ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of DEVELOPMENT RESEARCH

International Journal of Development Research
Vol. 06, Issue, 11, pp.10350-10354, November, 2016

Full Length Research Article

POTENTIAL OFTERPENOIDISOLATED FROM *MYRMECODIA PENDANS* AS ANTIBACTERIAL AGAINST *STREPTOCOCCUS MUTANS* ATCC 25175

[1,*]WIDYAWATI, [2]Yanwirasti, [3]Djong Hon Djong, [4]Hendra Dian Adhita, [5]Dikdik Kurnia and [2]Mieke H. Satari

[1]Department of Conservative Dentistry, Faculty of Dentistry, Baiturrahmah University-Padang, Indonesia
[2]Department of Phatology Anatomy, Faculty of Medical, Andalas University-Padang, Indonesia
[3]Department of Biology, Faculty of Mathematic and Natural Sains, Andalas University-Padang, Indonesia
[4]Department of Conservative Dentistry, Faculty of Dentistry, Padjadjaran University-Bandung, Indonesia
[5]Department of Chemistry, Faculty of Mathematic and Natural Sains, Padjadjaran University-Bandung, Indonesia

ARTICLE INFO

Article History:
Received 17th August, 2016
Received in revised form
22nd September, 2016
Accepted 11th October, 2016
Published online 30th November, 2016

Key Words:

Streptococcus mutans,
Myrmecodia pendans,
Terpenoid and caries dental.

ABSTRACT

Caries dentalis one of the most common human disease. One of the bacteria considered thata very important role in the mechanism of plaque formation and increased colonization of bacteria that cause caries is *Streptococcus mutans*. *S. mutans* is considered as bacteria that causes caries dentaldue to their ability to form biofilm on the tooth surface. Searching of bioactive compounds from natural materials is still the alternative, one of the plants that has potential is *Myrmecodia pendans*. In previous studies, the ethyl acetate extract of *M. pendans* reported can inhibit the growth of *S. mutans*. This study aims to isolate terpenoid compound from ethyl acetate fraction of*M. pendans* which has potential against *S. mutans*. Separation and purification of compounds using a combination of chromatographic techniques.Characterization of compounds using UV, IR, NMR and MS. Antibacterial activity test using Kirby Bauer and micro dillusion method. The results showed that the isolated compound was terpenoids type labdane diterpene. Terpenoid sensitivity test results of the concentration 10000; 5000 and 2000 µg/mL were 17.9; 16.8; 13.6 mm respectively. MIC test results and MBC are 78.125 and 625 µg/mL respectively.

INTRODUCTION

Caries dental is one of the most common human diseases. This disease is due to decrease of the hard tissues of teeth caused by *Streptococcus mutans*. The disease is an infection of dental cavities is dangerous because it can lead to degenerative disease (Joanne *et al*., 1996; katsura *et l*., 2001). Caries dental is characterized by the occurrence of demineralized tissue, causing damage to the organic matrix which can be caused by an imbalance of the factors contained in the caries. Caries dental is a disease associate with many factors which are interrelated (Singh and Pandeya, 2011). There are four causes ofCaries dental etiology, namely host, agent, substrates, and time. These factors are the main factors, these four main factors are interacting in a certain time and cause caries. In addition to the factors mentioned above there are also some factors that make a person risk of caries, they are fluoride, oral hygiene, saliva, diet, heredity, race andbacterias (Prabu *et al*., 2005; Patra *et al*., 2015).Therefore, we need a bioactive compound which can be an antibacterial agent, especially on S. mutans. Searching of bioactive compounds from natural materials is still the alternative, *Myrmecodia pendans* widely used by people in West Papua as a herb efficacious for the treatment of various diseases (Salib *et al*., 2013). In previous studies, it is known that ethyl acetate extract of *M. pendans* can inhibit the growth of *S. mutans*. In this study soxhletation method and ethyl acetate assolvent was used to pull the components inside the compound. *Myrmecodia pendans* plant is a plant from Papua, located in the eastern part of Indonesia. Sarang semut plant known by the people of Papua as a medicinal plant. *M. pendans* plantsis believed to cure various diseases including cancers, tumors, gout, diarrhea, fever and other diseases (Soekmanto *et al*., 2010; Supriatno, 2014)). This plant also spread from Malay Peniasula to the Philippines, Cambodia, Sumatra, Java, Papua, as well as the island

Corresponding author: Widyawati,
Department of Conservative Dentistry, Faculty of Dentistry, Baiturrahmah University-Padang, Indonesia.

