TRITERPENES FROM SARANG SEMUT TUBER (*Myrmecodia pendans*) AND THEIR ANTIBACTERIAL ACTIVITY TEST AGAINST *Escherichia coli*

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ABSTRACT

Infectious diseases are the third leading cause of death in the world with amount of 13.3%. In Indonesia, diarrheal disease caused by infection of Escherichia coli become the first cause of death in infants in the amount of 31.4%. Myrmecodia pendans empirically has been used by the people of Papua as a drug of various diseases. In previous study, extracts of M. pendans reported have activity as antibacterial, antioxidant, anticancer, and immudolatory. This research aims to isolation and antibacterial activity test against E. coli. Isolation and purification compounds used combination of several solvent and chromatography method. The structures of the isolated compounds were elucidated by FTIR, ^{1}H-NMR, ^{13}C-NMR, 2D-NMR as well as by mass spectroscopic (MS) and comparing with the literature. Antibacterial activity test was done by using Kirby- Bauer methods. Two triterpenoid type pentacyclic were isolated from etyl acetate extract of M. pendans tuber. The structures were obtained through spectral analysis as oleanolic acid and pomolic acid respectively. These compounds explain the medicinal value of M. pendans tuber. The average of inhibition zone of oleanolic acid at concentration 20.000 ppm, 10.000 ppm and positive control are 10.95; 8.72; 22.65 mm. Pomolic acid also shown inhibition zone at concentration 20.000 ppm, 10.000 ppm and positive control are 12.42; 6.81 and 22.60 mm. The isolation of oleanlic acid and pomolic acid from Mymecodia genus is being reported first time and antibacterial activity test against E.coli.

Keywords: *Myrmecodia pendans, oleanolic acid, pomolic acid, Escherichia coli.*

INTRODUCTION

In Indonesia, infection disease is the first major leading cause of death in kids under five years old. The type of infection which major leading cause of death in kids is diarrheal disease with the amount of 31.4%. diarrhea disease commonly caused by *E.coli* which could become ephidemic disease in some tropical regions. *E.coli* is a phatogenic bacteria [1,2]. Antibiotic use in clinical has made becomes resintant to some antibiotic groups so, the process to find new drugs from plants that is safe for the patient need to be done as an alternative options [1-3].

Sarang semut plant (*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 [2,3]. Sarang semut plants can treat various diseases including cancer, tumors, gout, diarrhea, fever and each other deseases [3]. This plant also scattered from Malay Peniasula to the Philiphines, Cambodia, Sumatera, Java, Papua, Cape York as well as the Solomom island. *M. pendans* is a member of Rubiaceae family with 5 genus, however only two which association with ant. They are *Myrmecodia* (45 species) and *Hypnophytum* (26 species) from those species, only *H. formicarum*, *M. pendans* and *M. tuberosa* are consider to have medicinall value [1,3-4].

Scientific publication on *M. pendans* is still difficult to obtain and generally only discussing about its ecology, taxonomy, ethnobotany and extract activity test [5]. In previous study, extract *M. pendans* contain flavonoid, tannin, phenolic, glucosidal and terpenoid. Engida *et al*, (2013) found five flavonoid compounds from *M. pendans* extract with analisis using HPLC method [6].

Ethanol extract of *Myrmecodia pendans* has antibacterial activity against *Escherichia coli*, at a concentration of 25 and 50%. The average of inhibition zone from ethanol extract of *M. pendans* 25%, 50%, stewed of *M. pendans*, negative control and positive control 10.3, 11.5, 6.67, 0, and 26.3 mm [8]. Ethanol extract also has antibacterial activity against *Shigella dysentriae* and *Klebisella pneumonia*. The MIC-MBC range against *S. dysentriae* lies between the extract concentration of 14-16%. Meanwhile MIC-MBC range against *K. pneumonia* lies between the extract concentration of 2.5- 5%. The antioxidant activity of chloroform and aqueous fraction also respectly with absorbances 0.161 and 0.112, whereas that of the control was 0.085 [9]. Triterpenoid compounds amyrin, ursolic acid, and betulinic acid reported has many antibacterial and antimicrobial activity [10-12]. So this is the reason why this research was estabilished, to isolated triterpenoid compounds from *M. pendans* plant and antibacterial testing against *E. coli*.

EXPERIMENTAL SECTION

Materials

The research was 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 were used including 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, amoxcicilin, milton media and cotton sticks.

