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Research Article

Formulation of Chewing Candy from Black Mulberry Extract (*Morus nigra* L.) as Anticaries Drug

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ABSTRACT

Objective: This study aims to evaluate the antibacterial activity of black mulberry extract (*Morus nigra* L.) in a chewing candy preparation against *Streptococcus mutans* (S *mutans*) and *Streptococcus sanguis (S sanguis)*. Methods: The antibacterial activity of the extract was determined by disc diffusion method. The extract dose was determined from minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values using the microdilution method. The extracts were formulated into three variations of the glucose-sucrose base: F1 (43.5%:8.7%), F2 (34.78%:8.7%), and F3 (26%:26%). The chewing candy of black mulberry extract was evaluated physically, including organoleptic, preference tests and antibacterial activity test against *S mutans* and *S sanguis*. Result: The results show that black mulberry extract has antibacterial activity with MIC 0.3125% and MBC 0.625% against *S mutans* and *S sanguis*. The best formulation of chewing candy consisted 26% w/w of sucrose, 26% w/w of glucose and 0.625% w/w of black mulberry extract. The chewing candy from black mulberry fruit extract has antibacterial activity with an 8.2 ± 0.269 mm inhibition zone against *S mutans* and one of 10.8 ± 0.878 mm against *S sanguis*. Conclusion: The chewing candy consisting of 26% w/w of sucrose, 26% w/w of glucose and 0.625% w/w of black mulberry extract has antibacterial activity against *S mutans* and *S sanguis*.

Keywords: *Morus nigra* L., chewing candy, *Streptococcus mutans, Streptococcus sanguis.*

INTRODUCTION

Medical treatment is a curative step to reduce dental and oral problems. According to a previous study, only 8.1% of the Indonesian population can afford medical dental care, and the less dental care people get, the more active caries they will have. There are more than 600 types of bacteria live in the oral cavity and saliva. The species of bacteria that caused dental caries are *S mutans* and *S sanguis*[1]. Dental caries begin with the adhesion process on the tooth surface, which causes dental plaque[2]. *Streptococcus* can produce acid on the tooth surface that contains extracellular polysaccharides (EPS), which play an active role in the cariogenic nature of bacteria[3].

Exploration of various dosage forms such as chewing candy is important to overcome oral disorders. Chewing candy has a chewy texture and longer contact time with the oral cavity[4].

Black mulberry (*Morus nigra* L.) is a highly watery plant containing phenolic compounds[5]. The content of phenolic compounds such as flavonoids has potential antibacterial activity. Souza et al. showed that mulberry has antibacterial activity against several microorganisms such as *Bacillus cereus, Escherichia coli, Escherichia faecalis, Salmonella choleraesuis,* and *Serratia marcescens*[6]. Another study showed that n-hexane, ethanol, and ethyl acetate extracts of mulberry fruit have antibacterial activity on *S mutans* and *S sanguis*[7]. All parts of the black mulberry plant contain phenolic compounds, especially the fruit, leaves, and roots[8].

The aim of this research is to evaluate the antibacterial activity of black mulberry fruit extracts against *S mutans* and *S sanguis* in a preparation of chewing candy as an alternative anticaries drug.

MATERIALS AND METHODS

Materials

The black mulberry fruit that was used in this study was obtained from plantations in Cibodas, Maribaya-Lembang.

Extraction

Black mulberry was dried in an oven at a temperature of 50 °C and extracted by maceration using 96% solvent ethanol for 24 h at room temperature. The extract was dried using a rotatory evaporator at 50 °C[9].

Black Mulberries Extract Phytochemical Screening

The ethanolic extracts of black mulberry (*M. nigra*) were screened for finding the presence of secondary metabolites such as tannins, alkaloids, flavonoids, terpenoids, steroids, and saponins[9].

Antibacterial activity of the extract

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Table 1: Formulation of chewing candy.

