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Immunomodulatory Compound from *Myrmecodia pendens* Merr & Perry

(Senyawa Imunomodulator dari *Myrmecodia pendens* Merr & Perry)

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Abstract: *Myrmecodia pendens* Merr & Perry (Rubiaceae) is widely used in Papua as traditional medicine against various diseases. Preliminary study revealed that ethyl acetate fraction has potency to increase mice Balb/c lymphocyte proliferation *in vitro*. This study aimed to investigate the active compound responsible for the activity. Dried powders of plant tubers were macerated in ethanol 95% followed by solvent evaporation. The crude extract was further fractionated by liquid-liquid partition with n-hexane, ethyl acetate and water, successively. The ethyl acetate fraction was subjected to a silica gel column and separated by vacuum. The subfraction yields were tested on mice Balb/c lymphocytes to observe the influence on lymphocyte proliferation by MTT assay. Total phenolic content of subfractions were determined by Folin-Ciocalteau method. Chemical characteristics of active compound was identified by GC-MS, UV-Vis spectroscopy, IR and H-NMR. The result showed that total phenolic content has no significant correlation to the lymphocyte proliferation activity. Subfraction 7 yielded from ethyl acetate-methanol (7:3v/v) contained a major constituent (78% by GCMS, 83.75% by Densitometry). GCMS and UV spectra suggested a phenolic substructure. IR and H-NMR suggested that the phenolic was glycosilated and had aliphatic moiety.

Keywords: *Myrmecodia pendens* Merr & Perry, lymphocyte proliferation, phenol derivative.

Abstract: *Myrmecodia pendens* Merr & Perry (Rubiaceae) banyak digunakan oleh masyarakat Papua sebagai obat tradisional untuk berbagai penyakit. Uji pendahuluan menunjukkan bahwa fraksi etil asetat berpotensi meningkatkan proliferasi sel limfosit mencit Balb/c. Penelitian ini bertujuan untuk menemukan senyawa aktif yang memiliki aktivitas imunomodulator. Serbuk kering dari tanaman sarang semut dimaserasi dengan etanol 95% diikuti dengan evaporasi pelarut. Ekstrak kental yang diperoleh difraksinasi dengan partisi cair-cair menggunakan n-heksan, etil asetat dan air. Fraksi etil asetat dimasukan dalam kolom *silica gel* dan dipisahkan oleh *vacuum*. Fraksi-fraksi yang dihasilkan diuji pengaruhnya terhadap proliferasi limfosit mencit Balb/c dengan MTT *assay*. Total senyawa fenolik dari fraksi ditentukan dengan metode Folin-Ciocalteau. Karakteristik kimia dari senyawa aktif diidentifikasi dengan GC-MS, spektroskopi UV-Vis, IR dan H-NMR. Hasil menunjukkan bahwa total senyawa fenolik tidak mempunyai korelasi yang signifikan pada aktivitas proliferasi limfosit. Fraksi 7 yang dihasilkan dari etil asetat-metanol (7:3 v/v) mengandung senyawa utama (78% dengan GCMS, 83,75% dengan densitometri). GC-MS dan Spektrum UV-Vis menunjukkan struktur fenolik. IR dan H-NMR menunjukkan bahwa senyawa fenolik itu adalah glikosida dan memiliki gugus alifatik.

Kata kunci: *Myrmecodia pendens* Merr & Perry, proliferasi limfosit, derivat fenol.

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INTRODUCTION

IMMUNE system plays an important role in human surveillance mechanism against various diseases[1]. It maintains homeostatic condition by monitoring host cells in order to detect and eliminate neoplastic cells as well as dead cells[2]. Internal and external factor influence the immune system modulation. In certain conditions such as stress and illnesses, human body requires external substances to help in maintaining the immune system function. These substances are called immunomodulators[3]. Based on the way it works, Katz has catagorized immunomodulator into three groups, i.e., immune restorator which restores disrupted immune system, immune stimulator which increases the immune system function and activity, while immune suppressor is used to suppress the functions and activity of immune system[4].

