

**Formulation and Antibacterial Activities of Chewable Lozenges of Celery  
(*Apium graveolens* L.) Leaf Ethanolic Extract against Dental Caries  
Causing *Streptococcus mutans***

**Formulasi dan Uji Anti Bakteri *Chewable Lozenges* Ekstrak Etanolik Daun  
Seledri (*Apium graveolens* L.) Terhadap *Streptococcus mutans* Pada Karies Gigi**

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**ABSTRACT**

Celery (*Apium graveolens* L.) is an herbal plant that has the chemical content flavonoid, saponin and tannin. The antibacterial activity of them have been investigated for many years. However, the antibacterial activity has not been evaluated in oral formulations. This study aims to formulate chewable lozenges celery leaf ethanolic extract, evaluate physical characterized and antibacterial activity of chewable lozenges. Celery leaf ethanolic extract is formulated into chewable lozenges using molded method with concentration of 20 g and 25 g. This formulation was characterized for organoleptic test, weigh uniformity, dissolution testing, thickness testing and pH determination. Antibacterial activity of chewable lozenges was carried out by diffusion method using *Streptococcus mutans*. From the *chewable lozenges* formula there are no significant physical differences between formulas and can be organoleptically acceptable. The results of the antibacterial activity test showed the value of *inhibition zone diameters* of *Streptococcus mutans* bacteria in F1, F2, F3, F4, F6 respectively 8,8 mm, 9 mm, 8,16 mm, 9,33 mm, 8 mm dan 9,13 mm. Formulation F4 shown better antibacterial activity as compared to its other formulations.

**Keywords:** antibacterial, *Apium graveolens* L., chewable lozenges, dental caries, *Streptococcus mutans*

## ABSTRAK

*Daun seledri (Apium graveolens L.) merupakan tanaman yang mengandung flavonoid, saponin dan tannin. Tanaman ini telah banyak diteliti sebagai anti bakteri. Namun, belum banyak penelitian mengenai aktivitas anti bakteri daun seledri dalam formulasi oral. Penelitian ini bertujuan untuk mengetahui formulasi chewable lozenges, mengevaluasi karakteristik fisik dan aktivitas anti bakteri chewable lozenges dari ekstrak etanol daun seledri. Chewable lozenges dibuat menggunakan metode peleburan (molded method) dengan konsentrasi ekstrak 20 g dan 25 g. Uji karakteristik fisik yang dilakukan meliputi uji organoleptik, keseragaman bobot, uji disolusi, uji ketebalan dan uji pH. Aktivitas antibakteri chewable lozenges dilakukan menggunakan bakteri Streptococcus mutans dengan metode difusi. Berdasarkan hasil penelitian diketahui bahwa tidak ada perbedaan yang signifikan antar formula dan dapat diterima secara organoleptik. Hasil uji aktivitas antibakteri menunjukkan nilai diameter zona hambat bakteri Streptococcus mutans pada F1, F2, F3, F4, F6 masing-masing 8,8 mm, 9 mm, 8,16 mm, 9,33 mm, 8 mm dan 9,13 mm. Formulasi F4 menunjukkan aktivitas antibakteri yang lebih baik dibandingkan formulasi lainnya.*

**Kata kunci:** antibakteri, *Apium graveolens* L., chewable lozenges, karies gigi, *Streptococcus mutans*

## Introduction

Dental caries is a dental tissue disease characterized by tissue damage tooth surface, enamel, and dentin, which spread to the pulp (Andini et al., 2018). It can be caused by bacteria that live in the mouth. *Streptococcus mutan* is a bacterium that can live in the tooth surface. That bacteria can make a complex biofilm commonly called "dental plaque" (Nbaia et al., 2018).

Dental caries is also a major public health problem in the world and is the most widespread non-communicable disease (WHO, 2017). Based on Riskesdas 2018, around 57.6% of Indonesia's population have oral and dental problems. Almost 93% of children aged 5-6 years old have dental caries. Caries can damage

children's enamel more quickly (Nbaia et al., 2018). This disease has a great impact on an individual's quality of life.

Celery leaf extract is known to have antibacterial activity against *Streptococcus mutans* at a concentration of 12.5% as it is the lowest concentration that still has an antibacterial effect (Majidah et al., 2014). Based on the results of phytochemical tests, the ethanol extract of celery leaf contains saponins, tannins and flavonoids (Khasanah, 2017).

Chewable lozenges are one drug delivery system for local action, especially in the mouth. They are safe and easy to use, can increase saliva production and do not disturb the balance of normal flora in the oral cavity. For that reason, this study aims

to formulate chewable lozenges of celery leaf ethanolic extract (*Apium graveolens* L.), evaluate physical characterized for organoleptic test, weigh uniformity, do dissolution and thickness testing and measure pH determination and antibacterial activity of chewable lozenges.

