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Application of mangrove (*Bruguiera gymnorrhyza*) leaves' extract on chewing gum as an inhibitory agent against dental caries causing bacteria

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Abstract

Mangrove *Bruguiera gymnorrhyza* contains bioactive compounds, such as tannin, saponin, and flavonoid. These are antimicrobial properties and have the potential to inhibit dental caries causing bacteria. Chewing gum incorporated with xylitol could inhibit plaque accumulation and demineralization at the early stages of dental caries and reduce the number of *Streptococcus mutans* in the mouth. This research was aimed to determine the inhibition power of *B. gymnorrhyza* leaves extract and its application on chewing gum towards *Steptococcus mutans* and *Streptococcus viridans*. The method used was experimental, consisting of the first stage with the treatment of different extract concentrations (5 mg/mL, 10 mg/mL, 15 mg/mL, 20 mg/mL, 25 mg/mL) and a second stage with the treatment of different extract concentration in the chewing gum (2 MIC, 3 MIC, 4 MIC, and 5 MIC). Results showed that *B. gymnorrhyza* leaves extract can be categorized as a strong inhibitor against dental caries causing bacteria with MIC value of 1.62 mg/mL towards *Steptococcus mutans* and 1.35 mg/mL towards *Streptococcus viridans*. The application of *B. gymnorrhyza* leaves extract at 3 MIC in chewing gum produced the most preferred chewing gum and has strong inhibitory activity against dental caries causing bacteria with inhibition diameter of 10.26 mm towards *Steptococcus mutans* and inhibition diameter of 12.51 mm towards *Streptococcus viridans*. It is also very safe to consume with an LC_{50} value of 10502 mg/mL.

1. Introduction

Bruguiera plant, which is locally known as "*lindur*", is found abundantly in tropical regions. In Indonesia, this plant is found in Java, Sumatra, Borneo, Mollucas, and Bali (Jacoeb *et al*., 2013). This type of mangrove has been used in several human needs, such as a food ingredient, as firewood, and also used in traditional medicine. Traditionally, mangrove *Bruguiera gymnorrhyza* fruit is used as medicine for curing fungal infection in the tongue (*Candidiasis*), baby stomach cleanser, and treating skin rashes (Abubakar *et al*., 2019). Mangrove *Bruguiera cylindrical* is used to treat eye pain, while the bark of *Bruguiera exaristata* is used to treat bleedings, and the *Bruguiera gymnorhyza* flower to treat ear infection (Purnobasuki, 2004).

The utilization of *Bruguiera* plant parts as traditional medicine indicates the presence of a bioactive compound that can cure diseases as it is antimicrobial. The root extract of *Bruguiera gymnorhyza* could inhibit *E. coli* and *S. aureus* (Audah *et al*., 2020), *Bruguiera gymnorhyza* leaves extract inhibits *Delftia* sp., *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Kurniasih and Halimah, 2019), polyisoprenoid from *Bruguiera gymnorhyza* leaves inhibits the growth of *E. coli* and *S. aureus* (Sumardi *et al*., 2018; Renaldi *et al*., 2018). Other types of mangroves also possess antimicrobial activity. For example, mangrove *Avicennia marina*, *Sonneratia caseolaris, Ceriops tagal, Rizhopora apiculata*, and *Sonneratia alba* have antibacterial activity against *Salmonella enterica* serovar Typhi and *Listeria monocytogenes* (Mustopa *et al*., 2015), mangrove *R. mucronata* leaves have antibacterial activity towards *Aeromonas hydrophila* (Mikchaell *et al*., 2018). Moreover, *Avicennia marina* leaves has antibacterial activity towards *E. coli* dan *S. aureus* (Audah *et al*., 2020), mangrove *Lumnitzera littorea* leaves have antimicrobial activity against *Bacillus cereus,*

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Pseudomonas aeruginosa, *Candida albicans*, *Cryptococcus neoformans* (Saad *et al*., 2011), mangrove *Avicennia marina* leaves have antibacterial activity towards *E. coli* and *S. aureus* (Renaldi *et al*., 2018), *Avicennia* sp. leaves inhibits *E. coli* and *S. aureus* (Alhaddad *et al*., 2019), the stem of *Nypa fructicans* inhibits *Bacillus subtilis*, whereas the *Nypa fructicans* leaves inhibit *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Kurniasih and Halimah, 2019). Other research has also reported that leaves, bark, and root of *Rhizophora apiculate* has the potential to inhibit *P. aeruginosa*, *S. aureus*, *Streptococcus* sp., and *E. coli* (Ciptaningrum and Putri, 2019), *L. racemosa* and *A. marina* has shown inhibitory activity against *S. aureus* and *Proteus* sp. which are resistant to antibiotics (Abeysinghe, 2010), polyisoprenoid from leaves of *Avicennia*, *Barringtonia*, *Calophyllum*, *Nypa*, and *Pandanus* inhibits the growth of *E. coli* and *S. aureus* (Sumardi *et al*., 2018).