Solomom. *M. pendans*plants is a family of Rubiaceae with 5 genus, but only two are inhabited by ants, which are*Myrmecodia* (45 species) and *Hypnophytum* (26 species), from this species only *H. formicarum*,*M. pendans* and *M. tuberosa* that has value for medicinal (Plummer, 2000; Jebb, 2009; Sulistyanngsih *et al*., 2011). The Scientific research on *M. pendans* is still few and generally only discusses about the ecology, taxonomy, ethnobotany and test of the extract activity (Prachayasittikul *et al*., 2008; Soekmanto *et al*., 2010). In a previous study, *M. pendans* extract contained flavonoids, tannins, phenolic, glukosidal and terpenoids. Engida *et al* (2013), found five flavonoid compounds from the ethyl acetate extracts of *M. pendans* by analysis using HPLC methods. Based on previous studies, it is necessary to isolate the terpenoid compound from *M. pendans* tubers which has antibacterial potention against *S. mutans*.

MATERIALS AND METHODS

The research is based on specimen of *M. pendans* tuber, collected from Ayawasi villages South Sorong, West Papua Province. This specimen was determined the material in department of plants, Taxonomy Laboratory of Biological Science, Universitas Padjadjaran. The following chemicals used were ethyl acetate, *n*-hexane,acetone, methanol, ethanol,aquadest, gel silica G60 (70-320 mesh), TLC plate silica and ODS, ODS RP-18, spraying 10% H_2SO_4-ethanol reagent followed by heating at 100°C for 1-2 min, alcohol 70%, bunsen burner, clorhexidin, Mueller Hilton medium and cotton sticks.

Instrumentation

The spectrum performed by using the following instruments of various spectroscopic: Fourier Transform Infrared Spectroscopy (FTIR)-Shimadzu prestige-21,^{1}H and ^{13}C-NMR spectra were obtained on JEOL JNM A-500, which works on 500 MHz (^{1}H-NMR) and 125 MHz (^{13}C-NMR). The solvent were acetone-D6. MS spectrum were recorded on GC-MS (Aglient MS). Laminar air flow, incubator Memmert, autoclavemachine HVE-50 Hirayama, jar and ELISA reader Diagnostic Automation Inc.

Extraction and Pre-Purification

Dried ground leaves of *M. pendans* tuber cutted into small pieces (with a diameter of ± 1 cm) as much as 1.5 kg. As much as 300 g sample extracted during 2x6 hours used soxhlet flask 5 L. Extracion repeatedly 5 times using ethyl acetate as solvent with soxletation methods. The soluted filtered and then evaporated by rotary evaporator at a temperature of 40°C to give a residu. Concentrate of ethyl acetate extract obtained as much as 15 g.

Isolation of Terpenoid

Ethyl acetate extract (15.0 g) is subjected using liquid chromatography liquid column method to the stationary phase silica gel G60 (70-230 mesh), eluent n-hexane and ethyl acetate, the solvent system and the increase in the polarity gradient of 10% (v / v). The total volume collected for each fraction is 500 mL. The separation of the ethyl acetate extract using chromatography column is first obtained 11 fractions (A01-11) and analyzed the stain separation by thin layer chromatography (TLC) silica G60, using a combination of n-hexane: ethyl acetate (7: 3). Fraction A07 (392 mg) shows two yellow and pink stain, then separated by chromatography column ODS RP-18 with isokrat using methanol eluent: H2O (3: 2). Fraction A07 (20-41) is analyzed the pattern of its separation with n-hexane: ethyl acetate (3: 2) and methanol (100%) shows a single spot on TLC silica and ODS, resulted terpenoid compound.

Antibacterial Assay

Rejuvenation Bacteria

Each number one loop of bacteria (*S. mutans*) from the stock is inoculated into a sterile test tube containing the suspension muller hilton much as 5 mL up to the level of turbidity 1/2 Mac Farland. Achievement of turbidity is done by comparing the standards then incubated for 48 hours at 37 ° C.

Testing of samples, negative and positive controls

Stick cotton is dipped in bacterial suspension then applied to the surface of medium until evenly distributed. Furthermore, as much as 50 µl samples, 50µl positive control (chlorhexidine) and a negative control (methanol) are dropped on Samir papers (disk) and placed on media then incubated at 37 ° C for 48 hours. After 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 inhibitory zone area (Filho *et al*., 2002; Roh *et al*., 2010).