Composition	Function	F1 (g)	F2 (g)	F3 (g)
Sucrose	Candy Base	100	80	60
Syrup	Candy Base	20	40	60
Heavy cream	Thickener	100	100	100
Soy lecithin	Stabilizer	10	10	10
Essence	Color and flavor	qs	qs	qs
Distilled water	Solvent	50 ml	50 ml	50 ml

Table 2: Antibacterial activity of black mulberry fruit extract against *Streptococcus mutans*.

Extract concentration (% b/v)	Inhibitory zone (mm)			Mean of inhibitory zone (mm)
	1	2	3	
10	0	0	0	0
20	9.2	9.4	9.6	9.4 ± 0.2
40	8.0	8.9	8.79	8.56 ± 0.49
80	13.01	12.4	13.8	13.07 ± 2.28

Table 3: Antibacterial activity of black mulberry fruit extract against *Streptococcus sanguis*.

Extract concentration (% b/v)	Inhibitory zone (mm)			Mean of inhibitory zone (mm)
	1	2	3	
10	9.5	8.95	9.11	9.19 ± 0.28
20	11.75	11.5	11.22	11.49 ± 0.265
40	13.34	13.1	13.13	13.19 ± 0.13
80	15.1	14.76	14.64	14.83 ± 0.23

An antibacterial activity test has been carried out using the disc diffusion method (Kirby Bauer). Mueller Hinton Agar (MHA) was mixed with 5% sterile sheep blood as a solid bacteria growth medium[10]. *S mutans* and *S sanguis* were dissolved separately in 0.5 McFarland of Tryptic Soya Broth (TSB)[11]. The dissolved bacteria were poured into solid media, then added by various concentrations of extract (80%; 40%; 20%; and 10%). Disc paper (6×10^{-3} m) was soaked in each concentration for 2 h. Then, dry disc paper was placed on solid media and incubated for about 24 h at 37 °C[12].

Determination of MIC and MBC

The minimum concentration of the inhibitory zone was diluted 10 times in a micro well. 10 µL of suspended bacteria were adjusted to 0.5 McFarland standards, dropped into each extract concentration, and incubated for 24 h at 37 °C[13]. Each concentration was subcultured on solid medium to determine the dose.

Formulation of chewing candy

Three variations of candy base (Table 1) were made with the following ratios of main ingredients sucrose to glucose: F1 (43.5%:8.7%), F2 (34.78%:8.7%), and F3 (26%:26%). Heavy cream was melted at 50 °C. Soy lecithin was dissolved in warm water, then mixed with cream. Glucose and sucrose were dissolved in 50 mL of water or liquid milk. After the base was formed, the temperature was increased to 120 °C. The heating temperature was controlled using a thermometer[14].*Antibacterial activity of chewing candy*

The antibacterial activity of chewing candy was tested by the disc diffusion method. The candy was dissolved in warm water, then disc paper was soaked in a solution for 2 h. Paper discs were dried in laminar air flow and placed on blood-MHA media, onto which 20 µL of bacterial suspension had been poured. The media was incubated at 37 °C for 24 h[15].

Table 4: Subculture of MIC and MBC against *Streptococcus mutans*.

Extract concentration (%b/v)	The growth of *Streptococcus mutans*.		
	1	2	3
20	-	-	-
10	-	-	-
5	-	-	-
2.5	-	-	-
1.25	-	-	-
0.625	-	-	-
0.3125	+	+	+
0.156	+	+	+
0.078	+	+	+
Medium control (TSB)	-	-	-
Positive control	+	+	+
Negative control	-	-	-

Evaluation and preference test

The quality of the base was observed on days 0, 1, 3, 7, 10, and 14. The parameters of observation were color, odor, taste, consistency, and homogeneity. A preference test was carried out on 21 panelists. All panelists rated the parameters of preference, i.e., smell, color, softness level, and aftertaste.

