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Antibacterial Activity of *Rhizophora mucronata* Leaves Extract and Its Application in Chewing Gum against *Streptococcus mutans* and *Streptococcus viridans*

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ABSTRACT

Rhizophora mucronata mangrove has antibacterial activity against pathogenic bacterial mostly dental caries-causing bacteria. This research aimed to determine the antibacterial activity of *R. mucronata* leaves extract and its application in chewing gum against dental caries-causing bacteria, which are *Streptococcus mutans* and *Streptococcus viridans*. The method used included inhibitory assay using different concentrations of *R. mucronata* leaves extract (5.0, 10.0, 15.0, 20.0, 25.0 mg/mL), followed by application of the extract in chewing gum formulation (2, 3, 4, 5 MIC/Minimum Inhibitory Concentration) to inhibit *S. mutans* and *S. viridans*. Results showed that *R. mucronata* leaves extract had strong antibacterial activity at a concentration of 25.0 mg/mL against *S. mutans* and *S. viridans* with inhibition zone diameters of 11.35 mm and 23.74 mm, respectively, and MIC of 0.78 and 0.65 mg/mL, respectively. Higher concentrations of *R. mucronata* extract resulted in higher inhibition, but lower acceptance in chewing gum, because of the astringent taste and dark green colour formed. The most preferred chewing gum was the one formulated with 2 MIC *R. mucronata* leaves extract with an acceptance level of 3.6 (slightly like) and inhibition zone diameter of 7.02 mm and 8.60 mm against *S. mutans* and *S. viridans*, respectively. Thus, chewing gum prepared with *R. mucronata* extract has the potential to prevent dental caries.

***Keywords*:** Antibacterial, Chewing gum, Dental caries, *R. mucronata*, Streptococcus.

Introduction

S. mutans and *S. viridans* are usually found in dental plaque and play roles in dental caries formation.[1] *S. viridans* in the oral cavity can synthesize polysaccharides, such as dextran from sucrose to form adhesions to other bacteria and colonize to form dental plaque. *S. mutans* ferment glucose in food and drink leftovers into acid which decreases pH in the oral cavity. Subsequently, the enamel demineralization process continues to form cavities. A decrease in pH in the oral cavity could reach 5.5 or lower, causing the enamel to dissolve easily. As a result, the hard tissues of the teeth are damaged and cause dental caries. Other bacteria that also contribute to dental caries formation are *Actinomyces*, *Lactobacillus*, *Streptococcus sanguis*, and *Staphylococcus aureus*. [2,3] Therefore, caries is the demineralization of enamel and dentine that is closely related to consumption of cariogenic foods. [4] Dental caries are still highly found in Indonesia in children and adults with a prevalence of about 85-99%. In 2017, WHO stated that the incidence of dental caries ranked first, and dental caries ranked the twelfth as the most common dental health problem in the world.[5] Therefore, prevention by inhibiting the growth of dental caries causing bacteria is required.[6] allergic reaction, and toxic reaction.[7] Therefore, a safer alternative antimicrobial agent is required. One of the natural resources that have potential antimicrobial properties is mangroves.

