth is caused by bacteria that can ferment carbohydrates, the final product of which is acid. Various species of bacteria colonize the oral cavity, especially in dental plaque and these bacteria can produce acid so that the process of demineralization of hard tooth tissue occurs (31). The components of microorganisms found in the mouth are *S. mutans* and *S. sobrinus*. These bacteria are acidogenic (capable of forming acid) and aciduric (survive in an acidic state) (5).

In the mouth, saliva is an essential element that can protect teeth against external influences, as well as from within the oral cavity itself. The food we eat can cause our saliva to be acidic or alkaline. The role of the salivary environment in the caries process depends on the microorganisms in the saliva (4). Acidic conditions are favored by bacteria; this can reduce the pH of saliva in the mouth. Normal saliva pH ranges from 6.7-7.3. The more acidic the pH of saliva, the easier it is for dental caries to occur. Salivary pH depends on the ratio of acid and its alkaline conjugate (32). In the initial pH measurement, the red phenol media was still neutral and red in color. Color changes in red phenol media from 0 h to 20 h can be seen in Fig. 3.

The measurement of acid production in *S. sobrinus* bacteria showed that at 0 h, the sample was seen to have an initial pH ranging from 6.5-6.7 (Fig. 3). This pH range value still shows a normal pH. In normal conditions, the degree of salivary acidity is between 5.6 and 7.0, with an average pH of 6.7. In subsequent measurements, namely 0 to 16 h, all samples were below the critical pH (pH < 5.5), including the positive control at pH 4.937. Some plants showed higher pH values than the positive control. These plants are *S. aromaticum* (pH = 5.233), *E. longifolia* (pH = 5.200) and *A. catechu* (pH = 5.195). This shows that these extracts could withstand acid production well compared to the positive control. Acid produced by *S. sobrinus* is different from that of *S. mutans.* In *S. mutans*, some samples are still able to inhibit at 16 h, while in *S. sobrinus*, there are no samples that can survive above the critical pH (pH < 5.5) both at 16 h and 20 h. This indicates that *S. sobrinus* is more acidogenic (able to form acid) and asiduric (survive in an acidic state) than *S. mutans*.

Streptococcus bacteria have the ability to attach to all surface locations in the oral cavity. The subsequent formation of

Fig. 3. The acid production by bacteria shown by changes in pH and colour in red phenol media.

dental caries is the culmination of a highly selective process of bacterial attachment and colonization of the tooth surface. If this continues, it leads to plaque formation (33). Dental plaque consists of a collection of large and small cells or individual cells of different bacterial species. Plaque formation and acid formation take place every time when sugar is consumed and for as long as the sugar is in the mouth. As a result of the plaque's limited susceptibility to saliva, the lactic acid that bacteria produce does not dissolve. Consequently, the tooth enamel in proximity to the connected plaque undergoes gradual softening (34). The attachment of *S. mutans* and *S. sobrinus* bacteria to the teeth can be seen in Fig. 4.

Fig. 5. (a) show that a few plant extracts can inhibit *S. mutans* related cell attachment. The percentage of this inhibitory activity ranged from 24.173% to 60.05%. The highest inhibition of cell attachment was by the extracts of *D. villosa* (60.051%) and *C. sumatranum* (57.252%). Positive control is under both (56.234%). Three plants, including *L. amoena* (52.163%), were above 50% inhibition, indicating the potential of the three plants to be new cell attachment inhibitors. There are five plants that have potential when compared to the mouthwash, namely *A. catechu* (38.422%), *E. longifolia* (36.896%), *C. papaya* (32.061%), *U. gambir* (31.298%) and *S. aromaticum* (24.173%). Meanwhile, *H. zeylanica* and *M. peltata* were not able to inhibit cell attachment caused by *S. mutans*.

Fig. 5 (b) shows the percentage value of attachment inhibition due to *S. sobrinus*, which ranged from 2.837% to 97.163%. All extract samples appeared to be able to inhibit bacterial attachment activity. The highest percentage was produced by the positive control at 97.163%, followed by the mouthwash (81.206%), whose inhibitory activity was close to that of the positive control. This indicates that the positive control and mouthwash were able to inhibit bacterial attachment activity to a high extent. This study also showed that all samples used had cell attachment inhibitory activity. The best inhibition was shown by the *A. catechu*, with a percentage value of 54.894%. *A. catechu* contains bioactive compounds, namely flavonoids, including tannins, which can strengthen teeth.