Instrumentation

The spectrum were performed by using the following instruments of various spectroscopic tools: 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).

Extraction and Pre-Purification

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

Isolation Compounds

Ethyl acetate extract (15 g) was subjected to coloumn chromatography over silica gel (70-320 mesh). Etyl acetate soluble part was gradiently with *n*-hexana-ethyl acetate mixtures (90:10-10:90)as eluent. A total of volume had been collected in every fraction as many as 500 mL. A total of 11 fractions (A01-11) were obtained and the progres of separation was monitored by thin layer chromatography (TLC) silica gel G 60 F_{254} TLC plates, using solvent system *n*-hexana:ethyl acetate (80:20). Fraction A07 (192 mg) showing two spot being colored pink and then subjected to coloumn chromatography over ODS RP-18 with gradiently using mixture methanol:H_2O (70:30-100:0) as eluent. A total of 24 fractions (B01-24) were obtained and the progres of separation was monitored by ODS TLC plates, using solvent methanol. Fraction B11-15 were combined (23 mg) and subjected to ODS coloumn chromatografy using mixture methanol-H_2O (75:25) as eluent solvent in isocratic to afford 40 fractions (C01-40). Fraction C20-34 eluted with *n*-hexana:ethyl acetate (70:30) and methanol (100%) showed single spot on TLC, afforded the compound 1 (13.7 mg). Fraction B07-10 were combined (28 mg) and subjected to ODS coloumn chromatografy using mixture methanol-H_2O (60:40) as eluent solvent in isocratic

to afford 35 fractions (D01-35). Fraction D18-31 eluted with *n*-hexana:ethyl acetate (70:30) and methanol (100%) showed single spot on TLC, afforded the compound 2 (11.3 mg).

Antibacterial testing With the Kirby-Bauer method

A number of one ose bacteria (*E. coli*) 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 1/2 Mac Farland. Achievement of turbidity is done by comparing with the standard then incubated for 16-18 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 (amoxcicilin) and negative control (methanol) dropped on paper disk and then placed on agar difution. Then incubated at 37°C for 24 hours. After 24 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 [8-9,13].

RESULTS AND DISCUSSION

Dried *M. pendans* extracted by soxhletation methods using etyl acetate soluble. Soxhletation methods were choosen because the time used more quickly and maximize the results obtained. It is based on several repetitions in the isolation of target compounds and compared with extraction by maceration. The etyl acetate extract of the tuber of *M. pendans* produced two triterpenoid type pentacylic oleanolic acid and pomolic acid. The structure of compounds was elucidated by extensive 1D and 2D NMR spectroscopy.

Compound 1 was obtained as white omorphous powder, gave positive Lieberfmann-Burchardt with colour as red and was solid in acetone soluble. UV (MeOH) λmax 202 nm explain no conjugated bond in compound. 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 [14-15]. 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 [15-16]

The ^{13}C-NMR and DEPT spectra confirmed presence of thirty carbon consisted of eight quartinery, five tertiary, ten secondary carbon and seven metyls resulted of

characteristic pentacyclic triterpenes [15]. The chemical shift at δ_C178.2, 122.2 and 144.2 ppm were the characteristic peaks for aleanolic acid type of skleton, assigned to C-28, C-12 and C-13 respectively. The oxygen deshielding chemical shift at δ_C 77,7 ppm was assigned to C-3 [16-18]. The ^{1}H-NMR and HMQC spectrum of compound 1 (Table 1) indicated resonances for an olefinik proton at δ_H 5.21 ppm (1H; *t*,*J*= 3.9 Hz) as α position. A hydroxyl proton at δ_H 3.17 ppm (1H; *dt*, *J*=11.0, *J*= 3.9 Hz) indicates that it is in axial position. Chemical shift at δ_H 2.90 ppm (1H; *dd*, *J*=9.7, *J*= 3.9 Hz) indicates α orientation [17]. Seven metyls singlet at δ_H 0.79 (3H), 0.98 (3H), 0.77(3H), 0.84 (3H), 1.17 (3H), 0.91 (3H), 0.98 (3H) ppm. These resonance characteristic of a triterpene with an olefin and an alcohol functualites [17-18].