Natural resources are good sources of new potential immune modulators. Plants with immunomodulatory effects have been used as part of immune therapy, i.e. effort to incorporate immune modulators into the conventional therapy to achieve maximum effect against various diseases[5]. Indonesia with its rich biodiversity and cultures offers a tremendous sources for new potential drugs from plant. One potential plant is *Myrmecodia pendens*, a plant which is popular as Ant Nest (sarang semut) by local people.

M. pendens grows an ephyphyte on big plants such as cajuput (*Melaleuca*), Cemara gunung (*Casuarina*), Kaha (*Castanopsis*) and Beech (*Nothophagus*)[6]. It grows mostly in association with ants. Its tuberous stem has specialized hollow structures, called domatia providing nesting cavities to ant colonies[7]. From ecological point of view, Ant Nest is potential to be developed as herbal medicines due to the ephyphytic nature, therefore can be cultured in a tropical forest without endangering the environment[8].

Soeksmanto *et al* has reported potential cytotoxic activities of *M. pendens* against cancer cell lines, HeLa and MCM-B2[6]. The active fraction contains saponin, alkaloid, tannin and flavonoid. Our previous study has revealed that the plants, extract and fractions have potential effects on Balb/c mice lymphocyte proliferation and macrophage phagocytosis *in vitro*[8]. It is interesting to find out the active constituents responsible for the activity in order to support its use as part of complementary medicine.

MATERIALS AND METHODS

MATERIALS. *M. pendens* was collected from Babo, Bintuni, West Papua, Indonesia, on March 2010. Plant's taxonomy was determined by Mr. Heri Sujadmiko (Laboratory of Plant Taxonomy, Faculty of Biology, Gadjah Mada University, Indonesia) with voucher specimen number 0275/T.Tb/V/2011. Chemicals for extraction and fractionation were technical grade solvents as follows: ethanol 95%, n-hexane, ethyl acetate and demineralized water (Brataco). Other material were precoated silica gel 60 F254 plates (Merck), formic acid, ethyl acetate, methanol (pro analyses grade, Merck), $AlCl_3$, $FeCl_3$, Cerium sulphate, Dragendorff. Materials for lymphocyte proliferation assay were lymphocyte cells from 2 months old Balb/c mice (Centre for Integrated Research and Assay, Gadjah Mada University), ethanol (Merck), RPMI 1640 media (Gibco), RPMI 1640 complete media plus FBS (Fetal Bovine Serum) (Caisson), PBS (Phosphate Buffer Saline) (Gibco), Hepatitis B vaccine (Engerix®), PHA (phytohemaglutinin, Gibco), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide, Sigma), sodium dodecyl sulphate (SDS, Merck), penicillin and streptomycin (Gibco), fungizon/amphoterisin B (Gibco), methanol (Merck). Materials for phenolic content assay were gallic acid (Wako Pure Chemical Industry Ltd.), Folin-Ciocaltaeu Reagent (Merck), $NaCO_3$, double purified water (Brataco). Instruments used were oven (Memmert), vacuum rotary evaporator (Heidolph® WE 2000), VLC column (vacuum liquid chromatography, Pyrex), UV lamps 254 nm and 366 nm, centrifuge (Sorvall), inverted microscope (Olympus), CO_2 incubator (Heraeus®), 96-wells microplates (Costar), microplate reader (Bio-Rad), and electric balance (Mettler Toledo), TLC scanner (CAMAG), UV Vis spectrophotometer (PerkinElmer) and Thermo Genesys 10 UV Scanning, FTIR 100 Perkin Elmer, GC-MS (Shimadzu, GC: 17A, MS QP 5000), LCMS Hitachi L6200 Mariner Biospectrometry, Delta2 Nuclear Magnetic Resonance (JEOL).

