

Effects of *Psidium guajava* Leaf Infusion on *Streptococci viridans*

Hing Yi Chen,¹ Ine Kuswardinah,² Laili Aznur³

¹Faculty of Medicine Universitas Padjadjaran ²Department of Microbiology and Parasitology Faculty of Medicine Universitas Padjadjaran, ³Department of Oral Health Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung

Abstract

Background: Dental caries is recognized as the most important oral burden. It is caused by the formation of lactate acid formed through reaction of bacteria and carbohydrates. *Streptococci viridans* has been proven as the primary etiologic agents for dental caries. Low accessibility in oral care services leads the Indonesian community to use plants in order to prevent dental caries. One of those plants is *Psidium guajava* (pink guava). The leaves were suggested to have antimicrobial effects on some gram-positive bacteria. When the organism is resistant to specific substance tested on media, a circular/inhibition zone around a disc containing antimicrobial substance was formed. The purpose of this study was to identify the presence of inhibition zones by infusion of *Psidium guajava* leaf on *Streptococci viridans* in vitro.

Methods: This laboratory experiment was carried out in September to October 2014 at the Microbiology Laboratory, Faculty of Medicine, Universitas Padjadjaran. Infusions of *Psidium guajava* leaf were made into four different concentrations (10%, 25%, 50% and 100%, respectively) and the identification of inhibition zones on *Streptococci viridans* obtained from the laboratory was tested using modified disk diffusion test. Distilled water acted as negative control. The results were then interpreted after 24 hours of incubation. Every procedure was repeated three times.

Results: All four concentrations of *Psidium guajava* leaf infusions have formed inhibition zones on the media, with the highest concentration (100%) producing largest average diameter.

Conclusions: The infusion of *Psidium guajava* leaf produces inhibition zones on *Streptococci viridans* in vitro. [AMJ,2016;3(3):345-8]

Keywords: Dental caries, *Psidium guajava*, *Streptococci viridans*.

Introduction

The World Health Organization (WHO) has identified dental caries as the most important oral burden especially in developing countries, affecting 60-90% of school-aged children and a majority of adults. This is mostly due to sociobehavioural and environmental factors.¹ Indonesia's Ministry of Health previously reported that the prevalence of health problems of teeth and oral cavity to be 23.4%, which occurred more frequently in suburban areas (24.4%). It may be consequence of the poor personal oral hygiene and lack of knowledge about oral health.²

Dental caries formed through acid by colonizing flora colonies in plaque on enamel later demineralize enamel and *Streptococci viridans* is the main etiologic agent. This

Streptococci species constitutes 39% in normal human oral cavity, making them opportunistic pathogens to human.³ Dental caries is both chronic and systemic diseases when untreated, can cause myocardial infarction, endocarditis and stroke.⁴

Furthermore, low accessibility to oral care services leads to treatment using traditional/herbal medicine, which is cheaper and more accessible. Most of the Indonesian community use *Psidium guajava* (pink guava).⁵ The fruits, leaves, bark and roots are parts of the plant that were utilized in oral care.⁶ The leaves were suggested to have antimicrobial effects on some gram-positive bacteria. These effects were attributable to the presence of essential oil in the leaves which can penetrate the lipid bilayer of cell membrane causes leakage of vital cell contents.⁷ When the organism

Correspondence: Hing Yi Chen, Faculty of Medicine, Universitas Padjadjaran, Jalan Raya Bandung-Sumedang Km.21, Jatinangor, Sumedang, Indonesia, Phone: +62 81394116717 Email: yichenhing@gmail.com

is resistant to specific substance tested on media, a circular/inhibition zone around a disc containing antimicrobial substance was formed. This study was conducted to illustrate the presence of inhibition zones by infusion of *Psidium guajava* leaf on *Streptococci viridans* in vitro. This study could provide the Indonesian community, especially those living in rural areas, as an alternative prevention action on dental caries which is cheaper and more accessible.

Methods

This study was conducted from September–October 2014 in the Microbiology Laboratory, Faculty of Medicine, Universitas Padjadjaran. All experiment procedures have been approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Padjadjaran. The leaves of *Psidium guajava* were obtained from Jatinangor area, Sumedang, West Java. The infusion of leaves was made into four different concentrations, which were 100%, 50%, 25% and 10%. According to The National Agency of Drug and Food Control, Republic of Indonesia, the concentration for herbal leaves in order to make infusion was supposed at 10%.⁸ The tested *Streptococci viridans* was obtained from the Microbiology Laboratory of Faculty of Medicine, Universitas Padjadjaran.