The ^{1}H-^{1}H COSY spectrum of compound 1 showed a correlation between H-11 (δ_H 1.32 ppm) and H-12 (δ_H 5.21 ppm), H-3 (δ_H 3.17 ppm) correlated with H-1 (δ_H 0.97, 1.55 ppm), H-18 (δ_H 1.33 ppm) to H-19 (δ_H 1.07 ppm). HMBC spectrum explains the position of the proton to carbon. Proton H-11 (δ_H 1.90) correlated with C-9 (δ_C 47.7 ppm), C-12 (δ_C 122.2 ppm) and C-13 (δ_C 144.2 ppm). Proton H-12 (δ_C 5.21 ppm) correlated with C-9 (δ_C 47.7 ppm), C-5 (δ_C 55.4 ppm) and C-12 (δ_C 122.2 ppm). This strengthens the position of the double bond in the compound of the internal double bond [17-20]. Proton H-23 and H-24 correlated with C-3 (δ_C 77.7 ppm) it indicates the position of the hidroxyl group in the compound 1. Position gem dimethyl showed by the mutual correlation between H-23 to C-24 (δ_C 27.9 ppm) and H-24 to C-23 (δ_C 15.5 ppm). Gem dimethyl second position is also showed by the mutual correlation between methyl H-29 to C-30 (δ_C 27.9 ppm) and H-30 to C-30 (δ_C 23.1 ppm). Mass spectral (MS/MS) presence of moleculer weight 456.66 for $C_{30}H_{48}O_3$ explain that compound 1 has double bond equivalent value is seven containing five siklik and two double bond [18-20]. This confirms the structures of compound 1, which was identified as 3*β*-hidroxy-12(13)-en, 28-oic acid (oleanolic acid) (figure 1). Comparation of the ^{1}H-NMR and ^{13}C-NMR spectral data of compound 1 and oleanolic acid suggestion similar resonance [19-20]. (Table 1).

Compound 2 was obtained as white omorphous powder, gave positive Lieberfmann-Burchardt with colour as red and was solid in acetone soluble. UV (MeOH) λmax 204 nm explain no conjugated bond in compound. The Infrared spectrum of these compound confirmed presence of hydroxyl group at 3436.9 cm^{-1}, which is a strain stretching of the C-OH group [14-15]. Carbonyl group at 1700 cm^{-1}, olefinic group C=C at 727.3 cm^{-1}. At wave number 2981.0 cm^{-1} there is a very strong attack of strain aliphatic C-H

stretching followed by absorption at 1460.4 cm^{-1} which is the C-H bending and at 1385.1 cm^{-1} which is the gem dimethyl stretch [14-16, 21-22].

The ^{13}C-NMR and DEPT spectra confirmed presence of thirty carbon consisted of eight quartinery, seven methin, nine methilen carbon and seven metyls resulted of characteristic pentacyclic triterpenes [21-22]. The chemical shift at δ_C179.2, 128.9 and 139.7ppm were the characteristic peaks type of ursan, assigned to C-28, C-12 and C-13 respectively. The oxygen deshielding chemical shift at δ_C 78,6 and 73.2 ppm was assigned to C-3 and C-19. These indicated the difference with compound 1. The ^{1}H-NMR and HMQC spectrum of compound 2 (Table 2) indicated for olefinik proton at δ_H 5.27 ppm (1H; *t*,*J*= 3.9 Hz) as α position. A hydroxyl proton at δ_H 3.16 ppm (1H; *dd*, *J*=11.1, *J*=4.5 Hz) indicates axial orientation, chemical shift at δ_H 2.64 ppm (1H; *td*, *J*= 8.5, *J*= 4.5 Hz) indicates α orientation. Six metyls singlet and one metyl dublet at δ_H 0.98 (3H), 0.79 (3H), 0.94 (3H), 0.84 (3H), 1.18 (3H), 1.13 (3H). and 0.95 (3H; *d*, *J*=4.6) ppm indicates α orientation [22-23].

HMBC spectrum explains the position of proton H-11 (δ_H 1.35 ppm) correlated with C-8 (δ_C 40.7 ppm), C-13 (δ_C 139.7 ppm) as double bond. Proton H-23 and H-24 correlated with carbon C-3 (δ_C 78.6 ppm) it indicates the position of the first hidroxyl group in the compound 2. The second hydroxyl group position showed correlated of proton H-29 and H-30 corelated with carbon C-19 (δ_C 73.2 ppm). Position gem dimethyl showed by the mutual correlation between H-23 to C-24 (δ_C 15.9 ppm) and H-24 to C-23 (δ_C 29.4 ppm). Mass spectral (MS/MS) presence of moleculer weight 473 explain that compound **1** has double bond equivalent value is seven containing five siklik and two double bond. This confirms the structures of compound **2**, which was identified as triterpenes pentacyclic 3,19-dihidroxy-12(13)-en, 28-ursolic acid (pomolic acid) (figure 2) [24]. Comparation of the ^{1}H-NMR and ^{13}C-NMR spectral data of compound **2** and pomolic acid suggestion similar resonance (Table 2).

