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RESEARCH ARTICLE

Anticaries activity of ethanolic extract from ant-nest plant (Myrmecodia Pendens Merr. & L.M Perry) against hyaluronic acid resistant *Streptococcus mutans*

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ABSTRACT

Background: *Streptococcus mutans* is known as primary microorganism in the formation of dental plaque and tooth decay. **Aims and Objectives:** To determine the antibacterial activity of ant-nest plant ethanol extract to inhibit *S. mutans* clinical isolates growth *in vitro*. **Materials and Methods:** The ethanol extracts obtained from the ant-nest plant were studied for antibacterial activity against cariogenic *S. mutans* obtained from patients with clinically identified carious lesions, using the agar diffusion method. Chlorhexidine gluconate was using as a comparative drug. The simplisia of ant-nest plant was extracted using a maceration method. The phytochemical screening was taken using standard methods. The minimum inhibitory concentration (MIC) test was performed by a macrodilution method and following by subculturing the overnight result onto the surface of agar media. **Results:** Phytochemical screening of ant-nest plant ethanol extract revealed the presence of polyphenols, flavonoids, tannins, and saponins. The extract showed intensive activity and totally inhibited the growth of *S. mutans* clinical isolates at 1.25% w/v. In comparison, the chlorhexidine gluconate, essential oil of plants and povidone-iodine in a ranged tested concentration also had given inhibition effect to all hyaluronic acid resistant *S. mutans* isolates. **Conclusion:** It can be reasoned that the ethanolic extract of ant-nest plant gave potent and direct antibacterial effect on *S. mutans*.

KEY WORDS: Ant Nest; Antibacterial; *Streptococcus mutans*; Caries; Hyaluronic Acid

INTRODUCTION

Biofilms can be defined as oral microbial-plaque communities and attached to a surface.[1,2] The microorganisms undergo transition from planktonic form two cells that attached to the host surface.[2] These bacteria communicate through physical interactions called segregation and coadhesion, as comfortably as other physiological and metabolic interactions.[3] Oral streptococci are present in great numbers in dental plaque, and several types interact with the enamel salivary pellicle to form a biofilm on tooth surfaces.[1,3,4]

Streptococcus mutans is the primary bacteria of oral streptococci that plays a significant part in the formation of dental plaque and caries.[3,5] Dental caries affects 60–90% of children in industrialized countries and approximately 10–15% of adults are affected by the severe periodontal disease. These plaques-mediated diseases lead to premature dental exfoliation and have a significant impact on the quality of life.[6-8] Periodontal disease has been also implicated with systemic chronic diseases such as cardiovascular disease.[9] An important element in both caries and periodontal disease is the oral microflora. As the primary etiological agent of

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human dental caries, *S. mutans* has developed multiple mechanisms to colonize the tooth surface and, under certain conditions, to become a numerically significant species in cryogenic biofilms.[10] Thus, biofilm control by mechanical debridement and use of adjunctive antimicrobials is of utmost importance in the prevention of plaque-mediated diseases.[11]

The use of mouthwashes and toothpastes containing antimicrobial components such as triclosan and chlorhexidine was purposed to inhibit the *S. mutans*. Among all active substances in mouthwash, chlorhexidine is the golden standard for inhibiting dental plaque formation of *S. mutans*.[12,13] Nonetheless, chlorhexidine has also been connected with adverse effects, including mucositis, altered taste, and staining of dental tissues and restorations.[14,15] Then, as regards the triclosan use in "everyday products" has been enhanced, as overexposure of microorganisms to this agent might propagate resistance.[15] Herbal mouthwash can be an efficient and secure treatment for tooth decay. Many studies had performed the antimicrobial effect of ant-nest plant ethanolic extract, because of containing secondary metabolites that can behave as natural antibiotics, such as the flavonoid, polyphenol, and tannin.[16] Those metabolites were also found in leaf and seed extracts of *Anisomeles indica* Linn. that performed anticaries activity against six clinical isolates of *S. mutans*.[17] Thus, this study was directed to evaluate the anticaries activity of ant-nest plant extract for the natural anticaries candidates in the hereafter.

MATERIALS AND METHODS

Material

The ant-nest plant (Myrmecodia Pendens Merr. & L.M. Perry) used in this study was obtained from Wamena, Papua, Indonesia. The ant-nest tuber had been defined in the Laboratory of Plant Taxonomy Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjadjaran, Bandung Sumedang Km 21 Jatinangor Sumedang, West Java. Several commercial mouthwashes containing chlorhexidine gluconate, a mixture of phenol with essential oils, povidone-iodine and hyaluronic acid, were applied as reference substance. The *S. mutans* was isolated from the dental plaque of patients at the Integrated Service Unit of Padjadjaran University, Bandung, Indonesia. The culture media that were used are blood agar base (Oxoid), fresh sheep blood, Nutrient Agar (NA-Oxoid), and nutrient Broth (NB-Oxoid). The chemicals that were used are ethanol 70%, distilled water, amyl alcohol, ammonia, dimethylsulfoxide (DMSO), chloroform, acetic acid solution, physiological NaCl solution 0.9%, barium chloride solution, sulfuric acid solution, Lugol's solution, n-butanol (Bratachem), ferric chloride reagent, Dragendorf reagents, and Lieberman - Burchard reagent Mayer.