## Research Methods

### Materials

Celery leaf simplicia was purchased from Herba Anugerah Alam, Bantul, Yogyakarta. Chewable lozenges formulation used ethanol 96%, gelatin, sorbitol, *aquadest*, natrium propionate, and *Peppermintt oil*. Antibacterial activity used *Streptococcus mutans*, Nutrient Agar (NA), Brain Heart Infusion (BHI), butanol, acetic acid, Pb acetic 10%, NaOH 20%, FeCl<sub>3</sub>, HCL 1%, DMSO 10%.

### Experiments

#### 1. Extraction

The celery leaf extract was made using the maceration method. Maceration was carried out using ethanol 96% with a ratio of 1: 5 w/v.

#### 2. Phytochemical screening

Phytochemical screening carried out on ethanolic includes examining the chemical secondary metabolites of flavonoids, saponin and tannins.

#### 3. Formulation of chewable lozenges

All materials were weighed. Aquadest was poured into gelatin, let stand for 10-15 minutes, and

then heated at a temperature of 65°C on a water bath. Sorbitol was added to the solution, stirred, and heated for 15 minutes until it dissolved. After that, aspartame was added and stirred until it dissolved. The thick extract of celery leaves was added and heated for 5 minutes. Sodium propionate and peppermint oil were added to the solution and then poured into chewable lozenges molds.

#### 4. Physical characterized of chewable lozenges

Chewable lozenges were evaluated for organoleptic test, weigh uniformity, homogeneity and pH.

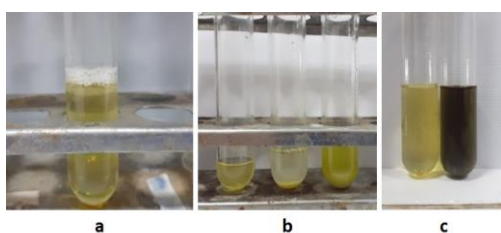
#### 5. Antibacterial activity

The antibacterial activity was tested by diffusion method against *Streptococcus mutans*. The method of antibacterial was assayed using the standard procedure assay. Antibacterial activity was identified by measuring zone of inhibition. They were divided into 6 group. Group I (positive control) was given mouthwash that contains of *chlorhexidine* 0,2%, group II (negative control) was given *chewable lozenges* without extract. Groups III, IV, V and VI was given chewable lozenges respectively for F1, F2, F3, F4, F5 and F6.

## Results and Discussion

Phytochemical screening of celery leaf ethanolic extract are presented in Figure 1 and Table 2. The

preliminary test results of phytochemical screening showed that the ethanol extract contained flavonoids, tannins and saponins. The compound was celery leaf extract which has antibacterial activity (Latifa et al., 2019).



**Figure 1.** (a) saponin, (b) flavonoids, (c) tannin

Based on the results of the organoleptic test, the resulting chewable lozenges was dark green in color. This is due to the dark color of the celery extract used so that it affects the color and brightness of the final product of chewable lozenges. The six chewable lozenges have the distinctive smell of celery mixed with peppermint oil, there

is no difference in aroma from the six formulas made. The addition of peppermint oil aims to cover the smell of celery. From the observations, the distinctive aroma of celery can be masked by the use of peppermint oil.

The chewable lozenges were tested for physical characteristics including organoleptic, pH, and weigh uniformity. The results of physical characteristics can be shown in Table 3. The pH value of an oral preparation greatly affects the growth of microorganisms. The pH of saliva is normally between 5.6-7.0 with an average of 6.7 (Soesilo et. al., 2006). The optimum pH of saliva for the growth of most microorganisms, especially bacteria, is around 6.5-7.5 (Soesilo et. al., 2016).

The pH value of chewable lozenges tested in this study was in the range of 5.49 - 5.75. This value is in the pH range of chewable lozenges according to Singh (2010) which is at pH 5-6.

**Table 1.** Design formulation of chewable lozenges

Materials (g)	Formulation of chewable lozenges					
	F1	F2	F3	F4	F5	F6
Celery extract	20	25	20	25	20	25
Gelatin	30	30	40	40	50	50
Sorbitol	13	13	17	17	20	20
Aspofam	0,1	0,1	0,1	0,1	0,1	0,1
Natrium propionate	0,2	0,2	0,2	0,2	0,2	0,2
Peppermint oil	0,1	0,1	0,1	0,1	0,1	0,1
Aquades	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100

**Table 1.** Phytochemical screening of ethanolic extract

Parameters	Identification	Result
Flavonoid	Precipitate, yellow	Positive
Saponin	Foam phase	Positive
Tannin	Dark green	Positive

The results of the evaluation show that the six chewable lozenges formulas meet the weight uniformity requirements. The requirements for the uniformity of the weight of chewable lozenges to the average weight of each formula refer to the Indonesian Pharmacopoeia.

