

## Antimicrobial Activity of Grape Skin (*Vitis vinifera*) Infusum on Salivary Mutans Streptococci

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### ABSTRACT

Dental caries was caused by *Streptococcus mutans*. Grape (*Vitis vinifera*) variety Blue Probolinggo have active substance: Polyphenol compound such as flavonoid, tannin, anthocyanin and resveratrol. One of its benefits is its capability to prevent dental caries. **Objectives:** The aim of this study was to determine the sensitivity of grape skin (*Vitis vinifera*) infusum on salivary mutans streptococci. **Methods:** Grape skin infusum of *Vitis vinifera* containing polyphenol compound was examined in vitro on the bacterial growth by determining the inhibition zone (agar diffusion method), Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The microorganisms tested of mutans of *Streptococcus mutans* was isolated from human harboring species in Jakarta Indonesia. Data obtained was done in a descriptive method. **Results:** grape skin infusum containing *Vitis vinifera* had effect on all of mutans of *Streptococcus mutans*: Inhibitory zone was inclined from 0.05 mm in concentration 20%/ml to 6.70 mm in concentration 90 %. MIC was made at 50% and MBC was made at 60%. **Conclusion:** The grape skin of *Vitis vinifera* showed antimicrobial activity against local strains of mutans of *Streptococcus mutans*, isolated from humans harboring species. It is expected that it can be used in preventing caries risk in the future.

**Key words:** Grape skin (*Vitis vinifera*), infusum, salivary mutans streptococci

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## INTRODUCTION

Dental caries, which is known the most common ailment of the mouth, is found to be caused by mutans streptococci. The presence of *Streptococcus mutans* is associated with the high number of salivary mutans streptococci<sup>1</sup> and caries activity of mutans streptococci of mutans streptococci in high caries prevalence<sup>2</sup>

For this reason early prevention is needed by controlling dental caries in the community has been done in many ways, such as by tooth brushing regularly using dentifrice and mouth rinsing. It is the most widely used and socially accepted form of oral hygiene. Mouth rinsing and tooth brushing are the principal ways for mechanical removal of plaque and to prevent *Streptococcus mutans* colonization on teeth.<sup>3,4</sup>

Grape (*Vitis vinifera*) variety Blue Probolinggo has active substance polyphenol compound such as flavonoid, tannin, anthocyanin and resveratrol known as antiseptic agent, which is beneficial for oral health. One of its benefits is its capability to prevent dental caries. The objective of the research is to determine the sensitivity of grape skin of *Vitis vinifera* infusum by measuring the inhibitory zone, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) on mutans streptococci, *in vitro*.

The result will provide information about grape skin *Vitis vinifera* and its

effectiveness in preventing dental caries for dentistry in particular and for researcher in general.

## MATERIALS AND METHODS

The material used in this research was grape skin of *Vitis vinifera* infusum. The bacteria used as analysis unit were local strains of mutans of salivary *Streptococcus mutans* isolated from human harbouring species in Jakarta Indonesia. These bacteria were cultivated in Tryptose - Yeast Extract - sucrose 20% - bacitracin ((TYS20B),<sup>5</sup> Diagnostic Sensitivity Test (DST) agar media and Brain Heart Broth (BHI) and incubated in anaerobic jar at 37<sup>o</sup> C for 3 x 24 hours.

Working method of Grape skin of *Vitis vinifera* infusum : 10 gram grape skin was weighed, then mixed with 500 ml of sterile aquadest in infusum pan and heated on the gas stove. After the temperature had reached 90<sup>o</sup> C, kept it still on the gas stove and stirred it once in a while.

While it was still hot, poured it into a 500 ml Erlenmeyer gourd using a glass funnel, which had been lined with filter paper and muslin, until the volume reached 500 ml. From this process, 10% concentration of infusum of grape skin solution was obtained .

Put the Erlenmeyer gourd into water-bath, which contained boiled water, kept it until the volume became 50 ml, from this

process 100% concentration of infusum of grape skin solution was obtained.

Kept the solution until the temperature decreased. Sterilization of grape skin infusum was done by using Tyndalisation method at temperature of 95<sup>o</sup> C for 30 minutes.