Extraction

The powdered of ant-nest plant simplisia were subjected to macerator using ethanol 70% as the solvent during 3 × 24 h. The extracts were then concentrated using a rotary evaporator in 40–50°C until dried extract with a constant weight was obtained.[18]

Phytochemical Screening

The screening of chemical elements was carried away to detect alkaloids, polyphenols, flavonoids, tannins, and saponins in the condensed extract. The screening was done using Farnsworth method.[19]

Bacterial Suspension Preparation

The bacteria were grown in NA medium at 37°C for 20 h. A loop full of the colonies was taken and suspended into a 5 ml of sterile physiological NaCl, then calibrate until the turbidity is equal to Mc Farland 0.5.

Antibacterial Activity

The antibacterial activity of the ant-nest plant extract was determined by the agar diffusion method. A 20 µL of standardized *S. mutans* suspension was put into a sterile Petri dish containing 20 ml of NA medium (40–45°C). The bacterial suspension was then homogenized manually using the agar and allowed to solidify. After the solid media achieved, the media were perforated to make holes as the storage of the extract and chlorhexidine gluconate. The extracts were diluted in several concentrations (10, 20, 40, and 60% w/v) using DMSO. For the standard, 0.1% chlorhexidine gluconate was used. Each of 50 µL ethanolic extracts of the ant-nest extract and 0.1% chlorhexidine gluconate was dropped into each hole using a micropipette. Control positive and the negative were made. The plates were incubated at 37°C for 20 h. The inhibition zone around each hole was observed, and the diameter was measured using calipers.

Resistance Test

The sensitivity of *S. mutans* clinical isolates against commercial mouthwashes was carried out by resistance test using the agar diffusion method. The mouthwashes contain several active ingredients, such as chlorhexidine gluconate, phenol mixture with essential oil, povidone-iodine, and hyaluronic acid. A volume of 20 ml NA and 20 µL standardized *S. mutans* suspension was put into a sterile Petri dish. The mixture then homogenized and allowed to solidify. The medium was perforated, and each of 50 µL commercial mouthwash put into each hole. All plates were incubated for 20 h at 37°C. The diameter of the inhibitory zone was measured using a caliper.

Determination of Minimum Inhibitory Concentration (MIC)

The determination of MIC value was assessed by serial dilution method. The broth dilution method was applied

to determine the lowest concentration of antimicrobial agent which inhibits the visible colony growth of test microorganisms being investigated.[20] The ethanol extracts of ant nest were dissolved in DMSO and then diluted with NB medium until achieved concentration with a range of 0.625–1.25% w/v. The volume of 10 µL standardized cell bacterial suspensions was dropped into each tube, followed by incubation at 37°C for 20 h. MIC was determined from the smallest concentration which did not show any turbidity. MBC was determined from the MIC range using the spread plate method. NA media in Petri dishes were subcultured from tubes without growth and incubated at 37°C for 20 h. The viable colonies on the agar surface were counted.[21]

RESULTS

The Yield of the Extraction

The maceration method was conducted by immersing the powders of simplisia in ethanol as an organic solvent. The solvents will penetrate the cell wall and into the cavity of the cell that contains the active substance so that the active substance will dissolve. Due to the difference between the solution concentrations of the active substance in the cell, the solution is pushed out.[22] The extraction process of 500,42 g of ant-nest simplicia yielded 77,47 g of thick extract; therefore, the rendemen value was 15.48%. The morphology of the extract was powder-shaped, reddish-brown color, typical smelt, and had sour taste. The water content value of the extract was 0.5% w/v. The value of water content was important to be determined because if its value more than 10%, the extract will be contaminated by a fungus.

Phytochemical Screening Result

The result of secondary metabolite analysis in the extract and simplisia showed that there were no differences of secondary metabolites contain. The class of secondary metabolite compounds found in simplicia and extract was flavonoids, tannins, polyphenols, and saponins.

Resistance Test Result

The resistance test result showed that both clinical isolates *S. mutans* were sensitive against several active substances containing in commercial mouthwash such as chlorhexidine gluconate, mixture of thymol, eukaliptol, menthol, and methyl salicylate, and povidone-iodine at minimal concentration 0.5%. The resistance test result can be seen in Table 1.

Antibacterial activity result

As shown in Table 2, the ant-nest extract possessed antibacterial activity against both of *S. mutans* clinical isolates at all tested concentration.