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A common way to decrease bacterial count is by antibiotic therapy, but the use of antibiotics creates new phenomena, such as resistance, It is based on several research which stated that *Avicennia marina*, *Sonneratia caseolaris*, *Ceriops tagal*, *Rhizophora apiculata*, and *Sonneratia alba* mangrove extracts had antibacterial activity towards *Salmonella typhi* and *Listeria monocytogenes*,[8] *R. mucronata* leaves extract had antibacterial activity towards *Aeromonas hydrophyla*,[9] *Bruguiera gymnorrhiza* root extract and *Avicennia marina* leaves extract had antibacterial activity towards *E. coli* and *S. aureus*,[10] *Lumnitzera littorea* leaves extract had antibacterial activity towards *Bacillus cereus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Cryptococcus neoformans*.[11] Other research also mentioned that *Avicennia marina* and *Bruguiera gymnorrhiza* had antibacterial activity towards *E. coli* and *S. aureus*,[12] *Avicennia* sp leaves extract inhibited *E. coli* and *S. aureus;*[13] *Bruguiera gymnorrhiza* inhibited bacteria *Delftia* sp; *Nypa fructicans*, stem extract inhibited *Bacillus subtilis*, while its leaves extract inhibited *Pseudomonas aeruginosa* and *Klebsiella pneumonia*.[14] Moreover, *Rhizophora apiculata* leaves, stem, and root extract could inhibit the growth of *P. aeruginosa*, *S. aureus*, *Streptococcus sp*, and *E. coli*,[15] *L. racemosa* and *A. marina* could inhibit bacteria *S. aureus* and *Proteus sp* which are resistant to antibiotics.[16] Polyisoprenoids from *Avicennia lanata*, *Av. marina*, *Av. officinalis*, *Barringtonia asiatica*, *Bruguiera gymnorrhiza*, *Calophyllum inophyllum*, *Nypa fruticans*, and *Pandanus odoratissimus* leaves extract could inhibit the growth of *E. coli* and *S. aureus*.[17]
From the antimicrobial activity research of various mangrove plants previously mentioned, antibacterial activity is still limited to food pathogenic and spoilage bacteria. The antibacterial activity of the mangrove plant is related to the presence of some phytochemical compounds, such as tannin, alkaloid, flavonoid, terpenoids, and saponin.[10,15] However, there is still limited report about the antibacterial activity of mangroves against pathogenic microbes in teeth. Some plants that have been reported to have antibacterial activity towards pathogenic bacteria in teeth were galangal extract,[18]

basil extract (*Ocimum basilicum*),[19] cloves extract,[20] white oyster mushroom extract,[21] against *S. mutans*, and tea tree oil towards *S. aureus*, *S. mutans*, and *S. viridans.*[22]

R. mucronata mangrove can be found abundantly in Indonesia and it is known to have antibacterial activity towards pathogenic bacteria. However, it has not been studied against bacteria that cause dental caries. Therefore, it is required to study its antibacterial activity towards dental caries-causing bacteria and its application to prevent dental caries.

Some common applications of antimicrobial agents to prevent toothache are on toothpaste and mouthwash.[18-22] However, in this research, an antibacterial extract of *R. mucronata* leaves was added on chewing gum, considering that candy is widely preferred despite can cause dental caries, and chewing gum usually stays longer in the mouth. Moreover, it could be added with other ingredients that can help to inhibit bacteria and increase consumer acceptance, so it can be more effective to inhibit dental caries-causing bacteria. Chewing gum can be useful to stimulate saliva secretion, increase the pH of plaque and saliva, therefore it can be used to clean the oral cavity. The addition of xylitol in chewing gum can inhibit plaque accumulation and enamel demineralization, increase remineralization during an early stage of caries and decrease the number of *Streptococcus mutans.*[23]

Thus, it is necessary to do research that aims to determine *R. mucronata* extract's ability in inhibiting the growth of *S. mutans* and *S. viridans*. This research aimed to determine the potency of *R. mucronata* extract in chewing gum in inhibiting dental caries-causing bacteria (*S. mutans* and *S. viridans).*

Materials and Methods

Sample collection

The sample used in this research were *Rhizophora mucronata* mangrove leaves, collected in May 2019. Mangrove leaves used were old leaves with dark green colour, with voucher number MA-0115-MIC-SBY obtained from Mangrove International Center, Surabaya, East Java, Indonesia. The bacterial culture used in this research were *S. mutans* and *S. viridans*, obtained from the culture collection of Laboratory of Dentistry, Faculty of Medicine, Brawijaya University. Other materials included ethanol (Merck) for mangrove leaves' extraction and materials for chewing gum making, such as gum erythritol powder (Niranbio brand) from 'Yosan' (a chewing gum company), Stevia sweetener (La store), glucose syrup (Rose Brand), mannitol and xylitol (La Store).