Before a disk diffusion test was carried out, leaves of *Psidium guajava* were prepared to be made into infusion. The leaves were first weighed to a total of 25 gram, before being washed and minced. The minced leaves were then put into an Erlenmeyer container and mixed with water of 25 ml. The container was later put into a pan that had been filled with boiling water. Then, the temperature was measured, and when the temperature has reached 90°C, it was maintained for 15 minutes. After 15 minutes, the container was removed from the pan and the mixture was poured and filtered through a flannel cloth to a bowl.⁸ A 100% concentration of *Psidium guajava* leaves infusion was formed. Next, the infusion was diluted with distilled water to obtain three concentrations of infusion, namely 50%, 25% and 10%. Those infusions were placed into 4 different test tubes.

Furthermore, the bacteria suspension was prepared using the direct colony suspension method where five samples from different areas of a colony of *S. viridans* had been cultured in blood agar for 24 hours at 35 ± 2 °C were mixed with 5 ml of sterile NaCl (8.5 g/L NaCl; 0.85% normal saline) 0.145 mol/L

for about 15 seconds. Then, the turbidity of the suspension was measured visually. The suitable turbidity was proportional to McFarland 0.5.⁹

The susceptibility effect procedure referred to the guidelines from the Clinical and Laboratory Standard Institute (formerly NCCLS) and the European Society of Clinical Microbiology and Infectious Disease (EUCAST). Prior to the inoculation of the testing of microbe was performed a petri dish containing blood agar was prepared. Then, the cotton swab was swapped on the surface of blood agar. The petri dish was left open for about 3 to 5 minutes to reduce humidity on the agar's surface before being closed by a cover lid.⁹ Five small areas on the media were marked with holes, at four sides and one at the center of the petry dish. The leaves infusions were applied into these 4 areas using different concentrations respectively. The one in the center acted as a negative control with the application of distilled water only. Those steps were repeated three times. Finally, those petri dishes were incubated at a temperature of 35 °C (±2°C) for 24 hours.

Furthermore, after incubation of the media for 20 to 24 hours, the diameters of inhibition zones were measured.⁹ The results were then analyzed and shown in a table.

Results

The test using distilled water showed negative results with no inhibition zones being formed in all three tests. The 10% of *Psidium guajava* leaf infusion showed the average diameter of inhibition zones to be 11.7 mm. While for the infusion of 25%, the average diameter was recorded as 18.3 mm. The leaf infusions of 50% and 100% were measured as 21.7 mm and 23.3 mm respectively.

The diameters of the inhibition zones presented increased from lowest at 10% concentration of leaf infusion and ranged highest when the leaf infusion concentration is at 100%. This result was consistent for all the three tests.

Discussion

Many studies had been conducted on the effects of *Psidium guajava* leaves as antimicrobial substance. A previous study using similar methods showed that different concentration of this plant extract had doubled acted as antimicrobial substance *Streptococcus* species.¹⁰ Another study used *Psidium*

Table Diameter of Inhibition Zones Results from Different Concentrations of Infusion of *Psidium guajava* on *Streptococci viridans* in vitro

Concentration (%)	Inhibition Zone (mm)			
	Test I	Test II	Test III	Average
Distilled Water	-	-	-	-
10	10.0	15.0	10.0	11.7
25	15.0	20.0	20.0	18.3
50	20.0	22.0	23.0	21.7
100	21.0	24.0	25.0	23.3

Note: *(-) is when there was no inhibition zone found

guajava ethanol-extract ranging from 10% to 100% on *Actinomyces* species to observe the morphological changes and inhibition zone's diameter. This study demonstrated that there were presence of inhibition zones and morphological changes.¹¹

In order to determine whether similar results could be revealed using *Psidium guajava* leaves, the infusion of 100% concentration was diluted with distilled water to obtain the remaining three concentrations of infusion, which were 50%, 25% and 10%. The presence of the inhibition zones was identified at the end of the study. The diameter of the inhibition zones formed by all the concentrations of infusion of *Psidium guajava*, increased in accordance to the level of concentrations used. The higher the concentration, the wider the inhibition zone was.

