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Antibacterial of Terpenoid A from Sarang Semut (Myrmecodia pendans) Against Streptococcus mutans

Meirina Gartika[1]*, Wartadewi[2], M.S. Mariam[3], D. Kurnia[4] and M.H. Satari[5]

[1]Department of Pediatric Dentistry,Faculty of Dentistry, Universitas Padjadjaran-Bandung, Indonesia.
[2,3,5]Department of Oral Biology, Faculty of Dentistry, UniversitasPadjadjaran, Bandung, Indonesia
[4]Laboratory of Natural Products Chemistry, Department of Chemistry, Faculty of Science, Universitas Padjadjaran-Bandung, Indonesia

Abstract : Caries is one of human diseases, which most commonly occur. This disease is the result of hard tooth tissue damage caused by *Streptococcus mutans*. Previous studies have introduced alternative antibacterial agent extracted from *Myrmecodia pendans* Merr & Perry, an indigenous plant from Papua. It has antibacterial-active phytochemical compounds and have been used empirically as natural medicine. This study was done to determine an active compound derived from *M. pendans* and to investigate its activity against *S. mutans* ATCC 25175. Ethyl-acetate soxhlet method was performed to extract of *M. pendans*, subsequently separated and purified through chromatography. The compound is determined as terpenoid A. Antibacterial activity of the compound was tested using Kirby-Bauer method with 0.5 Mc Farland in blood agar plate. The inhibition zones of terpenoid A after 48 hours incubation for 10.000, 5.000, 1.000 µg/mL were 13.7, 13.6, 11.8 and 14,6 mm respectively. Minimum Inhibitory Concentration (MIC) of terpenoid A against *S. mutans* was 39 µg/mL and Minimum Bactericide Concentration (MBC) was 312.5 µg/mL.
Keywords : *Streprococcus mutans*, Terpenoid A, *Mymecodia pendans, antibacterial activity*

Introduction

Sarang semut plants (*Myrmecodia pendans*) is the plant originated of local society in Papua island which is located in eastern Indonesia. Sarang semut plants are known by people of Papua as a medicinal plant[1,2]. Sarang semut plants can treat various diseases including cancer, tumors, gout, diarrhea, fever and each other deseases[1]. This plant also scattered from Malay Peniasula to the Philiphines, Cambodia, Sumatera, Java, Papua, Cape York as well as the Solomon island. *M. pendans* is a member of *Rubiaceae* family with 5 genus.. *Hypnophytum formicarum*, *Myrmecodia pendans* and *Myrmecodia tuberosa* are consider to have medicinal value[1,2].

Previous studies have introduced alternative antibacterial agent extracted from plants. *Myrmecodia pendans* Merr&Perry, an indigenous plant from Papua, possesses potential antibacterial-active phytochemical compounds and have been used empirically as natural medicine[3,4]. The using of herbal medicines is one of the solutions to this problem[5,6] . Ethanol extract of *Myrmecodia pendans* has antibacterial activity against *Escherichia coli*, at a concentration of 25 and 50%. Ethanol extract also has antibacterial activity against *Shigella dysentriae* and *Klebisella pneumonia*[6]. In our previous research found that EtOAc extract of *M.*

pendans can inhibit the growth of *Streptococcus mutans*. Terpenoid compounds include diterpenoid, monoterpenoid, and sesquiterpenoid reported has many antibacterial and antimicrobial activity[7-10]. Thus, we focused to find antibacterial of terpenoid against *S. mutans*.

One of the factors that support the occurrence of caries is the presence of cariogenic oral bacteria. Pathogenic bacteria in the plaque that became the main etiologic of tooth damage was *Streptococcus mutans*, often identified consistently as the most prominent bacteria[11]. *Streptococcus mutans* is thought to be the bacteria that cause dental caries, because of its ability to form biofilm known as plaque on the surface of the teeth[12]. Currently caries prevention is aimed at preventing dental plaque formation or reducing the amount of *Streptococcus mutans* in plaque. Some chemicals are often used in dental products that can suppress the growth of the bacteria. The option for caries prevention methods are antimicrobial compounds, artificial sweetener, therapy of cariogenic bacteria with avirulent strains or anti-mutans vaccine. Most attention is directed to the use of antibacterial compounds that can inhibit plaque formation[13]. Chlorhexidine is a gold standard of antiplaque mouthwash. It has a broad antimicrobial spectrum and long service life, making it potentially strong in the inhibition of plaque. Chlorhexidine provides side effects such as yellow-brown staining in teeth, burning sensation in soft tissues of the mouth, pain and dryness of the mouth tissue[14,15].