METHODS. Extraction and fractionation. Ant Nest tubers were washed, cut and dried in oven at 40-50°C, then were grounded to obtain dried pulverized samples. After immersing the powder in ethanol 95% (1:9 b/v), the residue was remacerated, then supernatants were combined and evaporated by vacuum rotary evaporator.

The ethanol extracts were fractionated using liquid-liquid partition with polarity gradient solvents as follows, n-hexane, ethyl acetate and water to yield n-hexane, ethyl acetate and water fractions, respectively.

Ethyl acetate fraction (2 g) was dried with 2 g of silica gel powder and subjected to a 7 cm silica gel column (12 g). Fractionation was carried out using gradient polarity eluent, 100 mL of each 100% n-hexane, n-hexane-ethyl acetate (1:1, 1:3 and 0:1

v/v), followed by ethyl acetate-methanol (1:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, and 0:1). Vacuum liquid chromatography was applied to assist band separation.

Several TLC systems were evaluated to observe the best spots separation. Eluent with the best separation was n-butanol - acetic acid – water (4:1:5 v/v). UV 254 and 366 nm lamps and cerium sulphate were used to detect constituents and to guide in combining the subfractions. Nine combined fractions were produced.

Determination of total phenolic content. Total phenolic content of extracts and fractions were determined in triplicate by the Folin-Ciocalteu method[9]. Each 0.1 mL of sample and standard solution (gallic acid 100 μL) was reacted with 0.1 mL of Folin-Ciocalteu reagent. After left for 4 min, the solutions were neutralized with 1 mL of 7.5% Na_2CO_3 solution. After 120 min incubation at room temperature, 3.8 mL distilled water was added. Absorbances were measured at 751 nm. Total phenolics contents were calculated as gallic acid.

Lymphocytes isolation and proliferation assay[10]. A spleen tissue from Balb/c mice was transferred to a 50 mm petri dish containing 10 mL of RPMI 1640. The suspension was centrifuged at 1,200 rpm 4°C for 5 min. Clumps were resuspended in 5 mL of tris ammonium chloride buffer and left in room temperature for 15 min. Centrifugation at 1,200 rpm 4°C was performed following addition of 5 mL RPMI. Clumps were separated from the supernatant, washed twice with RPMI and then diluted with complete medium. Lymphocytes cells were counted in haemocytometer. The cells were cultured in a CO_2 incubator at 37°C and ready for the assay. Suspensions of lymphocytes cells in 100 μL medium ($1.5x10^6$ cells/mL) were distributed into each of 96-wells micro plates. Each well was added 10 μL of hepatitis B vaccine and incubation was performed at 37°C for 24 hour with 5% CO_2 flow.

Series of samples were made to concentration of 10, 20, 50 and 100 μg mL^{-1} by using 0.5% tween 80. Afterward, 100 μL of sample ethyl acetate fraction was added and incubation was continued for 48 and 72 h. Into each well was added 10 μL of MTT (5 mg mL^{-1}) [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] then followed by 4 h of incubation at 37°C. Viable cells will react with MTT to form purple color. Stopper reagent of 50 μL 10% SDS in HCl 0.01 N was added into each well. Optical densities were measured by micro plate reader at 550 nm. Positive control was 10 μL of PHA 5 μg/μL.

Active compound identification fraction in ethyl acetate. TLC analyses of fraction 7 yielded from the VLC suggest that it has high purity. In order to support the suggestion, further purity tests was carried out by LCMS and GCMS analyses. TLC using silica gel F_{254} as stationary phase and 3 different polarity of eluents i.e. ethyl acetate-methanol-formic acid (1:1:0.1 v/v), (1:2:0.15 v/v), and (1:5:0.3 v/v). GC-MS system was 30 m of column Rastek Rxi-5MS, helium as mobile phase, EI 70 eV, and oven temperature from 0 to 300°C. LC-MS system was column C18 Supelco, eluent methanol-water (95:5 v/v), and positive mode ESI (Electrospray Ionization).