Meanwhile, a previous study on phytochemical analysis with the usage of four different extraction methods: n-hexane, ethanol and methanol, and distilled water was reported. The extraction with distilled water demonstrated as the only method that was able to demonstrate positive presence compared to the other chemical substances. These chemical contents were expected to contribute to antibacterial effect of the *Psidium guajava* leaves tested on gram-positive microorganisms, which were *Staphylococcus aureus* and *Bacillus cereus*. Through the action of flavanoids in penetrating cell membrane's lipid bilayer; saponins inhibiting gram-positive microorganisms and tannins interfere their production of protein, antibacterial effects were presented.⁵ Therefore, these chemical contents within the leaves have been suggested in producing inhibition zones in vitro.

The study has also illustrated that when *Psidium guajava* ethanol-extracts were tested on other microorganisms, for example *Streptococci mitis* and *Actinomyces* species, it

disrupted the morphology of these bacteria. Introduction of the extracts into the growth environment of these microbes has produced a hostile condition for them to grow. The bacteria needed a longer period of time in order to adapt and synthesize new essential enzymes to metabolise the substrates introduced into the growth medium. Therefore, the extract disrupted the physiological activities of the cells and may lead to cell death, contributing the presence of inhibition zones in tests.¹¹

This study was limited by the time provided in order to complete the whole study. The results should be further analyzed with statistical analysis in order to determine the antibacterial effect of infusion of *Psidium guajava* leaves on *Streptococci viridans* in vitro. The study had also been restricted when the validations of the results should be identified using other extraction methods, to compare whether similar results would be produced in the presence of inhibition zones when other extraction methods of *Psidium guajava* leaves were used.

Previous studies provided comprehensive explanations on the production of inhibition zones formed by *Psidium guajava* leaves chemical contents. These studies corresponded to the results of this study. Thus, from this study, it can be concluded that the infusion of leaves of *Psidium guajava* produces inhibition zones on *Streptococci viridans* in vitro.

The study can be further improved through comparing the inhibition zones formed with observation under microscopes in order to determine the colony affected by the infusion, thus comparing the numbers of bacteria affected and the morphological changes on *Streptococci viridans*. The results could be verified further with a statistical analysis in order to determine the antibacterial effect of infusion of *Psidium guajava* leaves on *Streptococci viridans* in vitro and in vivo, in

addition to determine the minimal inhibition of concentration.

References

1. Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. The global burden of oral diseases and risks to oral health. *Bull World Health Organ.* 2005;83(9):661-9.
2. Deprtemen Kesehatan RI. Riset Kesehatan Dasar (RISKESDAS) 2007. Jakarta: Badan Penelitian dan Pengembangan Kesehatan Departemen Kesehatan RI;2008.
3. Grusell EN, Dahlen G, Ruth M, Ny L, Quiding-Jarbrink M, Bergquist H, et al. Bacterial flora of the human oral cavity, and the upper and lower esophagus. *Dis Esophagus.* 2013;26(1):84-90.
4. Hujoel P. Dietary carbohydrates and dental-systemic diseases. *J Dent Res.* 2009;88(6):490-502.
5. Mailoa MN, Mahendradatta M, Laga A, Djide N. Tannin extract of guava leaves (*psidium guajava l*) variation with concentration solvents. *Int J Sci Technol Res.* 2013;2(8):106-10.
6. Kumar A. Importance for life 'psidium guava'. *Int J Res Pharm Biomed Sci.* 2012;3(1):137-43.
7. Biswas B, Rogers K, McLaughlin F, Daniels D, Yadav A. Antimicrobial ctivities of leaf extracts of guava (*Psidium guajava L.*) on two gram-negative and gram-positive bacteria. *Int J Microbiol.* 2013;2013:7.
8. Badan Pengawas Obat dan Makanan RI. Acuan sediaan herbal vol 5. 1st ed. Jakarta: Direktorat Obat Asli Indonesia, Deputi II, BPOMRI; 2010. p. 1-7.
9. Matuschek E, Brown DFJ, Kahlmeter G. Development of EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. *Clin Microbiol Infect.* 2014;20(4):255-66.
10. Nzeako BC, Al-Kharousi ZSN, Al-Mahrooqui Z. Antimicrobial Activities of Clove and Thyme Extracts. *Sultan Qaboos Univ Med J.* 2006;6(1):33-9.
11. Fathilah AR, Yusoff M, Rahim ZHA. The Effect of *Psidium guajava* and Piper betle extracts on the Morphology of Dental Plaque Bacteria. *Pak J Med Sci.* 2009;25(6):928-33.