Experimental

General experimental procedures – Dried of *Myrmecodia pendans* were supplied from Papua inland and were identified by Joko, Laboratory of Plants Taxonomi, Depatrment of Biology, Faculty of Science Universitas Padjadjaran, Bandung, Indonesia. Kiesel gel 60 silica gel resin was used for column chromatography (c.c.) (Merck, Darmstadt, Germany) and the ODS was a Li Chroprep RP-18 (Merck). TLC analysis was carried out using Kiesel gel 60 F_{254} and RP-18 F_{254S} (Merck). Deuterated solvents were purchased from Merck Co. Ltd. and Sigma Aldrich Co. Ltd. (St. Louis, MO, USA). *Streptococcus mutans* ATCC 25175 was used for the test, Muller Hinton broth and Muller Hinton agar as medium, and chlorhexidine as positive control.

Extraction and Isolation - The air-dried ground plant material (*Myrmecodia pendans*, 1.5 Kg), was extracted with EtOAc at 40°C on heating mantle of soxhlet extractor. The extract was evaporated to yield a residue (55.7 g). Residue (EtOAc extract) was subjected to column chromatography stationary phase silica gel 60 (300 g, 70-230 mesh, Merck, Munish, Germany) eluting with a gradient10% *n*-Hexane/EtOAc, to yield eleven fractions. Fraction 3 (5.7 g) was subjected to column chromatography stationary phase silica gel 60 (9 g, 70-230 mesh) eluting with a gradient 2.5% *n*-Hexane/EtOAc (100:0 to 25:75 v/v), to yield eleven fractions. Fraction 3.7 to fraction 3.9 (62.8 mg) was subjected to an RP-C18 column, eluting with a gradient 5% of H_2O/MeOH to yield **1** (33.2 mg).Rf value for compound **A** is 0.62 on TLC (Silica Gel60 F_{254S}) with eluting *n*-hexane/ethyl acetate(4:1). Isolated compound was characterization by infrared, Nuclear Magnetic Resonance JEOL ECA type and Mass Spectroscopy.

Antibacterial assay - Disk diffusion was conducted to determine antibacterial effects of compound on *S. mutans* ATCC 25175. The Kirby-Bauer disk diffusion susceptibility test was used to determine the sensitivity or resistance of *S. mutans* to compounds. Compounds (samples) were diluted with methanol, however chlorhexidine (controls) were diluted with water. A number of one ose bacteria of the stock was inoculated into a sterile test tube containing Muller Hilton suspension as much as 5 mL up to the level of turbidity 0.5 Mc Farland. Achievement of turbidity is done by comparing with the standard then incubated for 48 hours at 37°C. Cotton sticks dipped in bacterial suspension and applied on the surface of an agar medium until evenly distributed. Furthermore, as many as 50µL sample, positive control (chlorhexidine) and negative control (methanol) dropped on paper disk and then placed on agar diffusion. Then incubated at 37°C for 48 hours. The diameter of clear zone around the disk was observed. Inhibition zone around the disk was measured by using a caliper to determine the major inhibitory zone[16]. Tests were performed in triplicate.

Determining MIC value used micro dilution method. The bacterial cells were pre-cultured in Muller Hinton broth at 37°C under anaerobic conditions. They were incubated in the presence of compounds with the concentrations obtained by serial two-fold dilution at 37°C without shaking in the same broth for 48 hours on microplates as shown in procedure that used in Clinical and Laboratory Standards Institute, and their MICs were estimated as the lowest concentrations where the bacterial cells were not observed visually as reported previously, and given based on triplicate experiments. Water or MeOH used for dissolving compounds where

water and MeOH have no effect. The positive control, chlorhexidine was dissolved in water. The minimal inhibitory concentration values (MIC) and the minimal bactericidal concentration (MBC) of the pure compounds were determined by using the micro dilution broth method in 96-well micro plates[17]. Tests were performed in duplicate.

