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Antibacterial Effectiveness of *Jatropha* Leaf Extract (*Jatropha curcas L.*) against *Aggregatibacter actinomycetemcomitans*: In Vitro Study

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ABSTRACT

Background: Periodontal disease is one of the most common dental and oral health problems. Periodontal disease is characterized by inflammation as well as damage to the periodontal tissues, including the alveolar bone, cementum, gingiva, and the periodontal ligament. *Aggregatibacter actinomycetemcomitans* is the dominant bacterium in cases of periodontitis. *Jatropha curcas* plant is one of the ingredients that has been widely used for traditional medicine in the community, especially for the leaves. This plant is rich in active compounds such as alkaloids, tannins, phenols, flavonoids, and saponins, which are effective against gram-positive and gram-negative bacteria. This study aimed to determine the effectiveness of *Jatropha* extract (*Jatropha curcas L.*) as an antibacterial against *Aggregatibacter actinomycetemcomitans* in vitro. **Methods:** In vitro experimental studies. A total of 28 petri dishes contained bacterial colonies *Aggregatibacter actinomycetemcomitans* were used and divided into 7 test groups, treatment, and control groups. Obtained data were analyzed using SPSS program. **Results:** *Jatropha* extract treatment group with a concentration of 35% to 65% had optimal antibacterial abilities as the dose increased. This study also showed that at a concentration of 25%, *Jatropha curcas* extract did not have the antibacterial ability. **Conclusion:** *Jatropha curcas* extract has an antibacterial effect as the concentration of the extract increases against *Aggregatibacter actinomycetemcomitans*.

1. Introduction

Dental and oral health holds an important aspect in optimizing individual health. Healthy teeth and mouth will greatly determine the quality of life of each individual. Periodontal disease is one of the most common dental and oral health problems. Periodontal disease is characterized by inflammation as well as damage to the periodontal tissues, including the alveolar bone, cementum, gingiva, and the periodontal ligament. This case is considered a dental and oral health problem with a high incidence in the community. Gingivitis and periodontitis are examples of periodontal diseases.¹⁻⁵

Periodontal disease is caused by bacterial plaque present on the surface of the teeth, which is a group of harmful bacteria, including *Tannerella forsythia*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, dan *Fusobacterium nucleatum* and so on. *Aggregatibacter actinomycetemcomitans* is the dominant bacterium in cases of aggressive periodontitis with a high incidence. Disease activity by these bacteria can result in damage to the periodontal tissue. Chlorhexidine (CHX) is one of the most practical and popular antimicrobials for the treatment of patients with periodontal disease. Gram-positive

and gram-negative bacteria can be prevented from growing using 0.2% chlorhexidine gluconate. Chlorhexidine does have certain drawbacks, such as the potential for tooth discoloration when in direct contact with other irrigating materials as well as the possibility of an allergic tissue reaction. Therefore, it is necessary to explore new therapeutic modalities.⁶⁻¹⁰

Indonesia is a country with the second-largest biological wealth in the world. Indonesia's potential for natural materials as medicinal ingredients is very wide for exploration and development, including being used as a new therapeutic modality for the treatment of bacteria *Aggregatibacter actinomycetemcomitans*. *Jatropha curcas* plant is one of the ingredients that has been widely used for traditional medicine in the community, especially for the leaves. This plant is rich in active compounds such as alkaloids, tannins, phenols, flavonoids, and saponins, which are effective against gram-positive and gram-negative bacteria.¹¹⁻¹⁵ This study aimed to determine the effectiveness of *Jatropha curcas* extract (*Jatropha curcas* L.) as an antibacterial against *Aggregatibacter actinomycetemcomitans* in vitro.

2. Methods

This study was an in vitro experimental study with a post-test-only approach with a control group and used bacteria *Aggregatibacter actinomycetemcomitans* obtained from the Microbiology Laboratory Research Centre Universitas Airlangga. A total of 28 petri dishes containing bacterial colonies *Aggregatibacter*

actinomycetemcomitans were used in this study, which was further divided into 7 test groups, where each test group had 4 repetitions of the test, namely: Groups 1-5 (K1-K5) were the treatment group with *jatropha* extract with concentrations of 25%, 35%, 45%, 55% and 65%, K6, and K7 were the control groups, namely positive control (chlorhexidine) and negative control using DMSO. This study was approved by the medical and health research ethics committee, Faculty of Medicine, Dentistry and Health Sciences, Universitas Prima Indonesia, Medan, Indonesia.

Jatropha extract was carried out by maceration method using 70% ethanol solvent for 3x24 hours. Furthermore, the maceration resulting from the maceration process is carried out by an evaporation process using a rotary evaporator to obtain a thick extract. Each extract and control compound was put into a test petri dish, wherein the diameter of the inhibition zone in millimeters was assessed using a digital caliper. Inhibition zone diameter data were analyzed SPSS program for Windows 25.0 (IBM, USA). Univariate and bivariate analyzes were performed to compare the inhibition zone diameters of each treatment and control group.

