Efek Penghambatan Ekstrak Akuades Daun *Ocimum basilicum* terhadap *Streptococcus mutans* In Vitro

*Inhibitory Effect of Aqueous Extract of Ocimum basilicum Leaves on Streptococcus mutans In Vitro*

Paulus Paksi IL¹, David Sugihartana², Sri Larnani²

¹Program Studi Pendidikan Dokter Gigi Fakultas Kedokteran Gigi Universitas Gadjah Mada Yogyakarta
²Departemen Biomedika Kedokteran Gigi Fakultas Kedokteran Gigi, Universitas Gadjah Mada Yogyakarta

**ABSTRAK**

Daun kemangi (*Ocimum basilicum*) telah diteliti ekstraknya sebagai antibakteri patogen pangan. Penelitian eksperimental antibakteri dari ekstrak daun *Ocimum basilicum* terhadap patogen di rongga mulut, termasuk *Streptococcus mutans*, masih harus diperdalam. Penelitian ini bertujuan untuk mengetahui efek ekstrak daun *Ocimum basilicum* terhadap penghambatan pertumbuhan *Streptococcus mutans*. Daun *Ocimum basilicum* diekstraksi dengan metode maserasi menggunakan akuades steril. Ekstrak diuji penghambatannya terhadap pertumbuhan *Streptococcus mutans* dalam larutan NaCl fisiologis dengan metode dilusi dan spektrofotometri 550nm. Hasil penelitian menunjukkan terdapat pengaruh yang signifikanperlakuan ekstrak daun *Ocimum basilicum* terhadap penghambatan pertumbuhan *Streptococcus mutans* (p<0,05). Ekstrak daun kemangi (*Ocimum basilicum L.*) berpengaruh menghambat pertumbuhan *Streptococcus mutans*.

Kata Kunci: Ekstrak akuades, *Ocimum basilicum*, spektrofotometri, *Streptococcus mutans*

**ABSTRACT**

Basil (*Ocimum basilicum*) leaf extract has been studied as antibacterial food pathogens. An antibacterial experimental study of *Ocimum basilicum* leaf extract against pathogens in oral cavity, including *Streptococcus mutans*, still has to be explored. This study aimed to determine the effect of *Ocimum basilicum* leaf extract on the growth inhibition of *Streptococcus mutans*. *Ocimum basilicum* leaves were extracted by maceration method using sterile distilled water. The extract was tested for inhibition of the growth of *Streptococcus mutans* in physiological NaCl solution by dilution method and 550nm spectrophotometry. The results shows that there is a significant effect of *Ocimum basilicum* leaf extract on the growth inhibition of *Streptococcus mutans* (p <0.05). Basil (*Ocimum basilicum L.*) leaf extract has an effect on inhibiting the growth of *Streptococcus mutans*.

**Keywords**: Aqueous extract, *Ocimum basilicum*, spectrophotometry, *Streptococcus mutans*
PENDAHULUAN

Plants belonging to the Labiatae family have been traditionally used by the Indonesians as food crops and perfumers. One of the Labiatae plants used by the public is basil (Ocimum basilicum). Ocimum basilicum is used by the public for vegetables and body odor prevention (1). Ocimum basilicum leaves traditionally have been efficacious as a cure for diarrhea, dysentery, constipation, and intestinal worms (2).

Various modern studies on Ocimum basilicum leaves which are summarized in a review show that Ocimum basilicum leaves have pharmacological activity, including antibacterial (3). The research results by Ahonkhai et al. showed that the essential oil of Ocimum basilicum leaves made into a pure dosage form, toothpaste, and mouthwash could inhibit bacteria such as Klebsiella pneumonia, Staphylococcus albus, Pseudomonas aeruginosa, Proteus vulgaris, and Streptococcus viridans. Streptococcus viridans is found in gingivitis, periodontitis, and dental caries (4).

Streptococcus mutans belongs to the group of Viridans Streptococcus bacteria which can cause dental caries because it produces acid from carbohydrate fermentation which damages dental minerals (5,6). Research results show that antibacterial aqueous extract from Ocimum basilicum leaves can inhibit the growth of food pathogenic bacteria, namely Pseudomonas sp., Enterobacter sp., and Listeria monocytogenes. The biggest component of basil leaves is a water-soluble polar component (7). The effect of Ocimum basilicum leaf aqueous extract on oral pathogenic bacteria, including Streptococcus mutans, is still unknown. This study aimed to determine the inhibitory effect of Ocimum basilicum leaf extract on the growth of Streptococcus mutans.

