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Anti-microbial Activities of Syzigium cumini leaves against Periodontophatic Bacteria (*Porphyromonas gingivalis*)

Actividades antimicrobianas de las hojas de Syzigium cumini contra bacterias periodontopáticas (*Porphyromonas gingivalis*)

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SUMMARY

Introduction*: Syzigium cumini has been known to have an anti-microbial effect and is traditionally used as medicine for some human diseases. However, only a few studies were done on water extracts of these leaves. This study aimed to elucidate the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of water extract of Syzigium cumini leaves against Porphyromonas gingivalis that represent periodontopathic bacteria.*
Methods: *S. cumini leaves were collected from local farms. The leaves were washed and dried. Water extraction was performed to collect the compound, then was diluted into concentrations of 1 %, 2.5 %, 5 %, 7.5, 10 %, 12.5 %, 15 %, and 20 %. The bacteria were grown in triplicates agar blood and then put in the anaerobic jar to incubate for 48 hours at 37^{0}C. Disc Diffusion test, MIC, and MBC were performed.*
Result: *The anti-microbial disc diffusion test of S. cumini extract against P. gingivalis indicated by the presence of a 6.9 ± 0.14 mm clear area around the extract starting at a concentration of 7.5 %, and 13.6±0.32 mm at a concentration 20 %. The minimum inhibition concentration of S. cumini was 0.156 %. The clear sight starting from the 6th well indicates that there was no growth of bacteria. After incubation for 2x24 hours, there was no growth of bacteria on the agar blood with a 2.5 % concentration of S. cumini extract.*
Conclusion*: Aqueous extract of S. cumini leaves has an anti-microbial potential effect against Periodontophatic bacteria which was represented by Porphyromonas gingivalis. Further research on Syzigium cumini leaves at the molecular level is advisable.*

Keywords: *Syzigium cumini, Porphyromonas gingivalis, Human diseases.*

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RESUMEN

Introducción: *Se sabe que Syzigium cumini tiene un efecto antimicrobiano y se usa tradicionalmente como medicamento para algunas enfermedades humanas. Sin embargo, solo se han realizado unos pocos estudios en los extractos acuosas de estas hojas. Este estudio tuvo como objetivo dilucidar la concentración mínima de inhibición (CMI) y la concentración mínima bactericida (CMB) del extracto acuoso de hojas de Syzigium cumini contra Porphyromonas gingivalis que representan bacterias periodontopáticas.*

Métodos: *Se recolectaron hojas de S. cumini de fincas locales. Las hojas se lavaron y secaron. Se realizó extracción con agua para recolectar el compuesto, luego se diluyó en concentraciones de 1 %, 2,5 %, 5 %, 7,5, 10 %, 12,5 %, 15 % y 20 %. Las bacterias se cultivaron en sangre de agar por triplicado y luego se colocaron en la jarra anaerobia para incubar durante 48 horas a 37^{0}C. Se ha realizado la prueba de difusión en disco, MIC y MBC.*
Resultado: *La prueba de difusión de disco antimicrobiano del extracto de S. cumini contra P. gingivalis indicó la presencia de un área clara de 6,9 ± 0,14 mm alrededor del extracto a partir de una concentración de 7,5 % y de 13,6 ± 0,32 mm a una concentración de 20 %. La concentración mínima de inhibición de S. cumini fue de 0,156 %. La vista clara a partir de los pozos 6, indica que no hubo crecimiento de bacterias. Después de incubar durante 2x24 horas, no hubo crecimiento de bacterias en el agar sangre con una concentración del 2,5 % de extracto de S. cumini.*
Conclusión: *El extracto acuoso de las hojas de S. cumini tiene potencial efecto antimicrobiano contra las bacterias periodontófagas representadas por Porphyromonas gingivalis. Se recomienda realizar más investigaciones sobre las hojas de Syzigium cumini a nivel molecular.*

Palabras clave: *Syzigium cumini, Porphyromonas gingivalis, enfermedades humanas.*

INTRODUCTION

Globally gingivitis is inflammation of the gum that affects more than 90 % of the population and is found in 70.4 % of children ages 5-15 years old (1). According to an Indonesian basic health survey in 2018, the prevalence of periodontitis starting from age 15 to 65 -year-old was more than 67.8 % (2). The inflammation mainly occurs because of dental plaque as bacterial biofilm surrounding the gum. Untreated gingivitis will cause periodontitis, alveolar bone damage and tooth loss. Dental plaque is a biofilm that attaches to the dental surface due to neglect of oral hygiene (3). During the development of gingivitis, the microflora increases in the number of species (4).

To prevent the expansion of biofilm into subgingival, removal of the supragingival biofilm and rebalancing a microflora by adequate home oral hygiene and by professional cleaning will eliminate gingivitis (5). As an adjunct to other oral hygiene measures such as tooth brushing and flossing, antiseptic mouthwash has been used to prevent and treat gingivitis. Some medication against gingivitis bacteria, such as metronidazole gel has been used to treat periodontitis (6).