Determination of MIC and MBC

To determining MIC Value is used micro-dilution method. Bacteria rejuvenated in the Muller Hinton media at 37°C are diluted 0.5 Mac Farland. MIC test in this study is for determining the minimum concentration of a sarang semut compound which is able to inhibit the growth of bacteria. Thus the sensitivity test is performed on the smallest concentration of pure isolates. The test is performed by using a micro plate and pure isolates with different concentrations dissolved in methanol / water (3: 1). Aseptically it is inserted 0.15 ml of liquid broth medium which has been sterilized in an autoclave and 0.15 ml terpenoids of sarang semut into a micro plate. Comparison of turbidity is made a tube control not given the extract of the test. The lowest concentration of the extract in the test tube showing the same clarity as control tube is declared by MIC. Water or MeOH is used to dissolve the compound in which water and MeOH are no effect. Positive control of chlorhexidine is dissolved in water. Minimum rate of inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of pure compound are determined using a micro broth dilution method in 96-well microtiter plates (Urzua *et al*., 2008; Tomas *et al*., 2011). Tests are performed in duplicate.

RESULTS AND DISCUSSION

The *M. pendans*tuber is extracted by soxhletation methods using etyl acetate solvent. Soxhletation method is choosen because the time used more quickly and the result obtained is over maximum. It is based on several repetitions in the isolation of target compounds and compared with extraction by maceration. The ethyl acetate extract of M. Pendans tuber isolated is the kinds of terpenoid with a mass 130 mg.The structure of the compound is determined by 1D-NMR and 2D-

NMR.Terpenoids compound obtained is like yellow oilic. MS spectrum m/z indicates the peak of stabilization at molecular weight about 403.33. This suggests terpenoids type labdane diterpene with molecular formula $C_{25}H_{40}O_4$ (Roh *et al*., 2010; Souza *et al*., 2011). The Infrared spectrum of these compound confirmed presence of hydroxyl group at 3400 cm^{-1}, followed by absorption at wave number 1040.84 cm^{-1} which is a strain stretching of the C-OH group. Carbonyl group at 1688 cm^{-1}, olefinic group C=C at 655,78 cm^{-1}. At wave number 2987.10 cm^{-1} there is a very strong attack of strain aliphatic C-H stretching followed by absorption at 1456.32 cm^{-1} which is the C-H bending and at 1374.23 cm^{-1} which is the gem dimethyl stretch. ^{1}H-NMR (500 MHz, CD_3OD): δ_H 4.2 (*t*,*J*= 6.5;10.0 Hz, H-3)1.1(*d*, *J*=1.95 Hz confirmed olefinic proton H-15and16);2.5, (*m),* H-1,1.2 (*m)*confirmed proton metyl*,* H-2, 3.6 (*m)* also confirm metyl proton.H-5*,* 1.2 (*m)*confirmed methin proton. H-6, 1.9 (*m)* confirmed metilen proton. H-3 4.2 (1H) *t*; 6.5;10.0 confirmed hydroxylation methin.^{13}C-NMR (125 MHz, CD_3OD): δ_c 177.1 (C-20) indicated carboxyl group [19]. δ_c 39.5 (C-4) indicated carbon quartinary, δ_c 78.9 (C-3) indicated hydroxylation carbon afford to proton at δ_H 4.2. DEPT 135° spectrum indicated seven metyl signal, five metilen signal, six methin and nine quartinery carbon signal. Metilen signal at 39.4 (C-1), 24.0 (C-2), 33.1 (C-6), 42.3 (C-7). NMR date of these compound shown in table 1. The structure was determined by the analysis of NMR data as well as by comparison with literature. HMBC spectrum explains the position of the proton to carbon. Proton H-1 (δ_H 2,5 ppm) correlated with C-2 and C-9. Proton H-2 (δ_H 1,2 ppm) correlated with C-5, C-2 and C-3. Proton H-3 (δ_H 2,5 ppm) correlated with C-1. Proton H-3 (δ_H 4,2 ppm) correlated with C-1. Proton H-6 (δ_H 1,2 ppm) correlated with C-10. Proton H-15 (δ_H 1,1 ppm) correlated with C-16 and C-14. Proton H-16 (δ_H 1,1 ppm) correlated with C-15 and C-14, C-13. Proton H-17 (δ_H 4,5 ppm) correlated with C-8. Proton H-19 (δ_H 1,2 ppm) correlated with C-20 and C-3. Proton H-20' (δ_H 1,5 ppm) correlated with C-12, C-11 and C-10. This is confirms the structures of compound, which was identified as new terpenoid type labdane diterpen (Figure 1).