Extraction of Rhizophora mucronata Leaves

R. mucronata leaves extraction procedure was based on the maceration method at room temperature.[24] Mangrove leaves were washed and dried at room temperature (without exposure to sunlight to prevent degradation of compounds in samples by UV light) until the brown colour was formed. Dried samples were ground using a disc mill and further sieved using 60 mesh-sieve (pore diameter: 0.250 mm). Mangrove leaves powder (500 g) was macerated using ethanol solvent (2 L) with a ratio (w/v) of 1 : 4 (500 g of sample and 2 L of ethanol) for 72 hours. Samples were then filtered using filter paper and the filtrate was evaporated using a rotary evaporator at 40^{o}C until extract became concentrated. Afterwards, antimicrobial activity of extract was tested at concentration of 5.0, 10.0, 15.0, 20.0 and 25.0 mg/mL using well-diffusion method. MIC value against *S. mutans* and *S. viridans* was also calculated.

Preparation of Chewing Gum

Chewing gum making was based on previous research[25] that has been modified according to the formulation as shown in Table 1.

Gum erythritol powder, glucose syrup, stevia, xylitol, and mangrove leaves extract were put on top of a double boiler (a stacked pan, on which the pan below contains water to be heated and produces steam and the pan on top of it is smaller). The double boiler was heated with medium heat on a stove, and it was stirred until it became warm and sticky. Gum erythritol powder, citric acid, and mangrove leave extracts were poured into it and stirred using a spoon. After cooled down, mannitol was smeared onto hands and the dough was mixed thoroughly for 15 minutes. Afterwards, the dough was rolled and cut.

Antibacterial activity assay

Antibacterial activity assay was done using the well-diffusion method.[26,27] Before assay, both *S. mutans*, and *S. viridans* were rejuvenated in Nutrient Broth media by incubating them for 24 h at a temperature of 37°C. About 10 mL of sterile Nutrient Agar (NA) was poured into a petri dish and let solidify at room temperature. About 100 µL of each bacterial culture (*S. mutans* and *S. viridans*) was put onto NA media surface and spread using triangle and left for 5 minutes. Afterwards, wells with a diameter of 6 mm were made in the media using a sterilized cork borer. About 60 µL of mangrove leaves extracts with concentrations of 5.0, 10.0, 15.0, 20.0, and 25.0 mg/mL were put into the wells and the media were then incubated at 37^{o}C for 24 h. The clear zone formed around the well was the inhibition diameter measured in mm using a vernier calliper.

Minimum Inhibitory Concentration (MIC) value was calculated by plotting In Mo (In extract concentration) on the X-axis and square value of inhibition diameter on the Y-axis. The intersection of the equation with the X-axis is the Mt value. MIC is equal to 0.25 x Mt.

Toxicity assay

Toxicity assay was done using Brine Shrimp Lethality Test (BSLT) method.[28,29] The statistical calculation using probit (probability unit) was done to calculate the LC_{50} value. LC_{50} value shows the concentration of sample that can kill 50% of *A. salina* larvae. *Arthemia salina* Leach larvae were hatched inside a glass jar by immersing 1 g of larvae in 100 mL of seawater and aerated for 48 hours. The eggs will hatch, and their larvae, called nauplii, are ready for the assay. About 10-12 larvae of shrimp were prepared in 100 mL of seawater and given mangrove leaves extract concentrations of 10, 100, 200, 500, and 1000 ppm. Two drops of DMSO (dimethyl sulfoxide) were put into the larvae that have been mixed with the extract and incubated for 24 hours. Furthermore, the number of dead larvae at each extract concentration was counted and expressed as a percentage of mortality, with the following formula:

$$\%\ mortality = \frac{number\ of\ dead\ larvae}{number\ of\ total\ larvae} \times 100\%$$

% mortality was used to determine probit value from the probit table and linear regression was made. The following equation was used to calculate the LC_{50} value.

$$Y = a + bx$$

Y = probit value
a = regression concentration
b = slope
x = log concentration of sample

Hedonic test[30]

The hedonic test is a test in which the panellists decide whether they like or not the attributes of samples given.