Black-pigmented anaerobic gram-negative bacilli cause chronic gingivitis (7,8). It is characterized by brown or black pigment on agar blood. As an agent of periodontal disease (periodontopathic organism), one of the black-pigmented anaerobic is *Porphyromonas gingivalis* (5). The main colonization in the oropharynx and found almost solely at subgingival sites (5,6). *P. gingivalis* is non-motile, saccharolytic, and looks cox-shaped until it is short-lived. The initial stage of gingivitis is the colonization of this bacterium in the gingiva sulcus (7). At first, the bacteria colonize the periodontal environment and then attach to the layer of the surface of the tooth. *P. gingivalis* is found in the saliva layer on the surface of the tooth (7). This bacterium plays a very important role in virulence through the adhesion process with the human cells, able to inhibit the production of IL-8 by epithelial cells that can make the microorganisms avoid polymorphonuclear leukocytes, and the bacterial enzymes can facilitate tissue damage (7). There are some drugs to avoid the severity. However, antimicrobial resistance in patients worldwide is a global concern. Traditional medicine is the rising alternative medicine to overcome the hassle.

Eugenia Jambolana (*Syzygium cumini*) belongs to the family Myrtaceace. *Syzigium cumini* is a green tropical plant widely grown in Bangladesh, Pakistan, India, South America, Madagascar, Malaysia, Phlipina, and Indonesia (8). The plant has many names such as Duwet, Jamblang, Java plum, Jamun, Guava rivet, Indian Blackberry, and many more local names. The fruit taste is sweet-sour, and the color is purple when ripped. Based on empirical utilization, most of this plant has long been known as a traditional medicinal plant, especially for diabetes. The leaves are used to strengthen teeth and gums, treat vaginal discharge, abdominal pain, fever, gastropathy, dermopathy, and constipation, and inhibit the disposal of blood in the stool (9). *Syzigium cumini* fruits are edible, but seasonal. The leaves are available in all seasons, then it's easy to have as medicinal ingredients.

Some studies report that the stems, leaves, and fruits of *S. cumini* have activity as antioxidants, anti-inflammatories, antihelminthic, anticancer, antibacterial, and antidiabetic (10). The phytochemical and antioxidant effects of *S. cumini* show the presence of alkaloids, steroids, saponins, cardiac glycosides, carbohydrates, proteins, tannins, and phenols (11). Compared to other solvents in the percolation method, water extraction of these leaves resulted in the highest percentage of phenol and antioxidant capacity (12). *S. cumini* leaves have anti-microbial effects against gram-positive and gram-negative bacteria such as *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Staphylococcus aureus* (11,13,14) and some multidrug-resistant pathogenic bacteria (15). This study aimed to assess the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of the water extract leaves against *Porphyromonas gingivalis* as a periodontophatic microorganism.

METHODS

This *in vitro* study was an experimental study conducted at a microbiology laboratory. The variables were water extraction of *Syzigium cumini* leaves and oral microbiota representing periodontopathic bacteria *Porphyromonas gingivalis*.

Extraction

S. cumini leaves were collected from local farms in Sukorejo, Wonosobo, Central Java, Indonesia. The area longitude is 109.98, latitude -7.14, and elevation 3 527 feet. The leaves were washed under the tap water for 15 minutes to clean up, then dried in the oven at 50°C for 3 days. The dried leaves were crushed into powder.

The dried leaves powder was soaked in water at a concentration of 10 % w/v, equal to 25 g of powder in 250 mL of water in a cone flask, then shaken at 120 rpm, 27°C using an incubator shaker. After 24 hours, the solvent was filtered using Whatman No 1 and vacuumed filter to separate the extract from the solvent. The filtrate was centrifuged at 5 000 rpm for 10 minutes, at 27°C to separate the supernatant completely. The supernatant was then piped out, leaving residue on the bottom of the centrifuge tube. The collected solvents were then dried using a rotary evaporator (IKA RV8 model) until dry. The collected extract was left under the smoke hood for 2 days until the solvent completely evaporated. The yield was then collected and stored at -20°C before further analysis. The extract was diluted into concentrations of 1 %, 2.5 %, 5 %, 7.5 %, 10 %, 12.5 %, 15 % and 20 %.

Anti-microbial Disk Diffusion Test.

Freeze-dried cultures of *P. gingivalis* ATCCA 33277 have been purchased. The bacteria were grown in triplicates of agar blood and then put in the anaerobic jar to incubate for 48 hours at 37°C. The holes were made by punching the prepared agar blood using a sterile tube. Then it was placed each concentration of extract into each hole, then put all covered plates into an anaerobic jar. The agar blood was left in the anaerobic jar for 2 days. The data collection was done by determining the clear area from the bacteria-free around the hole. It was measured the diameter of the free area using a gauge on each plate. The hole with chlorhexidine was used as a control. Zones of microbial growth inhibitions were recorded at 24 and 48 hours.