Antibacterial Activity Test Result

Antibacterial activity test was done tested using Kirby-bauer methods against *Escherichia coli*. Triterpenes pentacyclic oleanolic acid and pomolic acid was measured antibacterial activity test with concentration 20.000 and 10.000 ppm. Positive control used was by amoxciciln with concentrated 1000 ppm. The average of inhibition zone of oleanolic acid at concentration 20.000 ppm, 10.000 ppm and positive control are 10.95; 8.72; 22.65 mm. Pomolic acid also shown inhibition zone at concentration 20.000 ppm, 10.000 ppm and

positive control are 12.42; 6.81 and 22.60 mm. Pomolic acid showed the active compound than oleanolic acid. In previous study, no research reported activity of oleanolic acid and pomolic acid against *E.coli*.

CONCLUSION

Sarang semut (*M. pendans*) originated from Papua island diagnosed to have an interesting chemical components in respons to the acute human disease such as infection diseases. Compound characterization using various spectroscopic techniques identified the final isolated compound as triterpenes type pentacyclic as oleanolic acid and pomolic acid. Antibacterial activity test shown that both of the compounds has activity against *E. coli.* Triterpenes type pentacyclic oleanolic acid and pomolic acid is being reported first time from genus *Myrmecodia* and antibacterial activity test against *E.coli*. In previous study, no research reported activity of oleanolic acid and pomolic acid against *E.coli*. In previous study, oleanolic acid had been isolation from plant *Scefflera odorata*, *Grewita optiva*, *Galium tortunense, Duroia macropylla, Calicarpa integerima* and *Euporbian microsciadin* with T-Cell proliferation activity with IC_{50} 0.5 mg/L [15,17-18,20-22]. The isolation of oleanlic acid from *Mymecodia* genus *and antibacterial activity test against E.coli* is being reported first time. In previous study, pomolic acid had been isolation from plants *Calicarpa integerima, Licania pittieri with activity anti-aggregating* and from *Euscaphis* with cytotoxit activity [22-24]. The isolation of pomolic acid from *Mymecodia* genus *and antibacterial activity test against E.coli* is being reported first time.

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Table 1 ^{13}C-NMR and ^{1}H-NMR data of compound 1 and comparation with literature.

Posisi C	δ_C (ppm)		δ_H (Int., mult., *J* = Hz)	
	Oleanolic acid (1)	Literature	Oleanolic acid (1)	literature
1	38.4	38.6	0.97 (1H; m); 1.55 (1H; *m*)	1.63 (2H; *m*)
2	27.2	26.7	1.52 (2H; *m*)	1.60 (2H; *m*)
3	77.7	78.5	3.17 (1H; *dt*,11.0; 3.9)	3.23 (1H; *dd*, 10.7; 4.7)
4	39.5	39.2	-	-
5	55.4	55.5	0.83 (1H; *m*)	0.74 (1H; *m*)
6	18.3	18.3	1.51 (1H; *m*); 1.42 (1H; *m*)	1.54 (1H; *m*)
7	33.1	32.6	1.33 (2H; *t* , 3.6)	1.49 (2H; *m*)
8	39.2	39.6	-	-
9	47.7	48.1	1.53 (1H; *m*)	1.54 (1H; *m*)
10	36.9	37.0	-	-
11	23.3	22.7	1.13 (2H; *m*)	0.94 (2H; *m*)
12	122.2	122.4	5.21 (1H; *t*, 3.9)	5.31 (1H; *dd*, 5.24; 3.6)
13	144.2	144.1	-	-
14	41.6	42.0	-	-
15	27.6	27.7	1.50 (2H; *m*)	1.60 (2H; *m*)
16	23.2	22.8	1.53 (1H; *m*); 1.92 (1H;*m*)	1.64 (2H; *m*)
17	42.0	46.7	-	-
18	41.4	41.5	2.90 (1dd; 9.7; 3.9)	2.82 (1H; *m*)
19	46.0	46.1	2.07 (2H; *m*)	2.87 (1H; *m*)
20	30.4	30.4	-	-
21	33.6	33.7	1.60 (2H; *m)*	1.62 (2H; *m*)
22	33.1	32.3	1.30 (2H; *m*)	1.30 (2H; *m*)
23	27.9	28.8	0.9 (3H; *s*)	1.00 (3H, *s*)
24	15.5	14.7	0.79 (3H; *s*)	0.79 (3H, *s*)
25	15.5	15.1	0.77 (3H; *s*)	0.93 (3H, *s*)
26	16.8	26.5	0.84 (3H; *s*)	0.79 (3H, *s*)
27	25.4	25.2	1.17 (3H; *s*)	1.16 (3H, *s*)
28	178.2	180.4	-	-
29	28.6	32.8	0.98 (3H; *s*)	0.92 (3H; *s*)
30	23.1	23.3	0.79 (3H; *s*)	0.94 (3H; *s*)