RESULTS AND DISCUSSION

Maceration was used to protect the compounds from degradation, especially the thermolabile compounds such as anthocyanin, contained in black mulberry fruit. Ethanol was used as a solvent because it can dissolve polar and nonpolar compounds, and it is safe for topical and oral application[9]. A confirmation test was needed to find out

Table 5: Subculture of MIC and MBC against *Streptococcus sanguis.*

Extract concentration (%b/v)	The growth of *Streptococcus sanguis*		
	1	2	3
10	-	-	-
5	-	-	-
2.5	-	-	-
1.25	-	-	-
0.625	-	-	-
0.3125	+	+	+
0.156	+	+	+
0.078	+	+	+
0.039	+	+	+
Medium control (TSB)	-	-	-
Positive control	+	+	+
Negative control	-	-	-

whether the black mulberry extract was still in good condition. The results show that total water-soluble content was 74.51% and total ethanol-soluble content was 78.80%. These values indicate that the extract had high solubility in two types of polar solvents. This occurred because black mulberry extract consists of a high number of hydroxyl and glycoside groups, thus increasing its polarity. The higher the concentration of dissolved extract, the greater the number of phenolic compounds in the extract and the The concentration of extract for *Streptococcus mutans* was 20%, while for *Streptococcus sanguis*, it was 10%. The MIC value for both bacteria was 0.3125%. The MBC value for both bacteria was 0.625%. Thus, the MIC–MBC range for both bacteria was 0.3125–0.625% (Tables 4 and 5).

The antibacterial activity of the chewing candy was tested using a 0.625% extract dose. Extracts were mixed in three formulas to see which formulation was most suitable for

higher the antioxidant activity[16,17]. All parameters, such as drying losses, moisture content, total ash content, and pH, indicated the compatibility of the extract. It can be concluded that the extract was suitable for use. Phytochemical screening was conducted to find the secondary metabolite contained in the ethanol black mulberry extract. The result of phytochemical screening was that black mulberry extract contained flavonoid, saponin, tannin, polyphenol, and mono sesquiterpene. Flavonoids and terpenoids were the most potent antimicrobials, more so than arylbenzofuran[18]. The phenolic compounds in mulberry extract are believed to denature bacterial cell proteins and inhibit the metabolism of the bacterial cell. Additionally, flavonoids and tannins destroy bacterial cell walls[19].

The results show that black mulberry extract had better antibacterial activity against *Streptococcus sanguis* (Tables 2 and 3). The different inhibitory activity between the two bacteria might have been caused by *Streptococcus mutans*' higher level of virulence. *Streptococcus mutans* is a dominant oral flora. This dominance happens because it directly metabolizes sugar substrate inside the mouth, while *Streptococcus sanguis* has the opposite mechanism for metabolizing sugar, even though both are correlated with dental caries[20]. *Streptococcus mutans* can produce three glucosyltransferases from sucrose, but *Streptococcus sanguis* only produces one[21].

supporting the antibacterial activity of the extract. The test showed that only F3 (26% sucrose, 26% glucose, 0.625% extract) had antibacterial activity. This is because F3 has less disaccharide composition (sucrose) than F1 and F2. It was known that one of *Streptococcus*'s virulence-increasing factors is the availability of sucrose; thus, when the formulation contains more sucrose, *Streptococcus*'s virulence is increased[21].