Antimicrobial activity of mangrove *Bruguiera* and other types of mangrove could inhibit pathogenic bacteria, particularly pathogenic bacteria in food products. Therefore, *Bruguiera* plant has the potential to be used as a natural antimicrobial source to be applied on several food products, such as fishery products (Hastarini *et al*., 2014). *Bruguiera* could be a natural antimicrobial agent because it contains bioactive compounds, such as steroid, triterpenoid, saponin, flavonoid, alkaloid and tannin (Purnobasuki, 2004; Doughari, 2012).

On the other hand, dental problems are a reoccurring problem in many countries including Indonesia. A common dental problem is dental caries. In Indonesia, dental caries is found in both children and adults with a prevalence of 85-99%. In 2017, WHO stated that permanent dental caries ranked the first and primary dental caries ranked the twelfth as the most common dental health problem in the world (Kassebaum *et al*., 2017). Bacteria that play roles in dental caries are *S. mutans* and *S. viridans* (Fatmawati, 2015).

There are 3 theories regarding the dental caries mechanisms, which are proteolysis theory, proteolytic-chelation theory, and chemo parasitic or acidogenic theory. The acidogenic theory explains that dental caries formation is caused by acid produced by microorganisms' action towards carbohydrates (Ramayanti and Purnakarya, 2013). *Streptococcus viridans* in the oral cavity could synthesize polysaccharides, such as dextran from sucrose to form attachment and form colonies with other bacteria, which subsequently form dental plaque. *Streptococcus mutans* could ferment carbohydrates into acid that causes enamel demineralization continuously to form cavities and cause dental caries (Ramayanti and Purnakarya, 2013; Haerazi *et al*., 2014). Negative effects from dental caries could be bleeding, infection, loss of tooth bone, wobbly tooth and even tooth loss (Andriani and Rizka, 2014). Furthermore, dental caries can indirectly cause cardiovascular diseases (Cotti *et al.*, 2011).

Prevention of dental caries can be done by inhibiting the bacteria causing it (Purnamasari *et al*., 2010). Inhibition towards dental causing bacteria can be done using antibiotics or natural antimicrobial agents. However, the use of antibiotics could cause new phenomena, such as resistance, allergic reaction, and toxic reaction (Newman *et al*., 2006). Meanwhile, mangrove *Bruguiera* has the potential to be used as a natural antibacterial agent to prevent dental caries. It is based on its capability to inhibit pathogenic bacteria. The leaves and bark of *Bruguiera gymnorryza* contain bioactive compounds, such as tannin, saponin, flavonoid, steroid, and terpenoid that possess antimicrobial properties (Anggraini *et al*., 2018).

A natural antimicrobial agent to prevent toothache has been applied in toothpaste and mouthwash (Siregar *et al*., 2011; Astuti and Meilawaty, 2013; Khairani *et al*., 2017; Paksi *et al*., 2018; Suhendar and Fathurrahman, 2019). Therefore, a more effective alternative to inhibit bacterial growth and is more preferred by consumers is required. One of the potential alternatives is the application of chewing gum. The consideration is that chewing gum could stimulate saliva secretion, increase the pH of plaque and saliva, using it as an oral cavity cleanser. The addition of xylitol in chewing gum inhibits plaque accumulation and enamel demineralization, increase remineralization in the early stage of dental caries and reduce the number of *S. mutans* (Hidayati *et al*., 2014). Another reason is that chewing gum is popular, it stays longer inside the mouth, and other ingredients assisting in the inhibition of bacteria can be added and increase consumers' acceptance.

Based on those considerations, research is required to determine *Bruguiera gymnorrhyza* leaves extract inhibitory activity against *S. mutans* and *S. viridans*, as well as to determine the potency of *Bruguiera gymnorrhyza* leaves extract in chewing gum to inhibit dental caries causing bacteria, namely *S. mutans* and *S. viridans*.