Table 1: Formulation of chewing gum making

Ingredients	Formulation			
	2 MIC	3 MIC	4 MIC	5 MIC
Extract (mg/mL)*	1.56	2.34	3.12	3.90
Erythritol (g)	25.0	25.0	25.0	25.0
Glucose(g)	27.0	27.0	27.0	27.0
Xylitol (g)	18.0	18.0	18.0	18.0
Stevia (mL)	0.134	0.134	0.134	0.134
Mannitol (g)	5.0	5.0	5.0	5.0

Note:* The MIC value of the extract was calculated based on inhibition diameter measurement

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In this test, panelists were asked to give their preference level spontaneously without comparing among samples.[30] In this research, a hedonic test was done to determine panellist preference level towards chewing gum that was added with *R. mucronata* leaves extract. The attributes were colour, taste, texture, and overall acceptance. This test used 70 panelists with 5-scale, in which 1 = dislike extremely, 2 = dislike, 3 = neutral, 4 = like, and 5 = like extremely

Statistical analysis

All data obtained in this research were analyzed using SPSS Program Version 20. Statistical analysis used was One-way Anova, followed by Tukey post hoc test with a significance level of $p < 0.05$.

Results and Discussion

Antibacterial activity of R. mucronata leaves extract

The inhibition diameter of *R. mucronata* leaves extract against *S. mutans* was about 6.15 mm to 11.35 mm, meanwhile, the inhibition diameter against *S. viridans* was about 11.86 mm to 23.74 mm. Both results show that the highest inhibition diameter was achieved at extract concentration of 25 mg/mL. To confirm this, statistical analysis using Anova on inhibition diameter data of *R. mucronata* leaves extract shows that extract concentration affects the inhibition diameter ($p<0.05$) towards *S. mutans* or *S. viridans* bacteria. Results of the post hoc test using Tukey can be observed in Figure 1.

Figure 1 shows the various concentration of extract that resulted in higher inhibition diameter against *S. mutans* or *S. viridans* bacteria. This phenomenon shows that a higher concentration of extract contains higher antimicrobial compounds.[31-32] Antibacterial activity of *R. mucronata* leaves extract is related to tannin, flavonoid, steroid, and triterpenoid compounds, which act as antimicrobial agent[33,34] and are found abundantly in a mangrove plant, specifically *Avicennia* and *Brugiera.*[12]

Based on its inhibition diameter, *S. viridans* was higher compared to *S. mutans.* This indicates that *R. mucronata* leaves extract inhibited *S. viridans* better than *S. mutans.* The difference in inhibitory activity could be related to the difference in cell wall composition or structure, therefore it gives different responses towards antibacterial compounds in *R. mucronata* leaves extract. *Rhizophora* mangrove leaves contain some antibacterial compounds, such as tannin, flavonoid, saponin, and triterpenoid.[10,15] Tannin forms a hydrophobic complex with protein, inactivates adhesion, enzyme, and protein transport of cell wall to disturb the growth of bacteria. Flavonoids inhibit nucleic acid synthesis, inhibit cell membrane function and energy metabolism, and inhibit the growth of bacteria by forming a complex with extracellular protein which disturbs cell membrane integrity. Furthermore, saponin causes lysis of bacterial cells. [10,15] Based on inhibition zone, there are 4 categories of antibacterial activity, which are very strong (inhibition zone $\geq$ 20 mm), strong (inhibition zone of 15-20 mm), moderate (inhibition zone of 10-15 mm), and weak (inhibition zone $\leq$ 10 mm).[35] Therefore, the extract concentration of 25 mg/mL, strongly inhibited *S. viridans*, and moderately inhibited *S. mutans.* Antibacterial activity of *R. mucronata* leaves extract towards *S. mutans* is still lower compared to noni extract with an inhibition diameter of 13.31 mm.[36] Meanwhile, the antibacterial activity of *R. mucronata* leaves extract *towards S. viridans* is comparable to green betel leaves extract, which has an inhibition diameter of 21.33 mm[37] but is higher compared to 80% shallot extract which has an inhibition diameter of 15.6 mm.[38]

Based on inhibition diameter data, the MIC (Minimum Inhibitory Concentration) value of *R. mucronata* leaves extract was calculated and the result obtained is shown in Table 2**.**