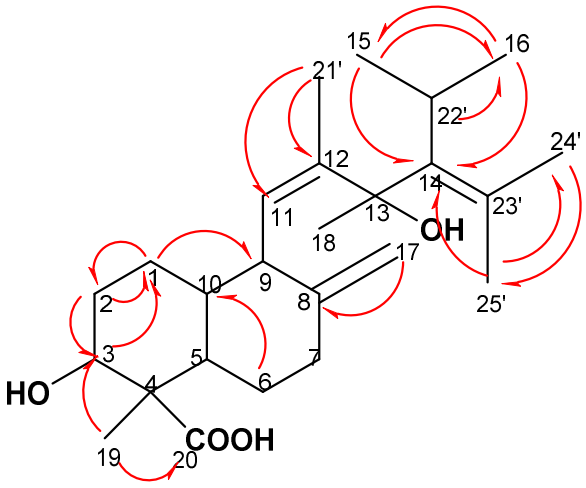

Figure 1. Structure diterpenoid type labdane

The Sensitivity Determination of Terpenoid against *S. mutans*ATCC 25175

Antibacterial activity test against*S. mutans*is used chlorhexidine as a positive control and methanol as a negative control. The use of chlorhexidine as a positive control due to the antibiotic commonly used to eliminate dental caries is chlorhexidine in a way gargled his mouth. The concentration of chlorhexidine used is 2.000 µg/mL. The use of methanol as a negative control because of the solvent used to dissolve the isolated terpenoids is methanol, so to determine the effect of solvent on bacterial growth is used a negative control test. Sample testing of isolated terpenoids is made in concentrations of 10.000; 5.000 and 2.000 µg/mL. The parameters used are the diameter of inhibition zone / nodes around the paper disk. Diameter of clear zone resulting from the antibacterial activity is measured by using a caliper and then compared to the negative and a positive control. The results of the sensitivity test terpenoids against bacteria *S. mutans* are shown in the following table.

Table 1. NMR data of isolated compound

Position	^{13}C-NMR δ_C (ppm)	DEPT 135°	^{1}H-NMR δ_H (*Int*., *mult*., *J*=Hz)	HMBC ^{1}H to ^{13}C
1	39.4	CH_2	2.5 (2H); *m*	C-2, 9
2	24.0	CH_2	1.2 (2H); *m*	C-5, 2, 3
3	78.9	CH	4.2 (1H) *t*; 6.5;10.0	C-1
4	39.5	Cq	-	-
5	32.5	CH	3.6 (1H); *m*	-
6	33.1	CH_2	1.2 (2H); *m*	C-10
7	42.3	CH_2	1.9 (2H); *m*	-
8	148.5	Cq	-	-
9	26.1	CH	2.7 (1H); *m*	-
10	48.6	CH	2.9 (1H); *m*	-
11	118.7	Cq	-	-
12	136.5	CH	4.9 (1H); *m*	-
13	71.2	Cq	-	-
14	42.6	CH	1.9 (2H); *m*	-
15	29.2	CH_3	1.1 (6H); *d*;1.95	C-16, 14, 13
16	29.2	CH_3	1.1 (6H); *d*;1.95	C-15, 14, 13
17	114.4	CH_2	4.5 (2H); *m*	C-8
18	14.7	CH_3	0.9 (3H), *t*;7.15;13.0	-
19	13.1	CH_3	1.2 (3H), *m*	C-20,3
20	177.1	Cq	-	-
21'	17.1	CH_3	1.5 (3H); *s*	C-12, 11
22'	42.6	CH	1.9 (2H); *m*	C-16
23'	104.6	Cq	-	-
24'	18.0	CH_3	0.9 (3H)	C-14, C-25
25'	26.1	CH_3	1.1 (3H)	C-14, C-24

Table1. Sensitivity test terpenoids against bacteria *S. mutans*ATCC 25175

Sample	Inhibition Zone of compound (mm) at Concentration (µg/mL)								
	10000			5000			1000		
	1	2	Average	1	2	Average	1	2	Average
terpenoid	17.8	17.9	17.9	16.8	16.7	16.8	10.7	10.7	10.7
klorheksidin*	td	td	td	td	td	td	14.6	14.6	14.6