Ocimum basilicum leaf extraction

Ocimum basilicum leaves were obtained from Sleman, Yogyakarta. Ocimum basilicum leaf extract was made at the Pharmacy-Biology Laboratory, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta. The extraction method used was maceration by extracting sterile distilled water and shaker 80 revolutions per minute for 24 hours. The extraction result was a thick extract of Ocimum basilicum leaves. The thick extract was then diluted with sterile distilled water to a concentration of 0.9375%, 1.875%, 3.75%, 7.5%, and 15%. The concentrations were chosen based on the concentration of Ocimum basilicum leaf extract with distilled water as a solvent which could inhibit Listeria monocytogenes (7).

Creating Streptococcus mutans Suspension

The suspension concentration of Streptococcus mutans bacteria used in this study was 10^6 colony-forming unit/ml in physiological NaCl and made from stock at the Health Laboratory Center Yogyakarta. The bacteria were cultured on a blood agar plate medium and then incubated for 24 hours at 37 °C. One bacterial colony was taken using an inoculating loop from the medium and then put into a test tube containing physiological NaCl.

Growth Inhibition Test of Streptococcus mutans

The growth inhibition test of Streptococcus mutans using dilution method and 550nm spectrophotometry was carried out at the Microbiology Laboratory and the Integrated Research Laboratory, Faculty of Dentistry, UGM. Sterile Brain Heart Infusion (BHI) broth as much as 3ml was put into six test tubes. The six test tubes were then filled with 1ml of six different solutions, namely Ocimum basilicum leaf extract 0% (as control) and Ocimum basilicum leaf extract concentration of 0.9375%, 1.875%, 3.75%, 7.5%, and 15% according to the dilution method (8). All the tubes of the solution were then given 1 ml Streptococcus mutans suspension. Tripliation was made to each solution.

As much as 1 ml from each test tube was inserted into cuvette for measuring the optical density before incubated (OD1) using 550nm spectrophotometer. Bacterial suspensions can be measured with optical densities using a spectrophotometer at 550nm wavelength (9). All the test tubes were then incubated for 24 hours at 37°C. Optical density measurements were carried out again after incubation (OD2). From the OD1 and OD2 values, the final optical density (ODf) was obtained. The ODf value was calculated using the equation ODf = OD2-OD1. ODf value as a result of the subtraction of spectrophotometric readings before and after incubation was compared to various treatment groups, namely Ocimum basilicum leaf extract concentrations of 0%, 0.9375%, 1.875%, 3.75%, 7.5%, and 15%. The ODf value states the increase in the turbidity intensity of the solution within 24 hours. The increasing turbidity intensity of the solution shows the addition of Streptococcus mutans bacterial colonies that grow within 24 hours. The higher the intensity of turbidity produced, the more colonies of Streptococcus mutans grow. On the contrary, the lower the increase in the turbidity intensity that is read, the fewer the Streptococcus mutans colonies that can grow in 24 hours.

Statistical analysis

Data on ODf values were expressed in terms of mean and standard deviation (X ± SD). The data were then analyzed statistically using IBM SPSS version 18. The distribution of ODf values was tested for normality using Shapiro-Wilk method, while the variance homogeneity was tested using the Levene method. Both methods were tested with a confidence level of 95%. If the distributions of normal ODf values and their variance were homogeneous (p> 0.05), the One Way ANOVA test was used with a confidence level of 95%. The significant effect of the Ocimum basilicum leaf extract group was proven by using the One Way ANOVA test with a confidence level of 95%. Additional, the significant differences among the treatment groups of the Ocimum basilicum leaf extract were analyzed by using the Least Significant Difference (LSD) test with a confidence level of 95%.