Compound identification was carried out by TLC, followed by densitometry method. System used was silica gel F_{254} as stationary phase, and ethyl acetate-methanol-formic acid (1:5:0.2 v/v) as the mobile phase. Chromatographic profiles were analyzed under UV 254 and 366 nm lamps and spray reagents $FeCl_3$ (phenolic), Dragendorff (alkaloid) and $AlCl_3$ (flavonoid). Absorption pattern was observed by UV-visible spectrophotometer. Fraction 7 was diluted 0.3 mg in 6 mL of ethanol and scanned at λ 200 to 800 nm.

Out of nine fractions tested, fraction 7 showed higher of lymphocyte proliferation activity. Active compound's functional group identification of this fraction was carried out by infra red spectrophotometer. Two mg of fraction 7 was grounded with KBr and pressed to yield a pellet for infra red spectrometric analyses. ^{1}H-NMR spectroscopy was carried out on sample in CD_3OD. Resonances were measured from 0 to16.0 ppm by using 500 MHz frequency.

Data analyses. Data of optical density from MTT assay and total phenolic contents were analyzed by Shapiro-wilk and Levenge-test for normality and homogeneity data. Parametric data were analysed by using one way Anova while non parametric data were analysed by Kruskal-Wallis and Friedman tests followed by Tamhane test. IR Spectroscopy data of active compound were analyzed by data comparison to available literatures.

RESULTS AND DISCUSSIONS

Recovery of crude extract was 14.78%, while n-hexane, ethyl acetate and water fractions recovery were 0.88%, 2.07% and 1.63%, successively. Previous report[8] concluded that the ethyl acetate fraction showed the highest potency as lymphocyte proliferation enhancer, while the n-hexane fraction has he lowest activity. In the same report[8], effect of extract and fractions on macrophage phagocytosis did not exhibit dose activity relationship. Therefore in this research, lymphocyte proliferation was chosen as bioactivity guidance.

Lymphocyte proliferation assay of VLC fractions. Fractination yielded 14 subfractions with 71.53% recovery (Table 1). Based on the TLC chromatogram profiles, several fractions were combined and resulted in 9 subfractions (Figure 1). Those sufractions were tested for the effect on lymphocyte proliferation of mice Balb/c by MTT assay (48 and 72 h incubation).

Results showed that all subfractions significantly enhanced lymphocyte proliferation in comparison to normal control ($p > 0.05$). Result of 48 h incubation (Table 2) showed that the highest activity was observed on subfraction 4 in 100 µg/mL (OD 1.020 ± 0.022), while the second highest activity showed by subfraction 7 in 50 µg/mL (OD 0.924 ± 0.033). Result of 72 h incubation showed similar profile (Table 3) but less proliferation activity, in general.

Total Phenolic Assay. Total phenolic assay showed that crude extract, fractions and sub fractions contained phenolics in different amounts (Table 4). Samples measurements were carried out at 730 nm based on gallic acid standard curve of $y = 0.001x + 0,051$ ($r= 0.991$). Correlation chart between total phenolic and lymphocyte proliferation activity of extract and fractions in 100 µg/mL (Figure 2) showed weak positive correlation but was not statistically significant. Fraction 4, which showed the highest total phenolic content was the fraction causing the highest lymphocyte proliferation effect. On the other hand subfraction 1 showed relatively high phenolic content but less lymphocyte proliferation effect. It is interesting to note that the subfractions having the highest activity, subfractions 4 and 7, have almost similar total phenolic content.

Purity analyses of isolate. Considering that subfraction 7 consisted of one major constituent and that it has potential effect on lymphocyte proliferation, this subfraction was analysed further to determine the purity of major compound. Three different degree of polarity eluents have been applied on subfraction 7 (isolate) resulted one spot on chromatograms with hRf 76, 72 and 69, successively (Fig. 3). The spot was identified as phenol derivative as shown by color change to blue-black spot with

Table 1. VLC subfractions yield.