MIC is the minimum concentration of a compound or extracts to inhibit the growth of microorganisms. A lower MIC value means higher inhibitory activity and vice versa. *R. mucronata* leaves extract has a MIC value of 0.78 mg/mL against *S. mutans* and 0.65 mg/mL against *S. viridans.* This means that *R. mucronata* leaves extract could inhibit *S. viridans* better than *S. mutans*. Compared to the MIC value of noni fruit extract against S. mutans, which was 0.375 mg/mL,36 inhibitory activity of noni fruit extract is higher than *R. mucronata* leaves extract. However, if compared to the MIC value of betel leaves extract against *S. viridans,* which was 15 mg/ml,[39] inhibitory activity of *R. mucronata* leaves extract is higher. These differences could be caused by the different antimicrobial compounds in extract and the difference in its concentration.

Antibacterial activity of chewing gum added with R. mucronata leaves extract

Inhibition diameter of *R. mucronata* leaves extract in chewing gum against *S. mutans* was about 7.02 mm to 11.22 mm, meanwhile, inhibition diameter against *S. viridans* was about 8.60 mm to 17.91 mm. Statistical analysis using ANOVA on inhibition diameter data shows that the concentration of *R. mucronata* leaves extract in chewing gum significantly affects inhibition towards *S. mutans* or *S. viridans*. Results of the post hoc test using Tukey can be observed in Figure 2.

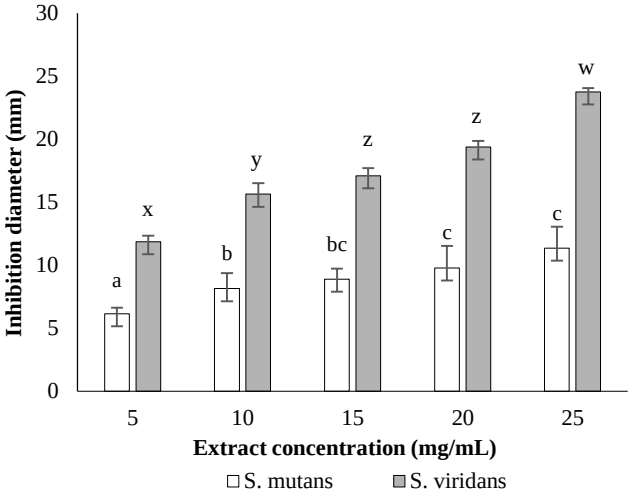

Figure 1: Inhibition diameter (mm) of *R. mucronata* leaves extract against *S. mutans* and *S. viridans.*

Notes: different letter notation in the same colour histogram shows significant difference at $p < 0.05$

Table 2: MIC value of *R. mucronata* leaves extract

Bacteria	MIC (mg/mL)
Streptococcus mutans	0.78
Streptococcus viridans	0.65

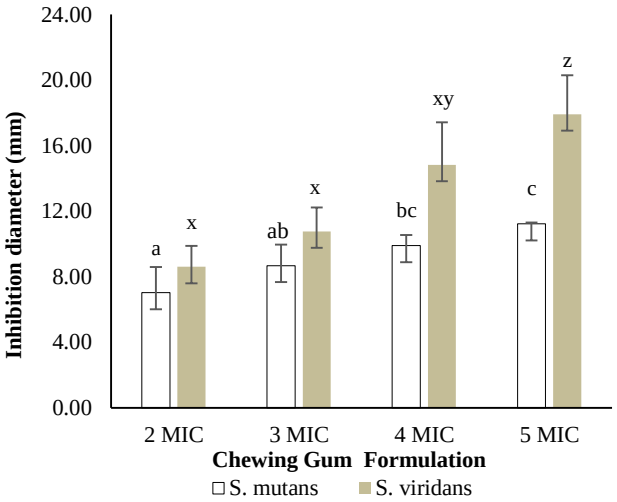

Figure 2: Inhibition diameter of chewing gum added with *R. mucronata* leaves extract towards *S. mutans* and *S. viridans*

Notes: different letter notation in the same colour histogram shows significant difference at $p < 0.05$.