RESULT

The results of the Streptococcus mutans growth inhibition test were in the form of ODf values expressed quantitatively in decimal from 3 replications in the control treatment group (Ocimum basilicum 0% leaf extract) and Ocimum basilicum leaf extract concentration of 0.9375%, 1.875%, 3.75%, 7.5%, and 15%. The ODf values from the three replications were then calculated for the mean and the standard deviation. Data of mean and standard deviation of ODf values are shown in Figure 1.
Turbidity measurement is one way to measure bacterial growth. The method is based on the principle of the higher the number of bacteria in liquid medium, the cloudier the medium. The turbidity intensity of the medium was measured by using a spectrophotometer. In a spectrophotometer, a beam is emitted through the bacterial suspension in the medium to be captured by the detector. If the number of bacteria increases, the light captured by the detector decreases. The amount of light captured by the detector is recorded in a spectrophotometer as transmission percentage. The logarithm value of transmission percentage is displayed by spectrophotometry in the form of decimal numbers. This numerical value is called optical density (OD). The OD values are used to monitor the increase in the number of bacteria in a certain time span (10). The OD value in this study was measured twice, namely before incubation (OD1) and after incubation (OD2), to find out ODf which showed the increase of bacteria. The mean ODf values in the treatment group of Ocimum basilicum 0% (control) leaf extract, Ocimum basilicum leaf extract 0.9375%, 1.875%, 3.75%, 7.5%, and 15% were positive (ODf value> 0).

This shows that there was an increase in the number of Streptococcus mutans bacteria during the 24-hour incubation process in all treatment groups. The increase in the number of Streptococcus mutans was different between one treatment group and others. The highest ODf value was found in the 0% extract group, which was 0.091±0.017, which showed that there were more number of Streptococcus mutans within 24 hours compared to other treatment groups. While the lowest value of ODf was in the treatment group of Ocimum basilicum leaf extract concentration of 15%, which was 0.006±0.005. This mean value showed that the least increase of Streptococcus mutans bacteria was in Ocimum basilicum leaf aqueous extract concentration of 15%.

The results revealed that the administration of Ocimum basilicum leaf extract showed an effect of lower ODf levels at doses of 3.75%, 7.5%, and 15%. The ODf value showed the increasing number of Streptococcus mutans bacteria within 24 hours. Significant differences in the increasing number of bacteria compared to control were thought to be due to the presence of the active ingredient in Ocimum basilicum leaf aqueous extract at concentrations of 3.75%, 7.5%, and 15%. These results indicate that Ocimum basilicum leaf extract has antibacterial activity thus it can inhibit the growth of Streptococcus mutans bacteria. This is in line with the research by Parhusip et al. that stated Ocimum basilicum leaf aqueous extract has antibacterial activity against Listeria monocytogenes bacteria (7). The results of this study also support the research of Mishra and Mishra regarding the antibacterial ability of Ocimum leaf aqueous extract against Staphylococcus aureus (11). Listeria monocytogenes, Staphylococcus aureus, and Streptococcus mutans bacteria are both Gram-positive and facultative anaerobes (10).

The results of research by Kaya et al. showed that the content of Ocimum basilicum leaf extract has antibacterial property against pathogenic bacteria, namely Pseudomonas aeruginosa, Shigella sp., Listeria monocytogenes, Staphylococcus aureus, and Escherichia coli (12). Bilal et al. in a review on phytochemical and pharmacological studies of Ocimum basilicum stated that the main compounds of phytochemical content of Ocimum basilicum leaf extract, which are most responsible for every pharmacological activity including antibacterial power, are still unclear (3). Hasan et al. in their research on the effect of Emblica officinalis fruit extract on the cariogenic nature of Streptococcus mutans bacteria showed that there was an effect of the synergistic work of various compounds in the extract on the reduction of the cariogenic nature of Streptococcus mutans, including inhibition of bacterial growth (13). In this study, the phytochemical content