Based on the results of the study, the chewable lozenges' preparation of celery leaf ethanol extract had antibacterial activity. According to table 4, the highest average value of antibacterial activity was observed for the positive control group 17.33 mm, followed by chewable lozenges F4 which was 9.33 mm with a sig value ( $p < 0.5$ ) as compared to the negative control group. Further, this value was significantly different from those for negative control and all of formula with  $p$  value 0,000 ( $p < 0,05$ ).

The higher the extract concentration, the larger the diameter of the inhibition zone (see F1 and F2, F3 and F4 and F5 and F6). Formula 1, 3 and 5 used the ethanol extract concentration of celery of 20%, while formula 2, 4 and 6 used the ethanol extract of celery with a concentration of 25%.

Chlorhexidine (CHX) has been commonly used as antiseptic agent for

dental practice since 1970, due to its long-lasting antibacterial activity with a broad-spectrum of action (Bescos et al., 2020). However, the long-term use of chlorhexidine cause side effect including brownish discoloration of teeth, restoration and tongue (Sajjan et al., 2016).

The ethanol extract of celery contained flavonoids, tannins and saponins as antibacterial compounds. Flavonoid compounds have 3 mechanisms as anti-bacterial: first, inhibiting nucleic acid synthesis in bacteria; second, inhibiting the function of the cytoplasmic membrane by reducing the fluidity of the membrane in the hydrophilic and hydrophobic regions so that the fluidity of the outer layer and the inner layer of the membrane decreases; and third, inhibiting energy metabolism. Flavonoids also have the ability to act as anti-glucosyltransferase (Vasconcelos et al, 2006).

Saponin compounds are believed to have antibacterial activity by interacting with cholesterol on the cell membrane and causing the cell membrane to undergo lipid modification, so that it interferes with the interaction of bacteria with the modified membrane. The disruption of these interactions disrupts the ability of bacteria to damage or interact with the host so that antibacterial substances will easily enter bacterial cells and interfere with metabolism, which ultimately leads to bacterial death. (Karlina et al, 2013).

**Table 3.** Physical characterized of chewable lozenges

Test	Formulation code					
	F1	F2	F3	F4	F5	F6
<b>Form</b>	Semi solid	Semi solid	Semi solid	Semi solid	Semi solid	Semi solid
<b>Smell</b>	Celery and peppermint oil	Celery and peppermint oil	Celery and peppermint oil	Celery and peppermint oil	Celery and peppermint oil	Celery and peppermint oil
<b>Color</b>	Dark greenish	Dark greenish	Dark greenish	Dark greenish	Dark greenish	Dark greenish
<b>pH</b>	5.52 ± 0.03	5.59 ± 0.03	5.63 ± 0.02	5.65 ± 0.03	5.69 ± 0.02	5.71 ± 0.04
<b>Weigh</b>	887.75 ±	874 ± 8.70	884.25 ±	886.6 12.46	893.2 ±	898.3 ±
<b>uniformity</b>	9.32		14.79		11.33	24.44

**Table 2.** The inhibition zone diameter against *Streptococcus mutans*

Replication (mm)	Formulation code							
	P	K-	F1	F2	F3	F4	F5	F6
<b>1</b>	18.2	7	9.2	8.2	7.2	9.8	9.2	10.1
<b>2</b>	17.4	6.6	8.8	10	9.1	9.4	7.9	8.1
<b>3</b>	16.4	6.8	8.4	8.8	8.2	8.8	8	9.2
<b>Average</b>	17.3	6.8	8.8	9	8.1	9.3	8	9.1
<b>SD</b>	0.9	0.2	0.4	0.9	0.9	0.5	0.7	1

Note: P = positive control (Chlorhexidine 0,2%), K- = negative control (chewable lozenges formula without celery extract), F1, F3, and F5 = chewable lozenges formula of celery extract at 20%; F2, F4, and F6 = chewable lozenges formula of celery extract at 25%

Besides flavonoids and saponins in the ethanol extract of celery, there was also tannin which has antibacterial activity. The mechanism of tannin as antibacterial can be seen from the bacterial cell membrane, which is the target of its action. According to Vasconcelos et al (2002), tannins are able to pass through cell membranes because they can precipitate on proteins. Tannin is also able to suppress enzymes like glucosyltransferase. In the research of Abdullahzadeh et. al. (2011), tannins are able to bind to lipoteic acid on the cell surface of *Streptococcus mutans* bacteria. This is what supports the antibacterial power of tannin compounds against *Streptococcus mutans* which causes dental caries.

## Conclusion

Chewable lozenges of celery ethanol extract inhibited the growth of *Streptococcus mutans* which causes dental caries.

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