Figure 2 shows that incorporation of *R. mucronata* leaves extract could potentially inhibit *S. mutans* and *S. viridans*. Figure 2 also shows that a higher concentration of *R. mucronata* leaves extracts added in chewing gum results in higher inhibition diameter against both *S. mutans* and *S. viridans.* This also indicates that the more extract added in chewing gum, the more active compounds from *R. mucronata* leaves extract are included. Therefore, it gives higher inhibition against *S. mutans* and *S. viridans.* Some compounds reported to have antibacterial activity from *Rhizophora* mangrove leaves were tannin, flavonoid, saponin, steroid, and triterpenoid.[10,15,33,34] Mechanisms of these organic compounds as antibacterial agents are by poisoning the protoplasm, destroying and penetrating the cell wall, and precipitating protein in microbial cells.[10,15] Moreover, the inhibition diameter of *R. mucronata* leaves extracts in chewing gum formulation against *S. viridans* was larger compared to *S. mutans.* This result shows a similar phenomenon to inhibition diameter of *R. mucronata* leaves extract, but in chewing gum formulation, there are several other ingredients added, such as glucose, erythritol, stevia, and xylitol. Among these ingredients, xylitol was reported to have antimicrobial activity.[23,25] Chewing gum added with 5 MIC of *R. mucronata* leaves extract (5 x 0.65 mg/mL = 3.65 mg/mL) gave inhibition diameter against *S. viridans* of 17.91 mm (Figure 2), whereas *R. mucronata* leaves extract gave an inhibition diameter of 17.1 mm (Figure 1) at a concentration of 15.0 mg/mL.

This shows that the addition of other ingredients in chewing gum increased the inhibitory activity of *R. mucronata* leaves extract against *S. mutans* and *S. viridans*. The ingredient that could increase the extract's inhibitory activity is xylitol. It is based on previous research which stated that chewing gum added with xylitol could inhibit plaque accumulation, enamel demineralization, increase pH in the oral cavity, and decrease the number of *S. mutans.* [23,25]

Antibacterial activity can be categorized as very strong if the inhibition zone is ≥ 20mm, strong if the inhibition zone is 15-20 mm, mild if the inhibition zone is 10-15 mm, and weak if the inhibition zone is ≤ 10 mm.[35] Based on its diameter inhibition, chewing gum that was added with *R. mucronata* leaves extract shows strong inhibition at an extract concentration of 5 MIC, inhibition diameter could reach about 11.22 mm towards *S. mutans* and 17.91 mm (3.9 mg/mL) towards *S. viridans*. When compared to hard candy that was added with ginger extract, chewing gum added with *R. mucronata* leaves extract had stronger inhibition, in which red ginger extract that was formulated in hard candy could inhibit *S. mutans* with an inhibition diameter of 10.08 mm at an extract concentration of 25 mg/mL.[40] The higher the extract concentration added into a product, the higher the inhibition diameter. The difference of inhibition diameter in each food product is influenced by extract type of bioactive compounds contained in extract, extract concentration, and interaction with other ingredients inside a food product. The higher the concentration of extract, the higher amount of bioactive compounds content. Therefore, the ability to inhibit the growth of bacteria is higher, shown by a larger inhibition diameter. In this research, the highest inhibition diameter was obtained at the treatment of chewing gum added with *R. mucronata* leaves extract with a concentration of 5 MIC (3.9 mg/mL which was 17.91 mm, higher compared to 80% basil leaves extract which only had inhibition diameter of 9.65 mm towards *S. mutans*.[41] This difference could be caused by several factors, such as inoculum concentration, incubation time, extract concentration, type of active compound, and antibacterial power of active compound. The higher concentration of inoculum could result in lower inhibition power, shown by smaller inhibition diameter. Extract concentration influences the diffusion rate of active compounds. Higher extract concentration results in a higher diffusion rate, therefore inhibition power is higher and subsequently, the inhibition diameter formed is larger.[42]