3. Results

Table 1 presents the results of the phytochemical screening test of *Jatropha curcas* extract. *Jatropha curcas* extract contains various secondary metabolites, namely: alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides.

Table 1 Phytochemical screening test.

Secondary metabolites	Reactor	Observation	Results
Alkaloid	Dragendroff	Turbid	+
	Bouchardart	Turbid	+
	Mayer	Turbid	+
	Wagner	-	-
Flavonoid	Mg _(s) +HCl _(p) +Amile Alcohol	Yellowish red	+
Saponin	Aquadest+Alcohol 96%	Foam	+
Tannin	FeCl ₃	Dark blue	+
Terpenoid/Steroid	Lieberman-Bourchat	Blue	+
	Salkowsky	-	-
Glycosides	H ₂ SO _{4(p)} +Molish	Purple ring	+

Table 2 presents the antibacterial effectiveness of *Jatropha* leaf extract (*Jatropha curcas* L.) against the bacteria *Aggregatibacter actinomycetemcomitans*. The *Jatropha* extract treatment group with a concentration

of 35% to 65% had optimal antibacterial abilities as the dose increased. This study also showed that at a concentration of 25%, *Jatropha curcas* extract did not have the antibacterial ability.

Table 2. Antibacterial effectiveness of *Jatropha* leaf extract (*Jatropha curcas* L.) against bacteria *Aggregatibacter actinomycetemcomitans*.

Group	Inhibition zone diameter (mm) Mean±SD	p-value*
K1	0	1,00
K2	10.39±0.17	0,00
K3	14.16±0.65	0,00
K4	16.75±0.50	0,00
K5	19.01±0,63	0,00
K6	24.24±0,45	0,00
K7	0	-

*Post hoc LSD VS K7.

4. Discussion

The presence of these active compounds caused the ethanol extract of *Jatropha* leaves at four concentrations, namely concentrations of 35%, 45%, 55%, and 65%, used in this study to inhibit bacterial growth. *Aggregatibacter actinomycetemcomitans* plays an important role in cases of aggressive periodontitis. Increasing the concentration of the extract resulted in a larger inhibition zone formed due to the higher levels of secondary metabolites in the ethanol extract of *Jatropha curcas* leaves. The results of other studies stated that the tannin content in *Jatropha curcas* leaves was 7.41-8.28%. This is supported by the results of phytochemical screening in other studies, which stated that there are alkaloid compounds, steroids as well as saponins in the dried and fresh leaves of *Jatropha curcas*.¹⁶⁻¹⁸

Phenol compounds are known to change the nature of cell proteins and damage cell membranes. Phenolic compounds can be bactericidal as well as bacteriostatic, depending on their concentration. Large amounts of phenolic compounds have the ability to penetrate and damage the bacterial cell wall, causing protein precipitation in the bacterial cell. The amount of phenolic chemicals at low concentrations causes the enzyme systems in the bacterial cells to become paralyzed. The susceptibility of bacterial liposomal membranes to steroid components that

inhibit bacterial growth is closely related to the mechanism of action of steroids as antibiotics. Because membrane phospholipids are permeable to lipophilic substances, steroids can interact with them to reduce membrane integrity and change the shape of the membrane, both of which can lead to cell fragility and eventual lysis.^{19,20}

Terpenoids are as soluble as phenolic substances. Terpenoid compounds have a way of working similar to phenolic compounds. Namely, both prevent the entry of essential ions into the bacterial cell by preventing their transport. Terpenoids function to provide bonds to carbohydrates and fats that can interfere with the permeability of the MRSA bacterial cell wall. The polarity of the flavonoid compounds makes it easier to pass through the polar peptidoglycan layer than the nonpolar lipid layer, allowing them to have a dominant inhibitory effect on gram-positive bacteria over gram-negative bacteria. Saponin compounds have the function of inhibiting protein synthesis due to accumulation and damage to the constituent components of bacterial cells that are owned by saponin compounds. Inhibition of peptidoglycan growth in bacterial cells where the poor cell wall layer causes cell damage is the ability of alkaloid compounds. Tannin compounds have the function of inhibiting DNA topoisomerase and reverse transcriptase enzymes as a mode of antibacterial

action that prevents bacterial cells from forming. In tannins, there are cell wall polypeptides that cause imperfect cell wall development. This causes the bacterial cells to lyse due to physical and osmotic pressure so that the bacterial cells will die.²¹⁻²⁴

5. Conclusion

Jatropha curcas extract (*Jatropha curcas L.*) has an antibacterial effect as the concentration of the extract increases against *Aggregatibacter actinomycetemcomitans*.

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