Subfraction	Collected from	Eluent used (v/v)	Yield (mg)	Recovery (% w/w)
VLC 1	Subfraction VLC 1	H:E (1:0)	10.0	0.5
VLC 2	Subfraction VLC 2	H:E (1:1)	54.1	2.7
VLC 3	Subfraction VLC 3	H:E (1:3)	71.3	3.6
VLC 4	Subfraction VLC 4	H:E (0:1)	432.4	21.6
VLC 5	Subfraction VLC 5	E:M (9:1)	59.0	2.9
VLC 6	Subfraction VLC 6	E:M (8:2)	380.2	19.0
VLC 7	Subfraction VLC 7	E:M (7:3)	85.2	4.3
VLC 8	Subfraction VLC 8	E:M (6:4)	264.9	13.2
VLC 9	Subfraction VLC 9-14	E:M (5:5) to (0:1)	74.2	3.7
	Total		1431.3	71.6

Note: H: n-hexane; E: ethyl acetate; M: methanol; eluents volume:100 mL. % w/w were calculated from weight of yield divide with weight of sample.

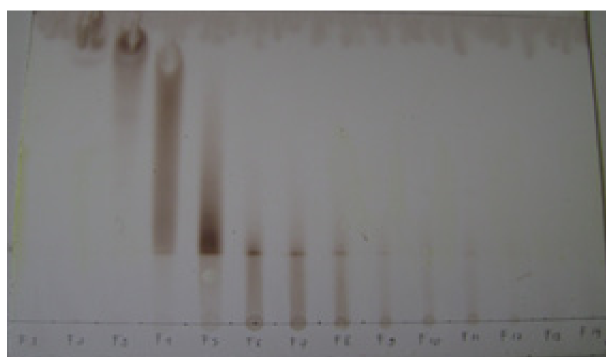

Figure 1. TLC profile of VLC subfractions. TLC system: silica gel 60 F_{254} and n-butanol - acetic acid - water (4:1:5 v/v), spray reagent: cerrium sulphate (Note : F1 to F14 are subfractions of ethyl acetate)

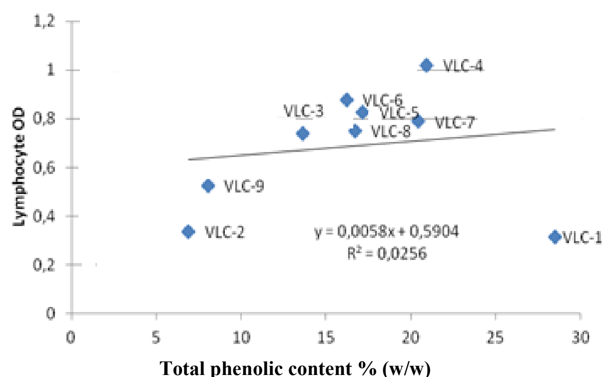

Figure 2. Correlation chart of total phenolic content vs lymphocyte proliferation activity after incubation with fractions (48h).

Table 2. Mean ± SD of lymphocyte optical density after incubation with subfractions for 48h.

Fractions	Concentration (μg/mL)			
	10	20	50	100
VLC-1	0.261±0.015	0.267±0.005	0.271±0.007	0.316±0.028
VLC-2	0.597±0.059	0.591±0.043	0.329±0.020	0.337±0.001
VLC-3	0.564±0.013	0.574±0.003	0.665±0.040	0.738±0.030
VLC-4	0.550±0.011	0.675±0.028	0.845±0.0019	1.020±0.022
VLC-5	0.507±0.019	0.598±0.030	0.687±0.030	0.828±0.027
VLC-6	0.561±0.013	0.584±0.013	0.711±0.020	0.877±0.020
VLC-7	0.646±0.015	0.725±0.007	0.924±0.033	0.789±0.020
VLC-8	0.594±0.031	0.600±0.055	0.700±0.046	0.751±0.070
VLC-9	0.592±0.016	0.615±0.022	0.606±0.052	0.526±0.027
Normal control	0.076±0.005			

Table 3. Mean ± SD of lymphocyte optical density after incubation with subfractions for 72h.