Hedonic Test of Chewing Gum

In general, a higher concentration of extract added into a product results in higher inhibition power. However, in a food product, too high an amount of extract cannot be added because of the limitation related to consumer acceptance or preference. Hedonic results of chewing gum added with *R. mucronata* leaves extract can be observed in Table 3. Table 3 shows that preference towards colour, taste, and overall decreases as the concentration of extract added into chewing gum increases. The decrease in acceptance towards colour is related to the colour of *R. mucronata* leaves extract, which was dark green.[43] Therefore, the higher the extract concentration added, the darker the colour of chewing gum. Thus, it was not preferred. The highest acceptance level towards colour was obtained at the addition of *R. mucronata* leaves extract with a concentration of 2 MIC, which was 3.4 (slightly like). The acceptance level towards the taste of chewing gum also decreases as the concentration of extract added increases. The highest acceptance level only reaches 3.6 (slightly like) at the addition of extract with a concentration of 2 MIC. The decrease of acceptance in terms of taste is related to the taste of extract that is very astringent. This astringency is related to high tannin content in *R. mucronata*.[44] Astringent taste from extract could mask the sweet taste of chewing gum.[45] Meanwhile, the acceptance level of panellists in terms of texture shows no significant difference, which means an addition of extract did not affect the texture of chewing gum. The texture of chewing gum is influenced by the types and amount of gum base and sugar used, whereas, in hard candy, the texture is related to the formation of large sugar crystals.[46] Overall acceptance level towards chewing gum added with *R. mucronata* leaves extract shows a similar trend to acceptance level towards taste and colour. Overall acceptance level decreases as the extract concentration added increases. The highest acceptance level was 3.6 (slightly like) at the addition of extract with a concentration of 2 MIC. Therefore, overall acceptance of chewing gum is influenced by astringent taste and colour of *R. mucronata* leaves extract.

Determination of Selected Treatment and Toxicity Assay of Chewing Gum

Based on Figure 2, the higher addition of *R. mucronata* leaves extract results in higher inhibition against *S. mutans* and *S. viridans.* However, Table 2 shows that the acceptance level of chewing gum decreases with the higher addition of *R. mucronata* leaves extract. It is caused by the astringent taste and the dark green colour of the extract. Therefore, the selected formulation was chewing gum added with *R. mucronata* leaves extract at a concentration of 2 MIC, as it had the highest hedonic score of 3.6 (slightly like) and also had inhibition diameter towards *S. mutans* of 7.02 mm and *S. viridans* of 8.60 mm.

Based on the BSLT method to determine the toxicity level of selected chewing gum, an LC_{50} value of 11685.8 mg/mL was obtained. A sample is considered toxic when it has an LC_{50} value of less than 1000 mg/mL.[29] Therefore, the selected chewing gum is safe to be consumed to inhibit dental caries causing bacteria.

Table 3: Hedonic results of chewing gum added with *R. mucronata* leaves extract

Extract Concentration	Hedonic Attributes score			
	Colour	**Taste**	**Texture**	**Overall**
2 MIC	3.40 ± 0.50^{c}	3.60 ± 0.60^{d}	3.10 ± 0.40^{a}	3.60 ± 0.50^{c}
3 MIC	2.90 ± 0.50^{b}	3.10 ± 0.40^{c}	3.20 ± 0.30^{a}	3.20 ± 0.40^{b}
4 MIC	2.50 ± 0.60^{a}	2.50 ± 0.60^{b}	3.10 ± 0.90^{a}	2.80 ± 0.60^{ab}
5 MIC	2.30 ± 0.70^{a}	1.90 ± 0.70^{a}	2.80 ± 1.00^{a}	2.50 ± 0.80^{a}

Notes:- different letter notation on the same column shows significant difference at $p < 0.05$
- Range of hedonic score 1 - 5, 1 = dislike extremely; 5 = like extremely

Conclusion

Antibacterial activity of *R. mucronata* leaves extract is categorized as strong with a MIC value of 0.78 mg/mL against *S. mutans* and 0.65-mg/mL against *S. viridans*. The addition of *R. mucronata* leaves extract of 2 MIC into chewing gum was the most preferred with an acceptance level of slightly like and inhibition zone diameter against *S. mutans* and *S. viridans* of 7.02 mm and 8.60 mm, respectively. The formulated chewing gum has the potential to be consumed to prevent dental caries.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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