2. Materials and methods

2.1 Materials and equipment

The material used was mangrove *Bruguiera gymnorrhyza* leaves which are old and green-coloured, obtained from MIC (Mangrove International Center), Surabaya, East Java, Indonesia. Materials for chewing

gum making were erythritol gum base powder "Niranbio", liquid Stevia sweetener, glucose syrup "Rose Brand", xylitol, mannitol, mangrove *B. gymnorrhyza* leaves extract and aquadest. Materials for analysis were *Steptococcus mutans* and *Streptococcus viridans* bacteria, obtained from the culture collection of Faculty of Medicine, Brawijaya University, *Artemia salina* Leach, Nutrient agar (NA), and dimethyl sulfoxide (DMSO).

Equipment used for chewing gum making were stove, pan, digital balance "Camry EK505", sieve, and tray. Equipment used for chemical analysis were incubator "Memmert", inoculating loop, 100 μL micropipette "ToPette Nesco", autoclave "E-Scientific", Petri dish and glassware.

2.2 Research method

The method used in this research was an experimental method that was performed in two-stage experiments. In the first stage of research, the treatment used was different concentrations of mangrove *B. gymnoryza* leaves extract from 5, 10, 15, 20, and 25 mg/mL against *S. mutans* and *S. viridans*. In the second stage of research, the experiment was done by adding the extract with concentrations of 2 MIC, 3 MIC, 4 MIC, 5 MIC into chewing gum and determining its inhibition towards *S. mutans* and *S. viridans*. The experimental design used was Randomized Full Factorial Design with 4 replications. Parameters observed were antibacterial activity in terms of inhibition zone and Minimum Inhibitory Concentration (MIC), hedonic organoleptic test, and toxicity of chewing gum. All research data were analyzed using Anova (Analysis of Variance) with Tukey Post Hoc test using SPSS Program, version 20.

2.2.1 Extraction of mangrove Bruguiera gymnorrhyza leaves

Extraction of mangrove *B. gymnorrhyza* leaves was done using the maceration method at room temperature (Adi and Usman, 2017). Initially, mangrove leaves were washed and dried at room temperature until the colour became brownish. The dried samples were reduced in size using a disc mill and sieved with a 60-mesh sieve. Mangrove leaves powder obtained was macerated using an ethanol solvent with a ratio of 1:4 (w/v) for 72 hrs. The macerate was then filtered using filter paper and the filtrate was evaporated using a rotary evaporator at 40°C until the concentrated extract was obtained.

2.2.2 Chewing gum making procedure

MIC value as the basis of chewing gum formulation was obtained from the diameter of inhibition shown in Table 1. The production of chewing gum is carried out

according to the research by Raithore and Peterson (2018) with slight modification. The formulation used can be observed in Table 2.

Table 1. MIC value of *B. gymnorrhyza* leaves extract

Bacteria	MIC (mg/mL)
Streptococcus mutans	1.62
Streptococcus viridians	1.35

Table 2. Formulation of chewing gum making

Ingredients	Treatment			
	2 MIC	3 MIC	4 MIC	5 MIC
Extract (mg/mL)*	3.24	4.86	6.48	8.10
Erythritol (g)	25.00	25.00	25.00	25.00
Glucose(g)	27.00	27.00	27.00	27.00
Xylitol (g)	18.00	18.00	18.00	18.00
Stevia (mL)	0.13	0.13	0.13	0.13
Mannitol (g)	5.00	5.00	5.00	5.00

*MIC value of extract was obtained from 1st stage research

Erythritol gum base powder, glucose syrup, stevia, xylitol, and mangrove *B. gymnorrhyza* leaves extract were placed inside a double boiler (a stacked pan, in which a bottom pan was filled with water to be boiled and a smaller size pan on the top of it). The double boiler was heated with medium heat on a stove and stirred until it became warm and viscous. Erythritol gum base powder, citric acid, and mangrove leave extract were put on top of it and stirred using a spoon. After it cooled down, mannitol was smeared onto both hands and the dough was mixed for about 15 mins. Afterwards, the dough was rolled, cut, and was ready for analysis.