Fractions	Concentration (μg/mL)			
	10	20	50	100
VLC-1	0.245±0.017	0.255±0.011	0.246±0.013	0.278±0.014
VLC-2	0.510±0.041	0.462±0.013	0.282±0.005	0.324±0.019
VLC-3	0.430±0.012	0.647±0.322	0.554±0.014	0.623±0.052
VLC-4	0.537±0.021	0.700±0.308	0.715±0.022	0.922±0.023
VLC-5	0.441±0.020	0.646±0.015	0.665±0.015	0.663±0.019
VLC-6	0.440±0.017	0.579±0.034	0.675±0.033	0.863±0.020
VLC-7	0.555±0.017	0.649±0.011	0.754±0.026	0.772±0.010
VLC-8	0.465±0.013	0.549±0.002	0.679±0.051	0.764±0.015
VLC-9	0.467±0.018	0.507±0.016	0.552±0.026	0.515±0.015
Normal control	0.044±0.004			

Table 4. Mean ± SD of total phenolic contents in samples (Folin Ciocalteau Method).

Samples	Mean±SD (% w/w)
Crude extract	12.373±0.278
n-hexane fraction	5.267±0.689
Non hexane fraction	23.141±0.838
Ethyl acetate fraction	18.657±0.624
Water fraction	10.222±0.227
Subfraction VLC 1	28.500±3.733
Subfraction VLC 2	6.896±0.373
Subfraction VLC 3	13.620±0.125
Subfraction VLC 4	20.941±1.198
Subfraction VLC 5	17.177±3.354
Subfraction VLC 6	16.204±0.112
Subfraction VLC 7	20.444±0.514
Subfraction VLC 8	16.696±0.341
Subfraction VLC 9	8.033±1.059

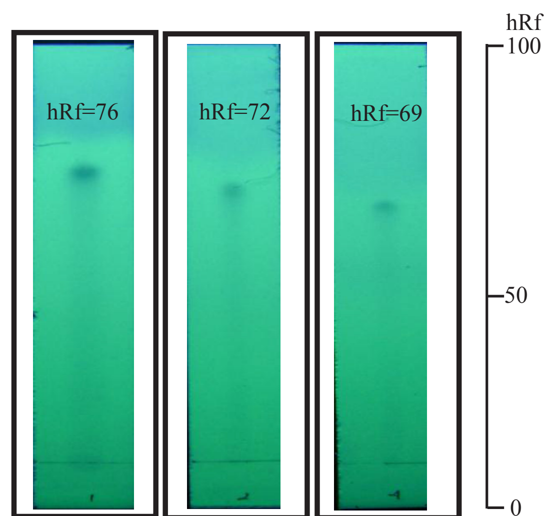

Figure 3. TLC profile of isolate detected by uv lamp 254 nm. TLC systems: silica gel 60F$_{254}$; mobile phases (left to right): ethyl acetate-methanol-formic acid (1:1:0.1 v/v), (1:2:0.15 v/v), (1:5:0.3 v/v)

$FeCl_3$. Densitometry measurement resulted 83.75% purity (detected at 201 nm).

GCMS analyses revealed 78.80% purity of isolate which occurred at 10.8 min (Fig. 4). EI fragmentation showed a stable ion fragment at 110 m/z with 100% abundance suggested a 1,3 Benzediol (Similarity Index: 81, Wiley 7 Library, Fig. 5).

LCMS in positive ion mode of measurement did not detect a stable pseudomolecular ion due to poor separation by the LC system. Acidic environment seems to be necessary for better separation.