Result and Discussion

Compound isolation – The active compound from Sarang semut is indicated by the appearance of a single stain on the chromatogram (Figure 1). The compound obtained was yellow and oilic and, was called terpenoid A.

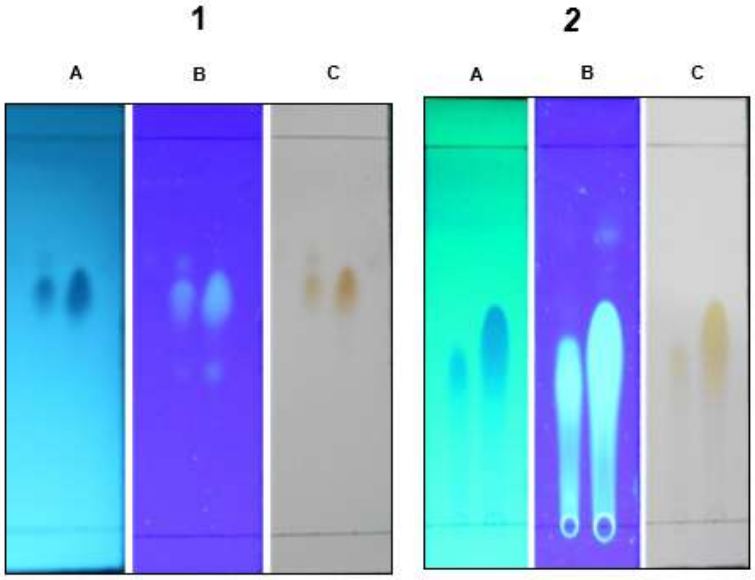

Figure 1. Chromatogram of Terpenoid A : the reversed phase, silika G_{60} RP-18 (MeOH 100%) and normal phase, silika G_{60} (n-hexane-EtOAc/ 3:2)

Antibacterial activity - Antibacterial activity test was done against *Streptococcus mutans* using disc diffusion method (also known as Kirby-Bauer method). The inhibition zone of terpenoid A against *S. mutans* was smaller than chlorhexidine as a positive control (Table 1).

Table 1. Antibacterial activity of Terpenoid A against *Streptococcus mutans*

Compound	Inhibition Zone of compound (mm) at Concentration (µg/mL)									MIC (µg/mL)	MBC (µg/mL)
	10000			5000			1000				
			Average			Average			Average		
Terpenoid A			13.7			13.6			11.8	39	312.5
Chlorhexidine*			**			16.7			**	1.9	31.2

*standard
**not yet

The inhibition zone of terpenoid A at 10,000, 5,000 and 1,000 µg/mL were 13.7, 13.6, and 11.8 mm respectively. Meanwhile the inhibition zone of Chlorhexidine as positive control were 16,7, 13,7, and 11,8 mm respectively (Figure 2). According to the protocol of Clinical Laboratory Standard International, the categories

of susceptibility on bacteria are susceptible (≥ 20 mm), intermediate (15-19 mm), and resistant (≤ 14 mm)[18]. Thus, based on these criteria, terpenoid A is resistent, while chlorhexidine is intermediate.