Table2. MIC value of terpenoids using ELISA reader against *S. mutans*

Plate	Concentration (ppm)											
	5000	2500	1250	625	312.5	156.25	78.12	39.06	19.53	9.76	4.88	2.44
M+S	1.19	1.165	0.964	0.807	0.661	0.429	0.201	0.116	0.084	0.06	0.055	0.05
	1.213	1.039	0.743	0.733	0.547	0.381	0.18	0.117	0.082	0.058	0.054	0.052
M+P	0.055	0.057	0.055	0.054	0.052	0.052	0.055	0.053	0.054	0.052	0.055	0.052
	0.107	0.06	0.053	0.053	0.05	0.051	0.053	0.053	0.05	0.051	0.048	0.05
M+S+B	1.236	1.091	0.842	0.749	0.583	0.478	0.214	0.133	0.113	0.135	0.09	0.092
	1.306	1.151	0.803	0.703	0.576	0.546	0.292	0.121	0.112	0.105	0.099	0.098
M+P+B	0.096	0.067	0.055	0.069	0.063	0.098	0.127	0.122	0.135	0.13	0.129	0.15
	0.096	0.067	0.056	0.072	0.071	0.108	0.13	0.125	0.146	0.137	0.148	0.148

Minimum inhibitory concentration (MIC) and Minimum Bacteroside Concentraton (MBC) of Terpenod against *S. mutans*ATCC 25175

To determine the minimum concentration of terpenoids against the growth of *bacteria S. mutans*, minimum inhibitory concentration (MIC) and minimum kill concentration (MBC) is tested. Determination of MIC seen from a comparison of the absorbance value on line C (media, test compounds and bacteria) with line D (media, solvents and bacteria), determination of MIC *S. mutans* shown in Table 2. Absorbance value of the solution in the wells marked with lines yellow indicates relative the same value. This shows the amount of bacteria that grow on lines C and D alike. While the value of absorbance of the solution in the well marked with a red line there are differences that indicate differences in the number of bacteria that grow on the C and D (Souza *et al*., 2008; Park *et al*., 2008). So that the MIC value for each test compound is the concentration of the test which is circled in red. MIC terpenoid value as 78.53 µg/mL. Based on the results of absorbance measurements using Elisa reader instrument, MIC values obtained as 78,125 µg/mL. MBC value wasdetermined withculture the plate start from 1-5 in Mueller Hiltonmedium and incubated at 37 ° C for 48 hours. The results of MBC value as 625 µg/mL, indicating at these concentrations there are no living bacteria. Determining MIC value also can taken on difference of OD control (Medium + bacteria) and OD sample (Medium + bacteria + sample). OD control is stable that shows no contaminant in well control or the other word that the bacteria grow well. In other hand, OD sample which contain medium bacteria and sample give decrease value significantly (Villinski *et al*., 2014). It means that sample has effect to bacteria growth and the lowest inhibition occurred well at concentration of sample 78.125 µg/mL as MIC value and 625 µg/mL as MBC value. Determination MBC value can be seen by visualization directly, the lowest concentration which are no growth bacteria defined as MBC value.

Conclution

Terpenoids sensitivity test results on the concentration 10000; 5000 and 2000 µg/mL were 17.9; 16.8; 13.6 mm respectively. MIC test results and MBC are 78.125 and 625 µg/mL respectively.

Terpenoid type labdane diterpenhas potential as antibacterial and antibacterial agent to be used as an alternative and a gold standard for mouthwash.

Acknowledgments

The authors would like to thank for Padjadjaran University research laboratorum for antibacterial activity testing, and Mrs Sofa Fajriah, M.Si and Dr. Ahmad, M.Si for NMR measurement.

REFERENCES

Engida, A.M., Kasim, N.S., Tsigie, Y.A., Ismadji, S., Huynh, L.H. and Ju, Y. 2013. Extraction, identification and quantitative HPLC analysis of flavonoids from sarang semut (*Myrmecodia pendans*). *JournalIndustrial Crops and Products.* 41, 392-396.

Filho, E,R., Magnani, R,F., Xie, W., Mirocha, C,J., Pathre, S,J., 2002. Hidroxilation Of Labdane Diterpene Cpresic acid *by Fusarum graminearum*. *J. Braz, Chem*, 13, 2, 266-269.

Jebb, M. 2009. A Revision of The Ant-plant Genus Hydnophytum (Rubiaceae).*NationaliBotaniciGardenIIreland.*ihttp://www.botanicgardens.ie/herb/research/hydnophytum.html.

Joanne. E., Dellar., Michael. D., Cole., Peter, G., and Waterman. 1996. Antimicrobial Abietane Diterpenoid From *Plectrantus Elegans.Departement of Pharmaceutical Sciences*. 003-9422(95)00694-X.