2.2.3 Antibacterial activity assay

Antibacterial activity assay was performed using the well-diffusion method (Bloomfield, 1991; Parhusip and Sitanggang, 2011). Initially, each culture of *S. mutans* and *S. viridans* was rejuvenated in Nutrient Broth for 24 hours at 37°C. About 10 mL of sterile Nutrient Agar (NA) was poured into a Petri dish and left to solidify at room temperature. Each culture (100 μL) was placed onto the surface of the media, aseptically spread thoroughly using a triangle and left for 5 mins. Wells with a diameter of 6 mm were made on the media that have been added with bacteria. Mangrove leaves extract with a volume of 60 μL with concentrations of 5 mg/mL, 10 mg/mL, 15 mg/mL, 20 mg/mL, 25 mg/mL were placed into the wells and incubated at 37°C for 24 hrs. The clear zone formed around the wells was the zone of inhibition and was measured using Vernier calliper. MIC (Minimum Inhibitory Concentration) value was calculated by plotting ln Mo (ln of extract concentration) on the X-axis and square of inhibition diameter on the Y-axis. The intersection of the equation on the X-axis was

the Mt value. MIC value is 0.25 × Mt.

2.2.4 Toxicity assay

Toxicity assay was done using Brine Shrimp Lethality Test (BSLT) method (Meyer *et al.*, 1982; Juniarti *et al.*, 2009; Muaja *et al.*, 2013; Puspitasari *et al.*, 2018). *A. salina* Leach larvae were hatched inside a glass jar by immersing 1 g of larvae into 100 mL of seawater and aerated for 48 hrs. Eggs were then hatched and the larvae (Nauplii) was ready for assay. About 10-12 shrimp larvae were immersed in 100 mL of seawater and extracted with a concentration of 10, 100, 200, 500, and 1000 ppm into each jar. As an addition, 2 drops of DMSO were also added into the jar and incubated for 24 hours. Furthermore, the number of dead larvae on each extract concentration that has been added into the jar was calculated and expressed as a percentage of mortality. Extract toxicity was then calculated using Probit.

2.2.5 Hedonic organoleptic test

A hedonic test is an organoleptic test in which panellists express their preference towards the given attributes. In this test, panellists were asked to determine their level of preference spontaneously without comparing among samples or comparing to standard (Utiarahman *et al.*, 2013). This test is used to determine panellists preference (Koch *et al.*, 2012) towards chewing gum that has been added with mangrove *B. gymnorrhyza* leaves extract in terms of several attributes, such as colour, taste, texture, and overall acceptance. Hedonic test was done using 5 scales (1= dislike extremely, 2 = dislike, 3 = neutral, 4 = like, 5 = like extremely) by 70 panelists.

3. Results and discussion

3.1 Antibacterial activity of mangrove Bruguiera gymnorrhyza leaves extract

Antibacterial activity of *B. gymnorrhyza* leaves extract was measured based on the zone of inhibition and MIC value towards *S. mutans* and *S. viridans*. ANOVA statistical results showed that extract concentration affected the inhibition zone towards *S. mutans* and *S. viridans* ($p<0.05$). Post hoc results using the Tukey test can be observed in Figure 1.

Figure 1 shows that the higher the extract concentration, the larger the zone of inhibition, for both *S. mutans* and *S. viridans*. This indicates that mangrove leaves extract contains antibacterial agent. According to Renaldi *et al.* (2018), strong antibacterial agents found in *Bruguiera* were steroid and triterpenoid. A strong antibacterial power of mangrove *B. gymnorrhyza* leaves extract is shown by an inhibition diameter of 21.4 mm at extract concentration of 25 mg/mL towards *S. viridans*. According to Kusmarwati and Indriati (2008) and Rahman *et al.* (2017), antibacterial activity with inhibition diameter >20-30 mm is categorized as very strong, diameter >10-20 mm is categorized as strong, the diameter of 5-10 mm is considered as moderate, and diameter less than 5 mm is categorized as weak.

Inhibition power of mangrove *B. gymnorrhyza* leaves extract towards *S. viridans* was much stronger compared to *S. mutans*. This might be caused by the difference in the cell wall structure, even though both bacteria are Gram-positive bacteria. However, the inhibition diameter of mangrove *B. gymnorrhyza* leaves extract towards *S. mutans* (6.76 mm per 25 mg/mL or 2.5 g/100 mL extract), is stronger than the angsana leaves extract (12.95 mm per 50% or 50 g/100 mL extract) (Asfrizal and Masyudi, 2018). Inhibition diameter of mangrove *B. gymnorrhyza* leaves extract towards *S. viridans* is also much larger (21.4 mm per 25 mg/mL extract) compared to bitter melon leaves extract with an inhibition diameter of 13.27 mm per 100% (100 g/100 mL) extract (Maharani *et al.,* 2012). Other than concentration, the difference in inhibition diameter is also related to its different bioactive compounds (Suciari *et al.*, 2017; Rahman *et al.,* 2017).