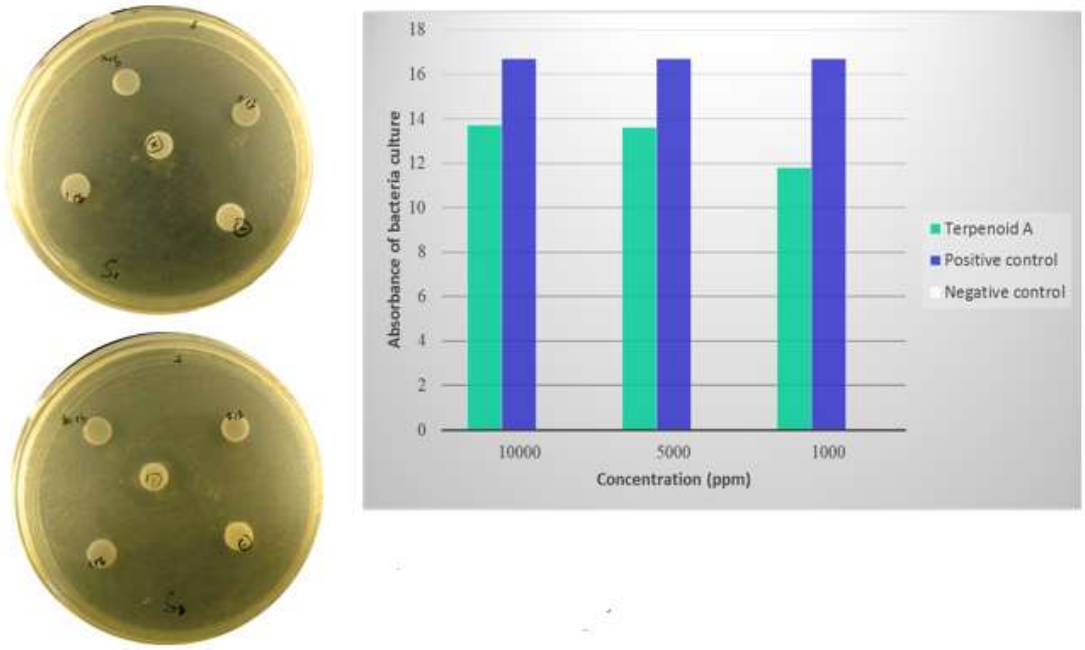

Figure 2. Susceptibility of Terpenoid A against *Streptococcus mutans*

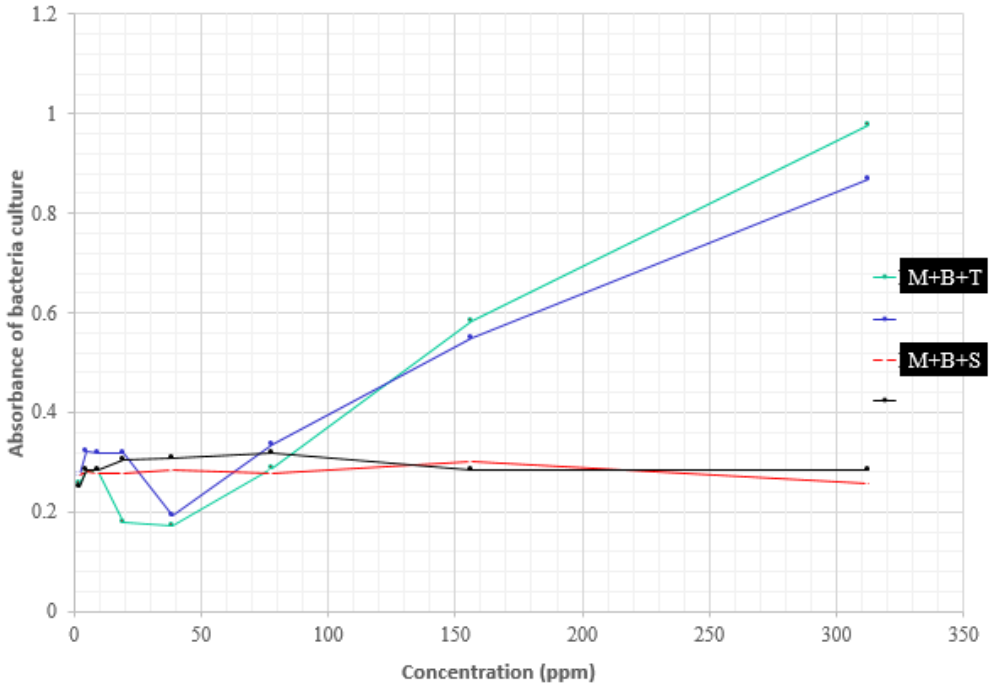

Figure 3. MIC and MBC value of Terpenoid A against *Streptococcus mutans*

The value of MIC and MBC measured by micro dilution method using microplate reader at 630 nm wave length were 39 and 312.5 µg/L. MIC value of OD sample (Medium + bacteria + sample) is determined only when the OD control (Medium + bacteria) is stable, as shown in Figure 3. The graphic reveals that there is

no increasing amount of contaminant in the well. In other words, the growth of bacteria is zero. On the other hand, the value of OD of the compound which contains medium, bacteria, and the compound decreases significantly. This means the compound has an effect on the growth of bacteria. The MIC value is on the lowest point of inhibition that occured in the well at a concentration of 39 μg/mL.