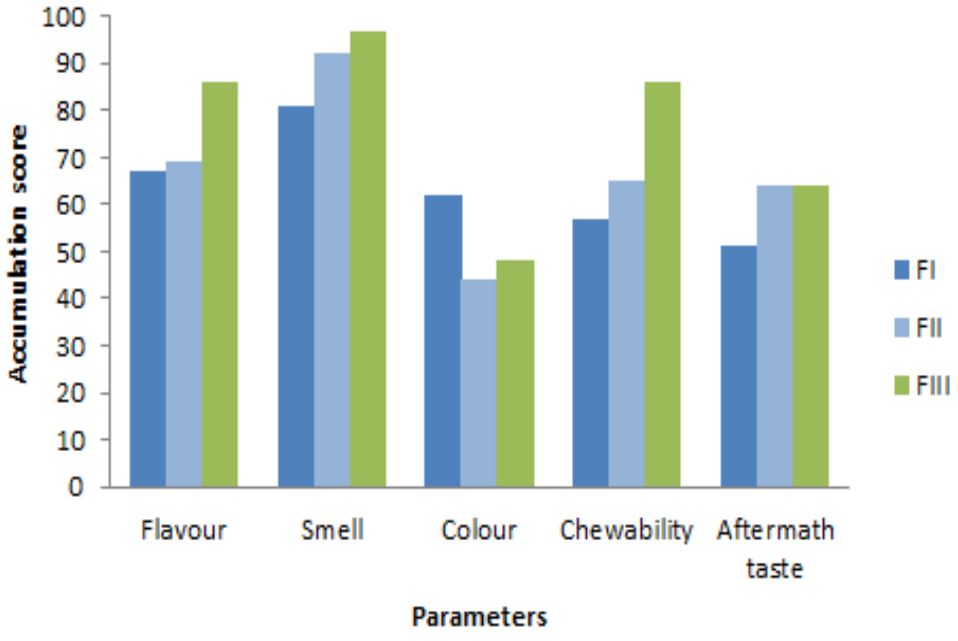

Figure 1: Preference score of the formulation

Table 6: Antibacterial activity of chewing candy against.

Formula	*Streptococcus mutans*		*Streptococcus sanguis*	
	Inhibitory zone	Diameter (mm)	Inhibitory zone	Diameter (mm)
F1	No	-	No	-
F2	No	-	No	-
F3	Yes	8.2 ± 0.269	Yes	10.8 ± 0.878

Disaccharides are the main constituents in bacterial metabolism. Oral bacteria use disaccharides to develop their ability to invade the surfaces of teeth. The maximal potential of the extract resulted from the lower sucrose content in the formula. F1 and F2 contain more sucrose, leading to the lower activity of the extract. The inhibitory zones of both bacteria show that chewing candy worked more effectively on *Streptococcus sanguis* than on *Streptococcus mutans* (Table 6). In addition to activity testing, all three formulations were tested for 14 days to determine the best formulation profiles. Test results on days 0, 1, 3, 7, 10, and 14 show that F3 had the best profile, with few changes during the testing period.

The activity of chewing candy was suspected due to an increase in the antibacterial compound penetration that diffuses into the test media. Tannin in black mulberry extract can disrupt the formation of bacterial cell wall and can be lysis[9,19]. Polyphenols in the extract can bind to surface components rather than entering the cells and inhibit extracellular. Polyphenols also bind metal ions and the reduction of metal ions by complexation with polyphenols may inhibit growth of bacterial. The extracts in chewing candy also inhibited adhesion of S. mutans with inhibited glucosyltransferase activity and inhibit glycan formation as has been shown for specific polyphenols. The chewing gum from mulberry extract may help in preventing plaque formation and inhibition of acid production may reduce damage to teeth by pre-formed plaque. Inhibition of S. mutans occurred in the presence of both sucrose and glucose, two of the main sugar components used in chewing candy and acid production was inhibited[22].

Organoleptic observation was done to all the formulations and resulting consistency profiles. F1 and F2 had white color, while F3 had a yellowish color. The greater amounts of sucrose in F1 and F2 did not exceed the saturation point, while F3 was yellowish due to a balanced mixture between liquid glucose monosaccharide and sucrose disaccharides to pass the saturation point and cause caramelization and chewiness. All dosage formulations showed good sweetness and homogeneity due to the heating process, which caused all ingredients to be evenly distributed.

CONCLUSION

The black mulberry extract has antibacterial activity with MIC 0.3125% and MBC 0.625% against *Streptococcus mutans* and *Streptococcus sanguis*. The best formulation of chewing candy consisted 26% w/w of sucrose, 26% w/w of glucose and 0.625% w/w of black mulberry extract. The chewing candy of black mulberry fruit extract has antibacterial activity with an 8.2 ± 0.269 mm inhibition zone against *Streptococcus mutans* and one of 10.8 ± 0.878 mm against *Streptococcus sanguis*.

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