Comparison of antibacterial power based on inhibition diameter might be difficult because it depends on the extract concentration used. Extract concentrations used are often not equal between those being compared. Therefore, a better comparison that can be used might be MIC and MBC (Minimum Bactericidal Concentration)
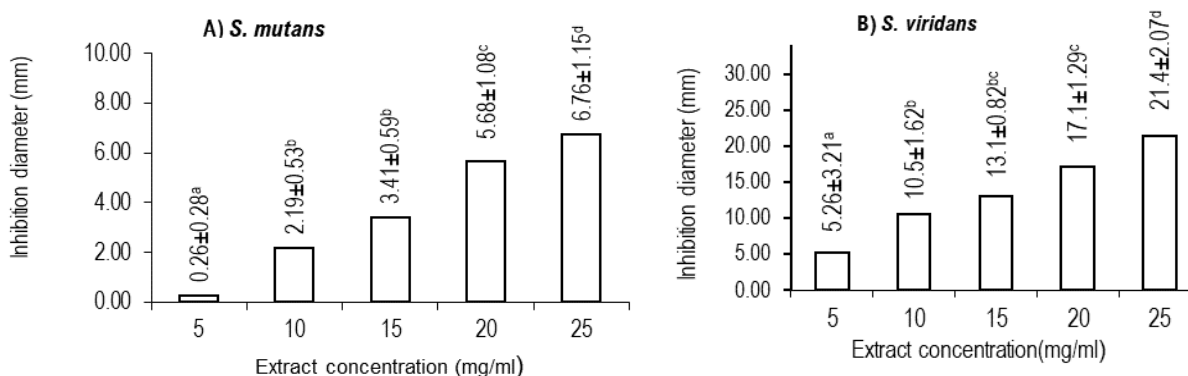

Figure 1. Inhibition diameter of *B. gymnorrhyza* leaves extract towards (A) *Streptococcus mutans* dan *S. viridans*.

value. Moreover, the antibacterial agent can be categorized as bacteriostatic or bactericidal depending on concentration per certain volume (Fitrial, 2011; Roihanah *et al.*, 2012). MIC results of mangrove *B. gymnorrhyza* leave extract can be seen in Table 1.

MIC values of mangrove *B. gymnorrhyza* leave sextract towards *S. mutans* and *S. viridans* is in accordance with their inhibition diameter. The larger the inhibition diameter, the lower the MIC value. MIC value of mangrove *B. gymnorrhyza* leaves extract towards *S. mutans* is higher compared to noni extract with MIC value of 0.375 mg/mL (Rifdayani *et al.,* 2014). MIC value of mangrove *B. gymnorrhyza* leaves extract towards *S. viridans* is also higher compared to bay leaf extract, with an MIC value of 0.12 mg/mL (Kurnia, 2018), but lower than betel leaf water extract, with MIC value of 15% (15 g/100 mL) (Owu *et al.,* 2020). The difference in MIC value or antimicrobial properties is influenced by the concentration of active compounds and properties of microorganisms (Fitrial, 2011). Even though the MIC value of mangrove *B. gymnorrhyza* leaves extract is higher compared to noni extract and bay leaf extract, it is still categorized as strong to inhibit dental caries causing bacteria. Thus, it has the potential to be applied to chewing gum.

3.2 Antibacterial activity of mangrove Bruguiera gymnorrhyza leaves extract chewing gum

According to Hidayati *et al.* (2014), chewing gum could stimulate the secretion of saliva, increase pH of plaque and saliva, making it suitable to be used as a cleanser for the oral cavity. Consumption of chewing gum that contains xylitol about 3 to 5 times a day by chewing it for at least 5 mins after eating could inhibit plaque accumulation and enamel demineralization, increase remineralization at the early stage of caries, and reduce the number of *S. mutans*.

ANOVA statistical results of inhibition diameter showed that mangrove *B. gymnorrhyza* leaves extract chewing gum affected the inhibition zone ($p<0.05$) towards *S. mutans* and *S. viridans*. Post hoc results using the Tukey test can be observed in Figure 2.