From the MIC value, MBC value can be determined. It was taken from the compound with the lowest concentration where there was no growth of bacteria, and the value was 312.5 μg/mL. The inhibition mechanism of bacterial growth can occurred by penetration of membrane cell. The surface layer of cell consists of four components: peptidoglycan, polysaccharide antigen, protein (glycoprotein) and glycerol from theicidic acid and lipoteichoic, also containing fimbria. Although the peptidoglycan of cell wall serves to protect bacteria from the high internal osmotic pressure, the open space between the polymers inside the wall can be broken down by proteolytic enzyme[19].

Freires[20] showed that menthol and eugenol were considered outstanding compounds demonstrating an antibacterial potential. Only L.sidoides mouthwash (1%) has shown antimicrobial effects against oral pathogens. Wassel[21] explained in corporating natural producst with fluoride into dental varnish can be effective for caries prevention especially miswak and propolis. In chemical analysis by Galvao mainly showed the presence of terpenes in essential oil. Essential oil showed activity at low concentration and their fractions were also effective against biofilm formed by *Streptococcus mutans*[22]. Leandro said that the activity of the isolated compounds pf oleoresins do not explain the strong activities of crude[23]. Sujatha studied that seaweeds have potential antibacterial substances which can be used against oral pathogens[24].

Natural products are used in caries prevention research. The effect of natural substances are on bacterial growth inhibition through cell wall biosynthesis and/or cell membrane permeability, inhibition of protein synthesis or nucleic acid metabolism and inhibition of enzyme activity such as glucosyltransferase or transcription level of genes[25].

In this study, a new terpenoid (which is classified as diterpene) was isolated from the *M. pendans*, and its antibacterial activity against *S. mutans* was shown for the first time. Based on comparison at *Scifinder*, terpenoid type labdane diterpeneis was a new compound isolated from plant. As a conclusion, this compound is a candidate as antibacterial agent, because it has potentials to become an antibacterial agent, and suggested to do a further research to find out whether it can prevent caries.

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References

1. Supriatno. Antitumor activity of Papua's Myrmecodia pendens in human oral tongue squamous cell carcinoma cell line through induction of cyclin-dependent kinase inhibitor p27Kp1 and suppression of cycklin E. J of Cancer Res and Ther, 2014, 2, 3: 48-53.
2. Soeksmanto A, Subroto, M, Wijaya, H, Simanjuntak, P. Anticancer activity test for extracts of sarang semut (*Myrmecodia pendens*) to HeLa and MCM-B2 cells. Pakistan Journal of Biological Sciences, 2010, 13, 3: 148-151.
3. Buang Y, Noya E, Ola P, and Cunha T. Antioxidabt activities of chloroform and aqueus fractions of Mrymecodia pendens extract : a preliminary study. *J. Applied chem. Sci*., 2013, 2, 1: 187-195.
4. Engida AM, Makonnea AM, Kasim NS, Tsigie YA, Ismady S, Suryadi, Huynh LH, Ju YH. Extraction, identification and quantitative HPLC analysis of flavonoids from sarang semut (Myrmecodia pendens). *Industrial Crop and Product*, 2013, 41: 392-396.
5. Roslizawati, Ramadhan NY, Fakrurrazi, Herrialfian. Aktivitas antibacterial ekstrak etanol dari rebusan sarang semut (*Myrmecodia Sp*.) terhadapa bakteri *Eschericia coli. Medika Vetenaria*, 2013, 7, 2: 0853-1943.

