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The effect of young pepaya leaf ethanol extract (*Carica papaya L.*) with concentration variants on the growth of pathogenic Streptococcus pyogenes bacteria causing pharyngitis through in vitro testing

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ABSTRACT

Background: Pharyngitis infection issues by *Streptococcus pyogenes* bacteria have been increasing worldwide, and antibiotic resistance to the bacterium treatment also continues to escalate. Young papaya leaves (*Carica papaya L.*) have a bactericidal and bacterostatic effect (include papain, flavonoids, alkaloids (karpain alkaloids), saponins, glycosides, phenols (tacophenol), and tannins) that can be an option for treatment of pharyngitis infections other than drugs. This study aims to determine the effectiveness of ethanol extract of young papaya leaves (*Carica papaya L.*) in inhibiting the growth of *Streptococcus pyogenes* bacteria through in vitro testing.

Methods: This study was a true-post experimental study using control group design. The sample was divided into 2 groups: positive and negative control groups as well as treatment groups with concentration variants of 25%, 50%, 75%, and 100%. Data were analyzed using SPSS version 17 software for windows.

Results: The result obtained from this study showed that papaya leaf extract (Carica papaya L.) was proven to significantly inhibit the growth of Streptococcus pyogenes bacteria at concentrations of 25% to 100% with an average diameter of the inhibition zone of 6.4 mm to 7.4 mm ($P<0.05$).

Conclusion: This evidences that the ethanol extract of young papaya leaves (Carica papaya L.) with various concentrations does exhibit an influence on the growth of Streptococcus pyogenes bacteria.

Keywords: young papaya leaves, ethanol, Streptococcus pyogenes, pharyngitis

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INTRODUCTION

Health issues in Indonesia are outright salient that they require special attention. There are a lot of epidemics owing to bacterial infection, either that of gram-positive or gram-negative bacteria. They develop clinical manifestations and eventually disrupt the life of the infected ones. In large parts of the world, *Streptococcus* bacterial infection keeps on increasing in number and affecting people of all ages.[1]

Streptococcus pyogenes belongs to *Group A Streptococcus* (GAS), the only species in the *group β-hemolytic strep-tococci*.[2] The bacteria which are often responsible for pharyngitis are *Group A Streptococcus*.[3] *Streptococcus pyogenes* is one of the most common bacteria that cause pharyngitis. Pharyngitis is an infection of the posterior pharynx or the tonsil and it might spread to the surrounding tissues. The sufferers usually experience symptoms of discomfort followed by sore throat as well as fever.[1,4] *Streptococcus pyogenes* secretes several virulence factors such as streptokinase, hyaluronidase, proteinase, hemolysin, polysaccharide capsules, cysteine proteases and *Streptococcal inhibitor of Complement* (SIC). *Streptococcus pyogenes* is one of the bacteria that are resistant to *erythromycin*.[1]

Antibiotic resistance has now been a global concern. There have been worrisome cases regarding an increase of antibiotic resistance to humans in recent years. Infection treatments by combining various antibiotics which were initially believed to be the remedy of bacterial infection have brought about a new health concern, that is the outgrowth of multi-resistance bacteria.[1] Owing to this reason, herbal-alternative-treatment-based experiments are conducted concomitant the increment of the resistance level, of one among those experiments includes the use of papaya leaves.

Papaya leaf extract *(Carica papaya L)* acts as an antibacterial, including papain, flavonoids, alkaloids (carpine alkaloids), saponins, glycosides, phenols *(tacophenol)*, and tannins.[4-8] The antimicrobial effect of tannins is by inactivating microbial adhesion and inactivating hydrolytic enzymes such as proteases and carbohydrolases

and inhibiting enzymes in enveloped transport proteins.[8-11]

MATERIALS AND METHODS

This is experimental research to determine the effect of young papaya leaf ethanol extract *(Carica papaya* L.) with concentration variants to the *Streptococcus pyogenes* bacterial outgrowth by *in vitro* testing employing *True Experimental Post Test with Control Group Design* method. Samples tested were divided into 2 major groups of the control group (K) and treatment group (P). The former is negative control (K1) and antibiotic *amoxicillin* control to *Streptococcus pyogenes* isolations (K2) while the latter is classified into 4 groups in accordance to the dosage usage in young papaya leaf ethanol extract (*Carica papaya* L.) to each *Streptococcus pyogenes* isolation, with concentration variants of 25% (P1), 50% (P2), 75% (P3), and 100% (P4).

The research was conducted at the Microbiology Laboratory of the Faculty of Medicine, Udayana University in October 2018. The production of young papaya leaf extract using maceration method was carried out at the Food Technology Laboratory of the Faculty of Agricultural Technology, Udayana University.