Minimum inhibitory concentration test (MIC)

The minimum inhibitory concentration (MIC) was performed by microdilution technique using 6 rows on 72 wells of microtiter plates and Mueller Hinton Broth (MHH) as a medium. According to the Clinical & Laboratory Standards Institute (CLSI), 100 mL MHB was added to all wells. On the first to third rows microtiter plates were added 100 mL of extract and then homogenized. The extract concentration on the first wells was 5 %, then reduce a half accordingly until the tenth wells. Therefore, the concentration of the extract was 5 %; 2.5 %; 1.25 %; 0.625 %; 0.3125 %, 0.156 %; 0.078 %, 0.039 %, 0.0195 %, and 0.00975 %, respectively. Next, from the fourth to the sixth rows microtiter plates added 100 mL of chlorhexidine. Inserted bacteria into the well's microtiter plates as much as 10^5

Mc Farland. The microplates were placed in an anaerobic jar and incubated for 48 hours at 37^{0}C. The visual examination was carried out to determine the MIC that can be seen from the absence of bacterial growth.

Minimum Bactericidal Concentration Test (MBC)

Planting the inoculum from the clear well into MHA and incubating anaerobically for 48 h at 37^{0}C. MBC was determined by assessing at the minimal concentration of extracts in which there was no growth of bacteria in the MHA.

RESULTS

The activity of *S. cumini* extracts against *P. gingivalis* was indicated by the presence of a 6.9 ±0.14mm clear area around the extract starting at a concentration of 7.5 %. At 20 % concentration, the clear zone was 13.6±0.32 mm. The results of the anti-bacterial test by disk diffusion test method (Kirby Bauer method) are presented in Table 1.

The result of the anti-microbial activity extract *S. cumini* against *P. gingivalis* can be seen in Table 2.

Table 1

The diameter of the clear zone area in response to Syzigium cumini (leaves)

Water extract (%)	R1	R2	R3	Mean	SD	Diameter of clear zone (mm)
1	0	0	0	-	-	-
2.5	0	0	0	-	-	-
5	0	0	0	-	-	-
7.5	7	6.8	0	6.9	0.14	6.9 ±0.14
10	11.2	11.1	11.8	11.4	0.38	11.4 ±0.38
12.5	12.6	12.5	12.9	12.7	0.21	12.7 ±0.21
15	12.9	12.3	13.2	12.8	0.46	12.78±0.46
20	13.8	13.2	13.7	13.6	0.32	13.6 ± 0.32
CHX %	R1	R2	R3	Mean	SD	Diameter of clear zone (mm)
0.2	22.1	20.5	22.8	21.8	1.18	21.8 ± 1.18

Table 2

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

Bacteria	Test Sample	Concentration (%) C(-)	C(+)	0.00975	0.0195	0.039	0.078125	0.15625	0.3125	0.625	1.25	2.5	5	MIC	MBC
	Water extract	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)		
		(-)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	0.15	2.5
		(-)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)		
P. Gingivalis	Test Sample	Concentration (%) C(-)	C(+)	9.77 E-05	0.000 195	0.000 391	0.000 781	0.001 563	0.003 125	0.006 25	0.01 25	0.025	0.05	MIC	MBC
	CHX	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)		
		(-)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	0.0004	0.0008
		(-)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)		

(+): bacteria growing.
(-): bacteria do not grow.

DISCUSSION

S. cumini leaves extract has anti-microbial effects on anaerobic bacteria and anaerobic facultative bacteria such as *Staphylococcus aureus* and *Escherichia coli* (15,16). However, the best way to extract *Syzigium cumini* has not been much explored (17-19). The potential water-based extraction was better than methanol extract and petroleum ether against four types of bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subitils*, and two types of fungi (*Aspergillus niger*, and *Candida albicans*) (17). This is reinforced by the results of Sukmasari et al. (12) which stated that out of some solvents in the percolation method, the water extract of *Sygyzium cumini* leaves has a total phenolic content and better antioxidant capacity. Supporting by a previous study on some oral microbiota (11,15). The aqueous extracts of *S. cumini* leaves in this study demonstrated antibacterial activity against *P. gingivalis* as one of the dominant black-pigmented or periodontopathic bacteria that cause periodontal diseases through the MIC and MBC test.

The extracts used in the disc diffusion test study exhibited antibacterial activity starting at a concentration of 7.5 % and 20 % concentration, the clear zone was 13.6±0.32 mm. To support the disc diffusion test, the broth microdilution test method found out the minimum concentration of *S. cumini* leaf extract against *P. gingivalis* bacteria was 0.156 % and the concentration of bactericide activity at least at a concentration of 2.5 %. Therefore, these results show that *S. cumini* leaves have antibacterial power, especially against anaerobic bacteria. This antibacterial activity may be attributed to the alkaloids, glycosides, steroids, terpenoids, saponins, flavonoids, and perhaps resins since these secondary metabolites were detected in the extracts (10,12,20,21).

The aqueous extract of *S. cumini* leaves has a potential effect to be used as an anti-microbial agent on black pigmented or periodontopathic bacteria which represent by *Porphyromonas gingivalis*, even though chlorhexidine solution presents a wide spectrum anti-microbial action (22). Therefore, further laboratory and clinical studies are required to determine its potency and safety at a molecular level.

CONCLUSION

Aqueous extract of *S. cumini* leaves has the potential effect to be used as an anti-microbial agent on Periodontophatic bacteria which represent by *Porphyromonas gingivalis*.

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