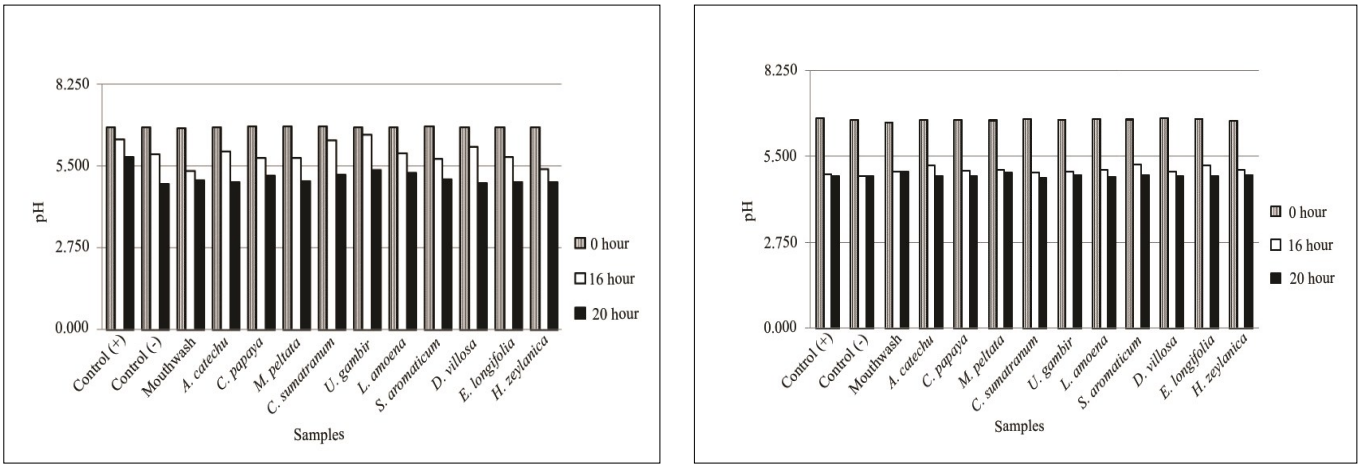

Fig. 4. Acid production from bacteria (a). *S. mutan*, (b). *S. sobrinus.*

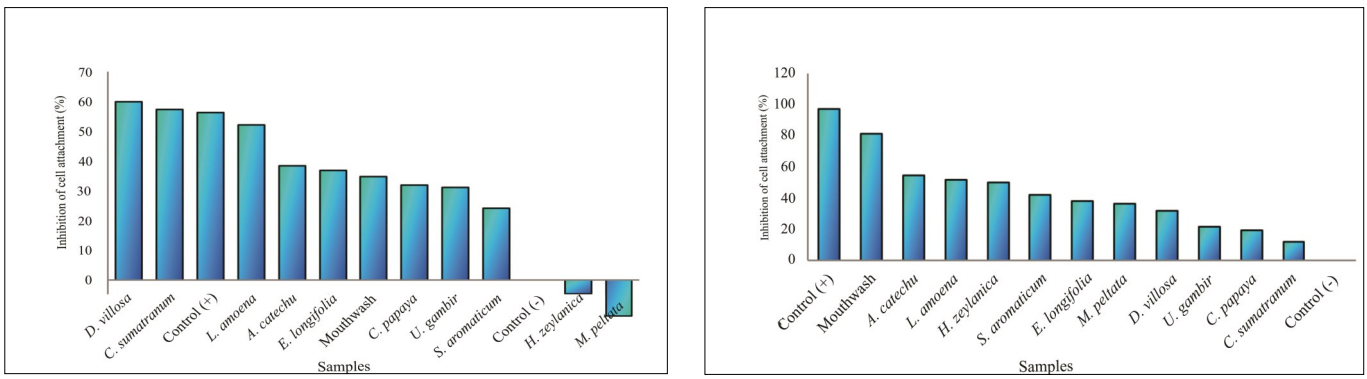

Fig. 5. Cell attachment of bacteria (a). *S. mutan,* (b). *S. sobrinus.*

L. amoena, H. zeylanica and *S. aromaticum* have the potential to inhibit cell attachment due to *S. sobrinus*. Eugenol compounds isolated from clove oil are commonly used for toothache medicine and mixed materials for tooth fillings (35). Various compounds that can control dental caries have been found, but there is still a limited availability of natural products. The high polyphenol content in plants can act as antibiofouling (preventing the attachment of microorganism colonies to teeth), antioxidants and antibacterials (36).

Medicinal plants have long been used in traditional medicine for maintaining dental health and preventing conditions such as dental caries. Numerous studies have explored the antimicrobial, anti-inflammatory and antioxidant properties of various plants like *Azadirachta indica* (neem) (37), *Salvadora persica* (miswak) (38) and *Camellia sinensis* (green tea) (39). These plants have been shown to inhibit the growth of cariogenic bacteria such as *Streptococcus mutans*, which are primarily responsible for tooth decay. Neem, for example, contains compounds like nimbidin and nimbin, which exhibit antibacterial effects and reduce plaque formation (40), while green tea's catechins help decrease acid production in the mouth (41).

In addition to antimicrobial activity, some medicinal plants promote enamel remineralization and improve oral hygiene. Miswak, traditionally used as a natural toothbrush, contains silica and resins that clean teeth and protect against plaque. Its regular use is associated with lower caries incidence and better overall oral health (42). Moreover, herbal mouthwashes made from plant extracts have been studied as alternatives to conventional chemical-based products, showing the potential to reduce oral bacteria and improve gum health without side effects. These findings suggest that medicinal plants could play a vital role in both preventing and treating dental caries, supporting their incorporation into modern oral care products.

Conclusion

In this study, we combined four methods to obtain traditional medicinal plants that have the potential as a source of components for alternative caries and plaque prevention treatments. Traditional medical plants from Temula Village, West Kutai, East Kalimantan province have potential for dental care. There are five samples in the non-toxic category: *C. sumatranum, M. peltata, U. gambir, S. aromaticum* and *L. amoena*. Then, only three samples could inhibit bacteria, namely *C. sumatranum, S. aromaticum* and *L. amoena*. These three samples contain total phenols, total tannins and total flavonoids, so they are also able to maintain the stability of acid production from bacteria and inhibit cell attachment. The balance of acids and alkaline substances in saliva plays a crucial role in oral health. A more acidic pH promotes dental caries, while a neutral or slightly basic pH helps protect the teeth. Understanding the factors that affect salivary pH can help in preventing tooth decay and maintaining dental health. By managing salivary pH, we can take the initiative to take steps toward better oral health and the prevention of dental issues.

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Authors' contributions

SE and HK performed conceptualization. HK, ASP and NAZ carried out the methodology. SE and MA handled the statistics. SE and HK have written the manuscript. KY and TM conducted the review and editing. SE provided funding, reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest.

Ethical issues: None

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