6. Sulistiyaningsih, Kusuma SAF, and Wira AS. Antibacterial activity test of antplant stem tuber ethanol extract (*Myrmecodia pendens Merr & L.M. Perry*) against *Shigella apoentriae* and *Klebsiella pneumonia. Proceedings of the 2nd International Seminar on Chemistry*, 2011: 397-400.
7. Wang X, Tang GH, Yuan CM, Zhang Y, Hou L, Zhao Q. Two new tirucallane triterpenoids from *Aphanamixis grandifolia*. Nat. Prod. Bioprospect, 2012, 2: 222-226.
8. Wang X, Tang G, Yuan,C, Zhang Y, Zou T, Yu Q, Hao X and He H. Aphagrandinoids A-D, cycloartene triterpenoids with antibacterial activities from *Aphanamixis grandifolia. Fitoterapia*, 2013, 85: 64-68.
9. Saha S, Subrahyaman,E, Kodangala C and Shastry S. Isolation and characterization of triterpenoids and fatty acid ester of triterpenoid from leaves of *Bauhinia varagata*. Der Pharma Chemica, 2011, 3, 4: 28-37.
10. Daisy P, Mathew S, Suveena S and Rayan N. A novel terpenoid from *Elephantosus scabur* – Antibacterial activity on *Staphylococcus aureus* : A substantiate computational approach. Int. J Biomed Sci, 2008, 4, 3: 196-203.
11. Chhour K, Nadjarni M, Brun R, Martin F, Jackues N, Huner N. Molecular analysis of microbial diversity in advanced caries. J Clin Microbiol, 2005, 43, 2: 843-848.
12. Yoshida A, Kuramitsu H. Multiple *Streptococcus mut*ans genes are involved in biofilm formation. Applied and Environmental Microbiology, 2002, 68, 12: 6283-6291.
13. Lester K. Zoocin A and lauricidin in combinatination selectively inhibit *Streptococcus mutans* in a biofilm model. Otago University, Dunedin, New Zealand, 2010.
14. Vrani E, La-Evi A, Mehmedagi A, Uzunovi A. Formulation ingredients for toothpastes and mouthwashes. Bosnian Journal of Basic Medical Sciences, 2004, 4, 4: 51-58.
15. Gold J. The role of chlorhexidine in caries prevention. Operative Denstistry, 2008, 33, 6:710-711.
16. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity : a review. J of Pharmaceutical Analysis, 2015, 6: 71-79.
17. Holla G, Yeluri R, Munshi AK. Evaluation of minimum inhibitory and minimum bactericidal concentration of nano-silver based inorganic anti-microbial agent (Novaron) against *Streptococcus mutans*. Contemp Clin Dent, 2012, 3, 3: 228-293.
18. Cockerill FR et al. Clinical and Laboratory Standards Institute, 2012, 29, 2: 20-21.
19. Hamada S, Slade H. Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiology Review, 1980: 331-384.
20. Freires IA, Denny C, Benso B, de Alencar SM, Rosalen PL. Antibacterial activity of essential oils and their isolated constituents against cariogenic bacteria : a systematic review. Molecules. 2015, 20: 7327-7358.
21. Wassel MO, Khattab MA. Antibacterial activity against *Streptococcus mutans* and inhibition of bacterial induced enamel demineralization of propolis, miswak, and chitosan nanoparticles based dental varnishes. Journal of Advanced Research, 2017, 8: 387-392.
22. Galvao et al. Antimicrobial activity of essential oils against Streptococcus mutans and their antiproliferative effects. Hindawi Publishing Corporation. Eviden-Based Complementary and Alternative s and Medicine, 2012.
23. Leandro et al. Chemistry and biological activities of terpenoids from *Copaiba* (*Copaifera spp.*) *aleoresins*. Molecules, 2012, 17: 3866-3889.
24. Sujatha L, Govardhan TL, Rongairah GS. Antibacterial activity of green seaweeds on oral bacteria. Indian Journal of Natural Product and Resources, 2012, 3, 3: 328-333.
25. Kalesinskas PTK, Ambrozaitis A, Peciuliene V, Ericson D. Reducing dental plaque formation and caries development, a review of current methods and implications for novel pharmaceuticals. Stomatologija, Baltic Dental and Maxillofacial Journal, 2014, 16, 2: 44-52.

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