The lab instruments equipped in this experiment include incubator, oven, autoclave, paper disk, Petri disk, micropipette, test tube, tweezers, stirring rod, aluminum foil, Erlenmeyer tube, wire gauze, analytic scales, medium glass bottles, small glass bottles, alcohol burner, cotton, tissue, sterile cotton sticks, sterile toothpicks, calipers, lights. Research materials in this study comprise of Muller Hinton Agar (MHA) media, ethanol 96%, antibiotic amoxicillin, Mc solution. Farland, NaCl 0.9%.

The making of young papaya leaf extract with young, fresh, and undamaged papaya was taken from the first stalk to the fourth from the first shoot. The leaves were washed down then dried for 4 days and mashed them up by blender until they became dry powder. The papaya leaf powder was soaked in ethanol 96% for 2 x 24 hours. Papaya leaf filtrate was concentrated with a rotary evaporator RE 300 until we obtained a concentrated extract in the form of a concentration of 100%. The concentrated extract of papaya leaves manufactured was then diluted by adding the solvent to each concentration of 25, 50, 75%.

The inhibition measurement of *Streptococcus pyogenes* bacterial growth was executed using the Kirby Bauer method where the bacterial suspension was thoroughly applied to the entire surface of the MHA. The suspension used was adjusted to the turbidity standard of McFarland solution. It was then followed by putting a *blank disk* which had been extracted from the young ethanol extract of papaya leaves with concentration variants of 25%, 50%, 75%, 100%, ethanol 96% as the negative control, and antibiotic *amoxicillin* as the positive control. Each treatment was repeated five times. It was afterward proceeded with the installation at 37°C in 24 hours in the incubator. After 24 hours, the inhibition zone was determined by measuring the clear zone generated around the *blank disk*. The inhibitory zone was measured by using a caliper in mm unit.

Data processing was initiated with a test of variance normality and homogeneity. In the *Shapiro Wilk* normality test, it was generated that the data distribution was not normal because it obtained a significant value of 0.006 ($p < 0.05$) at a concentration of 25%, 50%, 75%, 100% and also to those of positive controls. The homogeneity test result on *Levene* statistics obtained a significant value of 0,000 ($p < 0.05$). From the results of statistical tests the data showed that the distribution of data was not normal and the data variants were not homogeneous so ANOVA test could not be performed and carried on with non-parametric analysis *Kruskal Walis* to evaluate whether each concentration of young papaya leaf ethanol extract has a significant difference in the growth of *Streptococcus pyogenes* bacteria and acquires a significant value of 0,000 ($p < 0.05$) which might prove that ethanol extract of young papaya leaves in various concentrations affect the growth of *Streptococcus pyogenes* bacteria. Whereas to determine the difference between each concentration, *Post Hoc* analysis was employed using the *Mann Whitney* test.

RESULTS

The results of the inhibitory test results of young papaya leaf ethanol extract (*Carica papaya* L.) with concentration variants on the growth of *Streptococcus pyogenes* bacteria in the table are illustrated below.

Based on Table 1, the inhibition diameter was the lowest in extract 25% (6.4 mm), following by extract 50% (6.6 mm), extract 75% (7 mm), and extract 100% (7.4 mm) towards the growth of *Streptococcus pyogenes* bacteria. In addition, the significant difference was also found among extracts used in this study particularly in concentrations of 25% to 100% ($P<0.05$) (Table 2).

DISCUSSION

The obtained results from this study confirm the fact that young papaya leaves (*Carica papaya* L.) with concentration variants are capable of limiting the outgrowth of *Streptococcus pyogenes* bacteria.

Table 1 **An average inhibition diameter of ethanol extract of young papaya leaves *(Carica papaya* L.) towards the outgrowth of *Streptococcus pyogenes* bacteria**

Types of Treatment	Inhibition Zone Diameter					Average (mm)
	I	II	III	IV	V	
Extract 25%	7	6	7	6	6	6.4
Extract 50%	7	6	7	7	6	6.6
Extract 75%	7	7	7	7	7	7
Extract 100%	7	7	8	8	7	7.4
Control (+)	36	38	34	38	36	36.4
Control (-)	0	0	0	0	0	0

Table 2 **Analysis result on the differences among extract concentrations, positive controls, and negative controls**

Variables	Extract 25%	Extract 50%	Extract 75%	Extract 100%	Control (+)	Control (-)
Extract 25%	-	0.549	0.05	0.031*	0.008*	0.005*
Extract 50%	-	-	0.134	0.058	0.008*	0.005*
Extract 75%	-	-	-	0.134	0.005*	0.003*
Extract 100%	-	-	-	-	0.008*	0.005*
Control (+)	-	-	-	-	-	0.005*

Note : * = value p < 0,05 (significance)

At 25% concentration extract, it was produced an average inhibition diameter of 6.4 mm, 6.6 mm at 50%, 7mm at 76%, and 7.4mm at 100%.