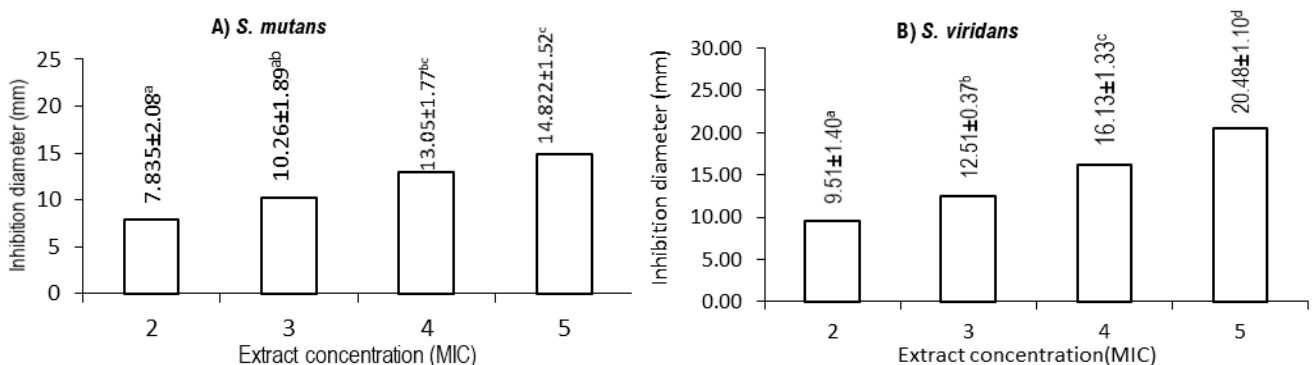Inhibition power of chewing gum that is added with mangrove *B. gymnorrhyza* leaves extract shows the same behaviour with its extract, in which higher extract concentration results in a larger inhibition zone. Based on its dosage or concentracus and its inhibition diameter, chewing gum has a better inhibition compared to the extract itself. Inhibition diameter of chewing gum added with 5 MIC extract concentration (5×1.62 mg/mL = 8.1 mg/mL) towards *S. viridans* was 20.48 mm, almost similar to that of extract concentration of 25 mg/mL which reached 21.40 mm. This result indicates that there is an interaction between extract and other ingredients of chewing gum, such as xylitol. It was also mentioned by Hidayati *et al.* (2014), chewing gum with xylitol could inhibit plaque accumulation and enamel demineralization, stimulate remineralization in the early stage of dental caries, and decrease the number of *S. mutans*. Inhibition power of chewing gum added with mangrove *B. gymnorrhyza* leaves extract is still stronger compared to chewing gum added with 75% xylitol which has an inhibition diameter of 9.68 mm towards *S. mutans* and 10.73 mm towards *S. viridans* (Kusumaningsari and Handajani, 2011). Based on its inhibition diameter, chewing gum added with mangrove *B. gymnorrhyza* leaves extract has a strong antibacterial activity towards *S. mutans* (with inhibition diameter of 14.82 mm at 5 MIC or 8.1 mg/mL) and a very strong antibacterial activity towards *S. viridans* (20.48 mm at 5 MIC). The inhibition diameter of this chewing gum is also larger compared to 500 mg/mL fingerroot extract which has an inhibition diameter of 8.54 mm towards *S. mutans* (Mahmudah and Atun, 2017) and 75% cherry leaves extract which has an inhibition diameter of 9.93 mm towards *S. viridans* (Sulaiman *et al.,* 2017). According to Mahardika *et al.* (2014), the difference in inhibition diameter at each concentration is caused by the different amounts of active compounds present in a certain concentration. A higher concentration of extract has a higher amount of active compound, therefore, the inhibition diameter formed would also be different at each concentration (Doughari, 2012).

Figure 2. Inhibition diameter of *B. gymnorrhyza* leaves extract chewing gum towards (A) *Streptococcus mutans* dan *S. viridans*.

3.3 Hedonic organoleptic results of chewing gum

An organoleptic hedonic test was done to determine the consumer's preference towards chewing gum that was added with mangrove *B. gymnorrhyza* leaves extract. This result can be seen in Table 3. The preference level towards the colour of chewing gum was not different up to 3 MIC concentrations but decreased at 4 MIC and 5 MIC. This decrease might be caused by the colour of the extract which was dark green which made the colour of chewing gum became darker too. However, up to 3 MIC extract addition, chewing gum was still preferred by panellists. Colour change is also related to different concentrations of mangrove extract added (Paputungan *et al*., 2014). Colour is important because colour plays a significant role in a food's appearance. Although a portion of food is delicious, if it has an unappealing appearance when served, it could decrease a person's appetite to consume it (Soeparno, 2005). The higher the concentration of extract, the colour of a food product will be more similar to the colour characteristics from the extract used.