MIC determination results

From MIC data presented in Table 3, it can be observed that the dilution of the extract showed the gradual sensitivity of both *S. mutans* isolates. The clearance of bacterial suspension was confirmed by observing the lines behind tubes are clearly visible, compared with the negative control. In this study, the suspensions in tube became clearly visible at a concentration of 1.25%. The MIC values for different isolates ranged from 0.925 to 1.25% w/v. The lowest MIC value of the extract

Table 1: The result of *S. mutans* isolates resistance test

The active substances of commercial mouthwashes	Diameter zone of inhibition (mm)	
	1st isolate	2nd isolate
Chlorhexidine gluconate 0.1%	18.4±0.002	16.4±0.006
Chlorhexidine gluconate 0.05%	16.5±0.006	15.3±0.006
Chlorhexidine gluconate 0.025%	15.1±0.006	14.1±0.006
Thymol 0.06%; eukaliptol 0.09%; menthol 0.04%; methyl salicylate 0.05%	10.3	10.8
Thymol 0.03%; eukaliptol 0,045%; menthol 0.02%; methyl salicylate 0.025%	10.0	10.6
Thymol 0.015%; eukaliptol 0.0225%; menthol 0.01%; metil salisilat 0.0125%	9.4	10.1
Povidone-iodine 1%	12.2±0.006	11.4±0.006
Povidone-iodine 0.5%	10.2±0.006	10.1±0.006
Povidone-iodine 0.25%	-	-
Hyaluronic acid 0.1%	-	-
Hyaluronic acid 0.05%	-	-
Hyaluronic acid 0.025%	-	-

Table 2: The inhibition diameter of ant-nest plant extract

Concentration (%w/v)	Diameter of inhibition (mm)	
	Ist isolate	2nd isolate
60	22.1±0.002	22.2±0.002
40	21.1±0.002	21.6±0.002
20	18.9±0.002	20.1±0.002
10	15.7±0.002	16.8±0.002
Chlorhexidine gluconate 0.025	18.2±0.002	17.8±0.002

Table 3: The MIC value of ant-nest extracts

Concentration % w/v	*S. aureus* colony	
	1st isolate	2nd isolate
1.25	-	-
0.925	+	+
0.825	+	+
0.725	+	+
0.625	+	+

showed the potent activity of the extract. The minimal concentration could minimize the unwanted of side effect probability.

DISCUSSION

The resistance test result showed that both clinical isolates *S. mutans* were sensitive against the extract and tested commercial mouthwash. Among those active substances, the chlorhexidine gluconate gave the best inhibition against both *S. mutans* isolates. This is in correlation with the study according to which chlorhexidine has been found to supressed *S. mutans* in saliva and dental plaque.[23,24] The same results also demonstrated in another research study, that chlorhexidine was found to be more effective as compared to povidone-iodine.[25] Only when compared with a commercial mouthwash containing a mixture of essential oils, povidone-iodine gave better inhibition result at a concentration above 0.5%. Both of *S. mutans* isolate did not produce inhibition zones around the well containing hyaluronic acid; it implies that both isolates had resistance. This is because of loss of effect of the mouthrinse. However, some research had reported that the effect of mouthrinse could be loss after a period of time. However, mouthrinse containing chlorhexidine gluconate performed a significant difference between post-restorative bacterial count and mean bacterial count in chlorhexidine group.[25] This can be attributed to chlorhexidine's superior antiplaque effect that can be explained in terms of its superior degree of persistence at the tooth surface or more correctly its superior persistence of antibacterial effect at the tooth surface.[26,27] However, the side effect, including mucositis, altered taste, and staining of dental tissues and restorations, and the need for frequent application of chlorhexidine, has promoted to search the alternatives that are more appropriate and save for consumers.[14,15,27,28] Interestingly, ant-nest extract was able to inhibit both of *S. mutans* isolates, which were known to be resistant to commercial mouthwashes containing hyaluronic acid. The inhibitory diameter zones of the extract were gradually increased as the increment of extract concentration.

The ant-nest extract's ability in inhibiting the growth rate of *S. mutans* was due to its active antimicrobial secondary metabolite contents, such as flavonoids, tannins, polyphenols and saponins. Phenol group such as tannin work destructively to the bacterial cell wall and interact with DNA. Tannin is a chemical substance in the polyphenol group which suspected to bind one of bacterial protein, which is adhesion and if occur it can damage receptor availability on the bacterial cell surface. Chlorhexidine gluconate is still effective against both isolates at a lower concentration than the extract. These data revealed that chlorhexidine gluconate remains the gold standard for anticaries activity. The chlorhexidine gluconate efficacy can be attributed to its bacteriostatic and bactericidal properties and its substantivity within the oral cavity. However, the administration of chlorhexidine requires a careful clinical evaluation of the clinical situation and an accurate diagnosis, hence, should be applied only under professional supervision.[29] Therefore, although the ant-nest extract is far less than that of chlorhexidine gluconate, it should be noted that the ant nest extract had been empirically safe for treating several diseases by Papua people in Indonesia.

CONCLUSION

Based on the result above, it can be concluded that the ethanolic of the ant-nest extract is moderately active for antibacterial activity against hyaluronic acid resistant *S. mutans*, especially for the treatment of caries.

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