Table 2 ^{13}C-NMR and ^{1}H-NMR data of compound 2 and comparation with literature.

Posisi C	δ_C (ppm)		δ_H (Int., mult., *J* = Hz)	
	Pomolic acid (2)	**literature**	**Pomolic acid (2)**	**Literature**
1	39.5	39.4	1.01 (2H; *m*)	-
2	27.1	28.1	1.04 (2H; *m*)	-
3	78.6	78.2	3.16 (1H; *dd*, 11.1; 4.5)	3.42 (1H; *dd*, 10.8; 5.2)
4	39.5	39.4	-	-
5	56.3	55.9	0.83 (1H; *m*)	-
6	15.2	18.9	1.51 (2H; *m*)	-
7	33.9	33.6	1.33 (2H; *t*, 4.2)	-
8	40.7	40.3	-	-
9	48.2	47.8	1.71 (1H; *m*)	-
10	37.9	37.3	-	-
11	24.4	24.0	1.35 (2H; *m*)	-
12	128.9	128.0	5,27 (1H; *t*, 3.9)	5.60 (1H, *t*)
13	139.7	140.0	-	-
14	42.3	42.1	-	-
15	28.1	29.3	1.13 (2H; *m*)	-
16	26.5	26.4	1.53 (1H; *m*); 1.92 (1H;*m*)	-
17	48.0	26.4	-	-
18	54.5	54.6	2.64 (1H; *td*, 8.5, 4.5)	3.06 (1H; *s*)
19	73.2	72.7	-	-
20	42.3	42.4	-	-
21	32.7	26.9	1.30 (2H; *m*)	-
22	38.5	37.3	1.64 (1H; *m*); 1.73 (1H; *m*)	-
23	29.4	28.8	0.98 (3H; *s*)	1.22 (3H; *s*)
24	15.9	16.8	0.79 (3H; *s*)	1.01 (3H; *s*)
25	16.4	16.5	0.94 (3H; *s*)	0.90 (3H; *s*)
26	17.4	17.4	0.84 (3H; *s*)	1.10 (3H; *s*)
27	24.7	24.7	1.68 (3H; *s*)	1.72 (3H; *s*)
28	179.2	180.8	-	-
29	27.3	27.1	1.13 (3H; *s*)	1.44 (3H; *s*)
30	16.7	16.5	0.95 (3H; *d*, 4.6)	1.10 (3H; *d*, 6.4)

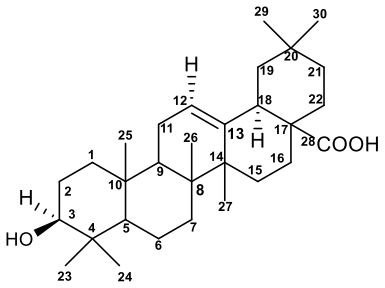

Figure 1. 3*β*-hidroxy-12(13)-en, 28-oic acid (oleanolic acid)

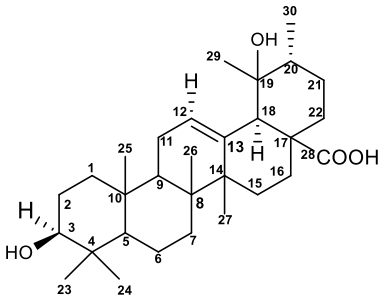

Figure 2. 3,19-dihidroxy-12(13)-en, 28-ursolic acid (pomolic acid).