Table 3. Hedonic value of *B. gymnorrhyza* leaves extract chewing gum

Extract concentration	Hedonic score			
	Colour	Taste	Texture	Overall
2 MIC	3.5±0.0[a]	3.2±0.3[a]	3.2±0.7[a]	3.4±0.0[a]
3 MIC	4.0±0.4[a]	4±0.5[a]	3.0±0.0[a]	4.0±0.5[b]
4 MIC	3.0±0.4[b]	2.9±0.5[b]	2.9±0.9[a]	3.2±0.3[c]
5 MIC	2.9±0.5[b]	2.8±0.4[b]	2.9±0.8[a]	3.1±0.5[c]

Values are presented as mean±SD. Values with different superscript within the same column are significantly different (p<0.05).

In terms of taste, the hedonic test showed a similar pattern of preference, in which up to 3 MIC extract was added into chewing gum, the preference is similar, but after 4 MIC, the preference towards chewing gum's taste decreases. A decrease of preference towards chewing gum is related to the astringent taste of extract, caused by high tannin content in mangrove plants (Hardoko *et al*., 2015). Taste is an important parameter to determine acceptance of a certain product. Even though a food product is highly nutritious, if it has an unwanted taste, it will be rejected by the panellists (Winarno, 2002). Moreover, astringency from extract could decrease the sweet taste of chewing gum (Palupi and Widyaningsih, 2015; Pujilestari and Agustin, 2017).

On the other hand, panellists' preference towards the texture of chewing gum was not significantly different, which means that extract addition did not affect the texture of chewing gum. The texture of chewing gum is more influenced by the type and amount of gum base and sucrose added, meanwhile hard candy is more influenced by sucrose crystal formation (Jalasena and Anjani, 2016), resulting from mixing sucrose and glucose syrup (Ward and Courts, 1977). Other factors that affect the texture of candy are the addition of gelatin, water, citric acid, heating process, and all ingredients should be added in the same amount at the same time and temperature (Riyawan *et al*., 2016).

The overall acceptance parameter is used in the hedonic test to determine and measure panellists' preference towards the whole attributes in a product (Gustiari, 2009). In terms of overall acceptance, chewing gum has increasing preference up to 3 MIC extract addition, then decreasing at a higher concentration of extract added. It was suspected that overall acceptance is also related to the taste and colour of chewing gum. The taste of chewing gum is related to the astringent taste from the tannin in mangrove *B. gymnorrhyza* extract. The dark green colour of chewing gum could be influenced by chlorophyll colour and brown colour from carotenoids and other pigments. A combination of those colours creates a dark colour (Ridlo *et al*., 2017).

3.3 The selected treatment and chewing gum toxicity

Although chewing gum in this research was intended to inhibit dental caries causing bacteria, inhibition power cannot be a determining factor, but acceptance or preference level was the determining factor to select the best treatment. Chewing gum with the highest preference was the one added with 3 MIC mangrove *B. gymnorrhyza* leaves extract (4.86 mg/mL).

On the selected chewing gum, a toxicity assay was done using BSLT to obtain LC_{50} value. Based on BSLT method, a sample is considered toxic if it has LC_{50} less than 100 mg/mL (Meyer *et al*., 1982). LC_{50} of chewing gum added with 3 MIC of mangrove *B. gymnorrhyza* leaves extract was 10502 mg/mL, therefore it is considered non-toxic.

4. Conclusion

Inhibition of mangrove *B. gymnorrhyza* leaves extract towards dental caries causing bacteria is categorized as strong with MIC value of 1.62 mg/mL towards *S. mutans* and 1.35 mg/mL towards S. *viridans.* The selected and most preferred addition of mangrove *B. gymnorrhyza* leaves extract in chewing gum is at 3 MIC concentration (4.86 mg/mL) at a preference level of 4.0 (out of 5.0) and inhibition diameter towards *S. mutans* of 10.26 mm and *S. viridans* of 12.51 mm, with LC_{50} value of 10502 mg/mL.

Conflict of interest

We declare no conflict of interest with the data contained in this manuscript. 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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