This research is in line with research probed by Tuntun et al. where the ethanol extract of papaya leaves was tested on *Staphylococcus aureus* bacteria. In this study, papaya leaf extract was tested in 10 concentration variants, such as: 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%.[11] Tests on concentration extracts of 10% and 20% failed to inhibit the growth of *Staphylococcus aureus* bacteria, however at concentrations of 30% to 100%, they successfully limited the growth of *Staphylococcus aureus* bacteria with an average diameter of inhibitory zone 7.9 mm.[11] Outcome differences between Tuntun's study comparing to this study are possible because in the making of the extraction of papaya leaves in Tuntun's study (2006) utilized old papaya leaves picked at the fifth and sixth stems from the bottom shoots.[11] This difference would certainly affect the content of the active substances between young and old papaya leaves.

Sudawarti has conducted an experimental study on the anti-bacterial effectiveness of papaya leaves towards gram-positive *Bacillus subtilis* bacteria.[12] Characteristics of *Bacillus subtilis* bacteria are similar to those of *Streptococcus pyogenes,* gram-positive and facultative anaerobic bacteria.[13] Sudarwati repeated tests on the extractions 6 times from an extract concentration of 20µg / ml to 100µg / ml. The average diameter of inhibition generated at a concentration of 20 µg / mL was 8,1 mm, 8,3 mm at 40 *µg / mL*, 8,4 at 80 *µg / mL*, 8,6 mm at 100 *µg / mL*. It was found as well that the average diameter of the inhibitory power might be due to the age difference of papaya leaves, the length of maceration time, and the media used. It used old papaya leaves, macerated within 5 days, and using NA (Nutrient Agar) media.[12] NA is a bacterial culture medium recommended for the cultivation of non-fastidious microorganisms.[14]

Research studies on the antibacterial effectiveness of papaya leaves were also investigated on *E. Coli* bacteria by Suwandi and Sugito wherein the extract concentration of papaya leaves was divided into 5 concentration variants of 12%, 14%, 16%, 18%, and 20% with 5 repetition times.[15] The average diameter of inhibition was obtained by 14.2 mm at a concentration of 20%. Comparing the research conducted by Sugito and Suwandi, the inhibition of the ethanol extract of papaya leaves produced higher than this study.[15] This might be due to differences in the types of bacteria tested. In the research conducted by Sugito and Suwandi, the ethanol extract of papaya leaves was testified on *E. coli* bacteria which are gram-negative bacteria that there are certainly nonconformities in bacterial cell structure influencing the antibacterial mechanism on the ethanol extract of papaya leaves.

Antibacterial compounds on papaya leaves *(Carica papaya*) are composed of papains, flavonoids, alkaloids (carpine alkaloids), saponins, phenols *(tacophenol*), and tannins.[8-11]

Saponin forms foams which are durable during the extraction process and works by damaging the structure of cell walls and permeability of bacterial cells to create leakages on nutrients, proteins, and enzymes of the bacterial cells.[16,17] Phenol works by breaking off the peptidoglycan bonds of the cell walls through the destruction of hydrophobic bonds on cell membrane components such as proteins and phospholipids while dissolving all components that are hydrophobically bonded and causing an increase of membrane permeability and cell wall leakage occurs.[11] Carpine alkaloids take effect by disrupting bacterial DNA which brings about damage to the nucleus of bacterial cells. Damage to the cell nucleus will stop the process of bacterial metabolism so that the bacteria will be lysis or inactive.[11]

On cell wall membranes, Flavonoids take part in denaturation and coagulation of proteins.[18,19] In parts of the leaves or parts of plants there are several types of flavonoids such as flavonol, *flavone*, and glycosides.[20,21]

Tannins inhibit *the reverse transcriptase enzyme* and the topoisomerase DNA, preventing bacterial cells from forming.[19] Tannin also activates adhesion in microbial cell and enzymes, disrupting protein transports in the bacterial cell layers.[18]

CONCLUSION

Based on this study report, it can be concluded that the ethanol extract of young papaya leaves has the potential to inhibit the growth of Streptococcus pyogenes bacteria with a minimum inhibitory concentration at a concentration of 25% with an inhibition diameter of 6.4 mm. The biggest inhibition zone is produced at 100% extract concentration with an inhibition zone of 7.4mm. There are significant differences between extract concentrations of 25%, 50%, 75%, and 100% for positive controls with antibiotic amoxicillin and negative controls with ethanol 96%.

ETHICAL CLEARANCE

This study has received ethical approval from the Ethics Committee Board, Faculty of Medicine, Udayana University, Bali, Indonesia prior to the study being conducted.

CONFLICT OF INTEREST

The authors declare that there is no competing interest regarding the manuscript.

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AUTHOR'S CONTRIBUTION

All authors are equally contributing to the study from data gathering, data analysis, until reporting the result of study.

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