Spectrophotometric analyses was performed on the isolate in ethanol which showed absorbance peak at 217 nm (Fig. 6). This finding is in accordance to

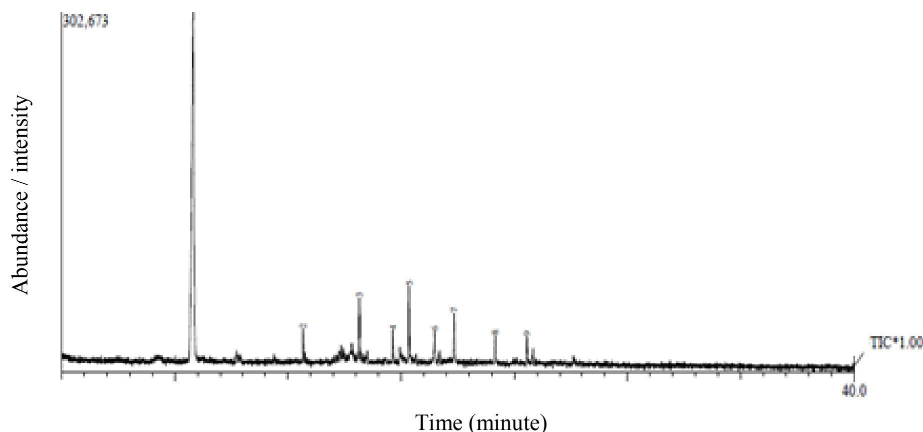

Figure 4. GCMS chromatogram of isolate.

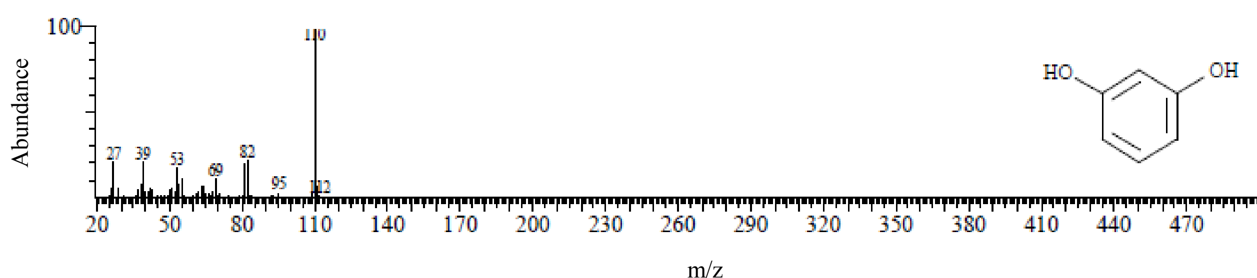

Figure 5. Proposed EIMS fragmentation of isolate.

the previous obtained data, stated that a compound having an absorbance peak at 211 nm can be considered as a phenol derivate[(11)]. An auxochrom substituent can influence the absorbance intensity and wavelength of the phenolic compound. This effect could be a bathochromic (shifted to higher wavelength) or hypsochromic (shifted to lower wavelength).

IR spectra (Fig. 7) supported a phenol moiety as shown by strong and broad bands at 3369 cm^{-1} (OH stretching), 1308 cm^{-1} (OH bend) and 1200 cm^{-1} (C-O stretching). Strong bands at 1709 cm^{-1} (C=O stretching) and 1615 cm^{-1} (C=C stretching) were consistent to an aromatic ring. At fingerprint region, strong bands at 840 cm^{-1} suggested a para substitution. Aliphatic moiety was shown by bands at 2925 and 2854 cm^{-1} (C-H stretching) and at 1452 cm^{-1}($-CH_2-$)

Further analyses by ^{1}H-NMR (MeOD, 500 MHz) revealed a presence of resonances in aromatic region i.e., 6.35-7.14 ppm. Resonances in the upperfield region suggested an aliphatic moiety. A characteristic