Figure 1 shows that the highest ODf value was in Ocimum basilicum leaf extract of 0%, and the lowest ODf value was in Ocimum basilicum leaf extract concentration of 15%. The ODf value decreases as the concentration of Ocimum basilicum leaf extract increases. The results of Shapiro-Wilk analysis showed that the distribution of ODf values was normal (p = 0.433 for extract of 0%, p = 0.485 for extract of 0.9375%, p = 0.726 for extract of 1.875%, p = 0.298 for extract of 3.75%, p = 0.605 for extract of 7.5%, p = 0.510 for extract of 15%). The results of Levene test showed that the variance of the data was homogeneous (p = 0.088). ODf value data were then analyzed using One Way ANOVA test. The result showed that there were significant differences (p = 0,000) of ODf values among treatment groups. The LSD test showed that significant differences (p <0.05) were found between 0% Ocimum basilicum leaf extract group and Ocimum basilicum leaf extract groups of 3.75%, 7.5%, and 15%, between 0.9375% concentration extract group and concentrations of 7.5% and 15%, and between 1.875% concentration extract group and concentrations of 7.5% and 15%. There were no significant differences (p > 0.05) between 0% Ocimum basilicum leaf extract group and the extract group concentrations of 0.9375% and 1.875%, between the extract group concentration of 0.9375% and concentrations of 1.875% and 3.75%, between the extract group concentration of 1.875% with concentration of 3.75%, and among groups of Ocimum basilicum leaf extract 3.75%, 7.5%, and 15%.

**DISCUSSION**

Turbidity measurement is one way to measure bacterial growth. The method is based on the principle of the higher the number of bacteria in liquid medium, the cloudier the medium. The turbidity intensity of the medium was measured by using a spectrophotometer. In a spectrophotometer, the beam is emitted through the bacterial suspension in the medium to be captured by the detector. If the number of bacteria increases, the light captured by the detector decreases. The amount of light captured by the detector is recorded in a spectrophotometer as transmission percentage. The logarithm value of transmission percentage is displayed by spectrophotometry in the form of decimal numbers. This numerical value is called optical density (OD). The OD values are used to monitor the increase in the number of bacteria in a certain time span (10). The OD value in this study was measured twice, namely before incubation (OD1) and after incubation (OD2), to find out ODf which showed the increase of bacteria.
contained in Ocimum basilicum leaf extract was thought to work synergistically in inhibiting the growth of Streptococcus mutans bacteria.

The research results of Edriss et al. showed that the phytochemical contents of Ocimum basilicum leaf aqueous extract are flavonoids, saponins, and alkaloids (14). Flavonoids are known to inhibit the growth of Streptococcus mutans (15). With its antioxidant activity, flavonoids damage bacterial cell walls (16). Besides, flavonoids inhibit cell wall formation, damage and inhibit cell membrane formation, and inhibit the formation of bacterial nucleic acids (17,18). Saponins are soap-like compounds that are antiseptic and have antibacterial effects (19). Saponins reduce the surface tension of bacterial cell membranes by acting on phospholipid phosphate groups from cell membranes. These saponins enter the cell and denature cell proteins, so bacterial cells swell and break (20). Alkaloids inhibit bacterial growth by interfering the bacterial cell division process (21). Alkaloids also damage cell walls and membranes and inhibit the formation of protein and bacterial DNA (22).

Other results from this study showed that there were no significant differences in the ODf mean value of Streptococcus mutans between 0% Ocimum basilicum leaf extract group (control) and Ocimum basilicum leaf extract group concentrations of 0.9375% and 1.875%. There were no significant differences between the extract group concentrations of 0.9375% and 1.875% with the concentration of 3.75%, and among groups of Ocimum basilicum leaf extract concentrations of 3.75%, 7.5%, and 15%. This proves that the aqueous extract of Ocimum basilicum leaves starting at 7.5% has antibacterial property against Streptococcus mutans. Parhusip et al. showed different research results that stated the same extract at 15% concentration inhibits Listeria monocytogenes bacteria (7). This may be due to differences in the genus and bacterial species and differences in bacterial growth inhibition test methods that were used.

Ocimum basilicum leaf aqueous extract starting from 7.5% concentration has the effect of inhibiting the growth of Streptococcus mutans bacteria. The extract is thought to inhibit bacterial growth through damaging bacterial cell walls which affect bacterial cell metabolism. This study requires supporting research that can examine the percentage of antibacterial component of Ocimum basilicum leaf aqueous extract, the isolation of these components, and the cellular working mechanism of these components against Streptococcus mutans bacteria cells.

The research results show that the extract of Ocimum basilicum leaves at concentrations of 7.5% and 15% have an inhibitory effect on Streptococcus mutans bacteria, namely by suppressing the growth of the number of bacteria in a 24-hour period. This shows the potential of Ocimum basilicum leaves as an herbal ingredient from Indonesian environment that can be processed, researched, and utilized in the health sector, including dental and oral health.

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