Katsura, H., Tsukiyama, R.I., Suzuki, A.andKobayashi, M. 2001. *In vitro* antimicrobial activities of bakuchiol against oral microorganisms. *Antimicrobial Agents Chemotheraphy.* 45, 3009-13.

Park, M., Bae, J. and Lee, D.S. 2008. Antibacterial activity of [10]-gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria. *Phytotheraphy Research*. 22, 1446-49.

Patra, J.K., Kim, E. S., Oh, K. Kim, H.J., Dhakal, R., Kim, Y. Dan Baek, K.H. 2015. Bactericidal effect of extracts and metabolites of *Robinia pseudoacacia* L. On *Streptococcus mutans* and *Porphyromonas gingivalis* causing dental plaque and periodontal inflammatory diseases. *Molecules.* 20. 6128-6139.

Plumer, N. 2000. Cultivation of The Epiphytic Ant-Plants Hydnophytum and Myrmecodia. *Cactus and Succulent Journal*.72, 142-147.

Prabu, G.R., Gnanamani., and Sadulla, S. 2005. Guaijaverin – a plant flavonoid as potential antiplaque agent against *Streptococcus mutans*. *Journal of Applied Microbiology*. 101: 487–495.

Prachayasittikul, S., Buraparuangsang, P., Worachartcheewan, A., Ayudhya, C.I.N., Ruchirawat, S. and Prachayasittikul, V. 2008. Antimicrobial and antioxidative activities of bioactive constituents from *Hydnophytum formicarum* Jack. *Molecules*. 13, 904-921.

Roh, S, S., Park, K, M., Kim, Y., 2010. Abeitic acid from *Resina pini* of Pinus Species as a Testosteron 5α-Reductase inhibitor. *J of health science*., 56, 4, 451-455.

Salib, J.Y., Nabila H.S., Helana N.M., and Emad F.E. 2013. Antibacterial activity of *Barleria cristata* bark extracts.*Journal of Applied Sciences Research*. 9;3: 2156-2159.

Singh, G.S., and Pandeya, S.N. 2011. Natural product in discovery of potential and safer antibacterial agent. *Natural product in medicinal chemistry*. 63-101: 978-81-308-0448-4.

Soeksmanto, A., Subroto, M. A., Wijaya, H and Simanjuntak, P. 2010. Anticancer Activity Test for Extract of Sarang Semut Plant (*Myrmecodia pendans*) to HeLa and MCM-B2 Cells. *Pakistan Journal of Biological Science.* 13(3), 148-151.

Souza, A.B., Souza, M.G.M., Moreira, M.A., Moreira, M.R., Furtado, N.A.J.C., Martins, C.H.G., Bastos, J.K., Santos, R.A., Heleno, V.C.G., Ambrosio, S.R. and Veneziani, R.C.S. 2011. Antimicrobial evaluation of diterpenes from *Copaifera langsdorffii* Oleoresin against periodontal anaerobic bacteria. *Molecules*. 16, 9611-9619.

Sulistiyaningsih, Sri, A.F.K.and Arif, S.W.K. 2011. Antibacterial activity test of antplant stem tuber ethanol extract (*Myrmecodia pendens* Merr. and L.M. Perry) against *Shigella dysentriae* and *Klebsiella pneumonia. Proceedings of the 2nd International Seminar on Chemistry*. Jatinangor, 397-400.

Supriatno. 2014. Antitumor Activity of Papua's *Myrmecodia pendans* in Human OralTongueSquamous Cell Carcinoma Cell Line Through Induction of CyclinDependent Kinase Inhibitor p27Kip1 and Suppression of CyclinE. *JournalofCancer Research and Therapy*. 2(3), 48-53.

Tomas, I., Rubido, SandDonos, N. 2011. In situ antimicrobial activity of chlorhexidine in the oral cavity. Formatex. 10, 530-537

Urzúa, A., Rezende, M.C., Mascayano, C. and Vasquez, L. 2008. A structure-activity study of antibacterial diterpenoids. *Molecules*.13, 882-891.

Villinski, J.R., Bergeron, C., Cannistra, J.C., Gloer, J.B., Coleman, C.M., Ferreira, D., Azelmat, J. Grenier, D and Gafner, S. 2014. Pyrano-isoflavans from Glycyrrhiza uralensis with Antibacterial Activity against *Streptococcus mutans* and *Porphyromonas gingivalis*. *Journal Natural Product*. (77), 521 526.

****