Sensitivity Test to grape skin of *Vitis vinifera* infusum could be done in two ways<sup>6</sup>:

#### Drug serial dilution method

The Bacteria Culture Medium of mutans streptococci. A portion of the strain of mutans streptococci in TYS20B media was taken to be cultivated in BHI agar. Then, put the cultivated bacteria inside an incubator at 37<sup>o</sup>C for 48–72 hours in anaerobic condition.

After 2–4 days, added the cultivated mutans streptococci, little by little into a physiologic salt solution until the turbidity was equal to McFarland II standard solution. As the turbidity of culture bacteria medium had been equal to McFarland II standard solution, the number of bacteria cells/milliliter would be counted, their number was  $0.5 \times 10^9$  bacteria/milliliter. If equalization process had been done culture of mutans streptococci, shall be diluted as follows:

Prepared 7 tubes each containing 9 ml physiologic saline solution and prepared 1 tube containing 5 ml physiologic saline solution. Took 1 ml bacteria from the culture bacteria medium which had been equal to

McFarland II standard solution then dilute culture medium bacteria until 10<sup>7</sup> times.

The bacteria dilution needed 7 times until the number of bacteria in the last tube was estimated at 50 cells/ml. Each tube should be labeled. Sensitivity test of bacteria to grape skin *Vitis vinifera* infusum: Prepared 8 tubes, each of the tubes was filled with 2 ml of BHI agar. Each tube was labeled 1–8. Added 2 ml of grape skin *Vitis vinifera* infusum into the first tube, mixed them until homogeneous,

Took 2 ml from the first tube, added it into the second tube, mixed them until

homogeneous. Did the same procedure through the 8<sup>th</sup> tube. After dilution was finished, then put 1 ml of the diluted bacteria from the homogeneous solution in those eight test tube. All test tubes put in anaerobic jar at 37<sup>o</sup> C for 2 x 24 hours.

After 72 hours, macroscopically it could be seen in which tube, the bacteria could not grow. Recorded the result to determine the MIC.

#### Method using disc with drug in solid media

Poured diluted bacteria into the Diagnostic Sensitivity Test (DST) agar petri disc, the bacteria suspension should wet the DST agar thoroughly. Then put grape skin *Vitis vinifera* infusum on the surface of a disc and put it on the surface of DST agar.

Those petri discs were incubated in anaerobic jar at 37°C for 3 x 24 hours. Inhibition zone would appear around the disc and it should be measured i.e the diameter of the bacteria zone around the samples.

## RESULTS

The results of sensitivity test of mutans streptococci to *Vitis vinifera* grape skin infusum with serial dilution method and the inhibitory zone can be seen on Table 1.

**Table 1.** Result of sensitivity test of mutans of *Streptococcus mutans* to Grape skin of *Vitis vinifera* infusum

The concentration of grape skin of <i>Vitis vinifera</i> (%/ml)	Inhibitory zone in mm
20%	0.50
30%	0.73
40%	1.67
50%	2.06
60%	2.95
70%	3.68
80%	5.26
90%	6.70

N = 120 specimen

## DISCUSSION

The results showed that all of laboratory strains of mutans of *Streptococcus mutans* were sensitive to concentration 20 % to 90% grape skin *Vitis vinifera* infusum . Inhibitory zone of mutans of *Streptococcus mutans* was 0.50 mm,0.73 mm, 1.67 mm, 2.06 mm, 2.95 mm,3.68 mm, 5.26 mm, and 6.70 mm.

Therefore grape skin of *Vitis vinifera* infusum can be accepted inductively as an active substance that can inhibit the growth of mutans of *Streptococcus mutans* in vitro. In the previous study it has been proven that the grape juice of *Vitis vinifera* can inhibit the growth of *Listeria monocytogenes*<sup>7</sup> and has antimicrobial activity against mutans of *Streptococcus mutans* isolated from human harboring species.<sup>8</sup>

Grape fruit contains several chemical substance classified as an active substance polyphenol compound such as flavonoid, tannin, anthocyanin and resveratrol. Grape fruit has been considered as having property of anti microbial effect against mutans of *Streptococcus mutans*. And the most important effect of grape fruit is its capability to inhibit the growth of the salivary mutans streptococci .With proper futher study, grape fruit has potential role in inhibiting caries.

## CONCLUSION

The result showed that grape skin of *Vitis vinifera* had bacterial activity against local strains of mutans of *Streptococcus mutans* isolated from human harbouring species in Jakarta Indonesia. Therefore, by consuming grape fruit of *Vitis vinifera* can prevented caries.

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