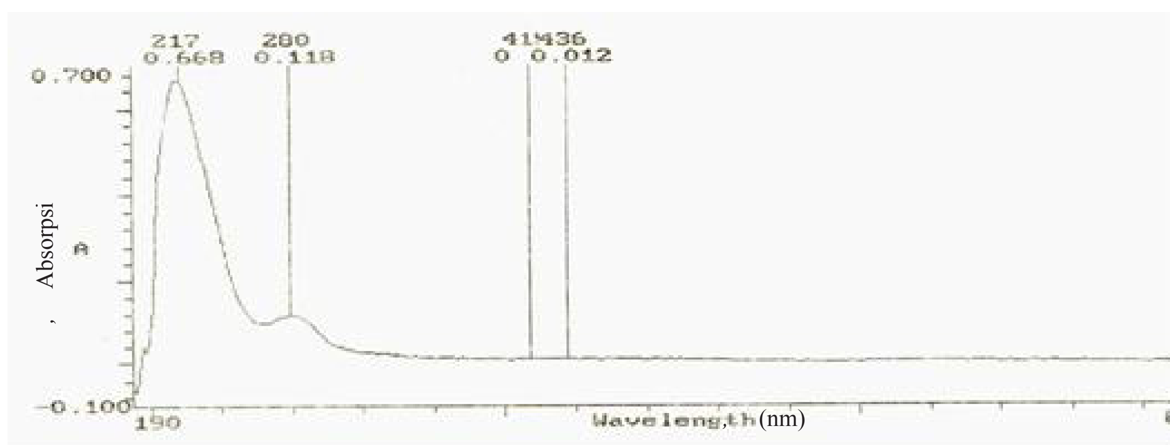

Figure 6. UV-Vis spectrum of isolate in EtOH.

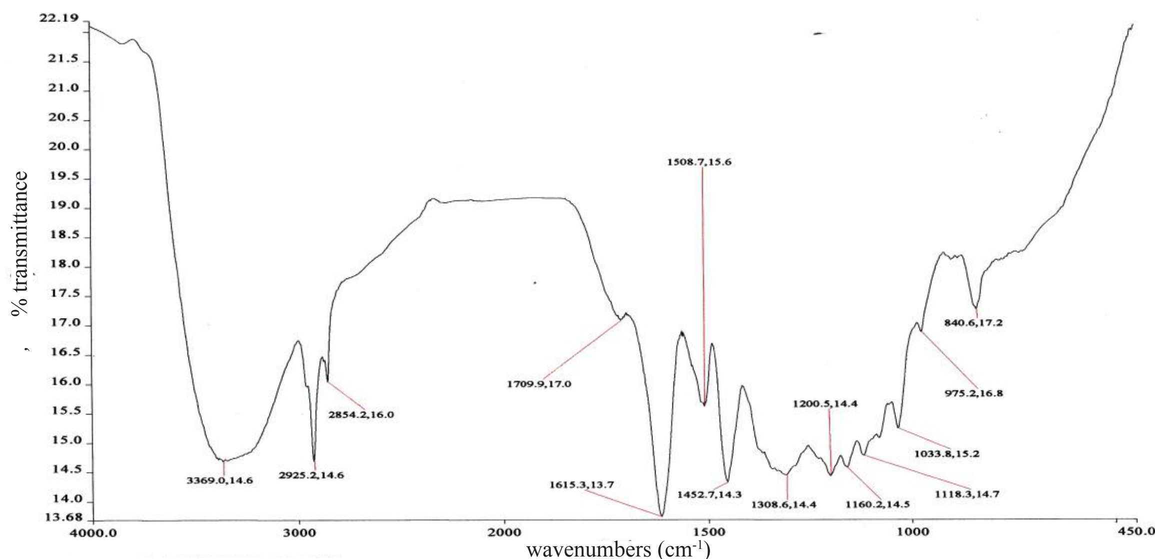

Figure 7. IR spectra of isolate (KBr).

of an anomeric proton resonance at around 4.40 ppm as well as CH-OHs at 3.32-3.43 ppm suggested that the isolate was glycosylated (Fig. 8).

Several phenolic compounds have been reported to possess immunomodulatory effect, e.g., phenolic compound from Ginger (*Zingiber oficinale* Roscoe[12] increased lymphocyte proliferation, while polyphenol from green tea leaves (*Camellia sinensis*) was reported to increase the amount and phagocytic activity of macrophage[13]. Considering that the subfraction 7 was identified further as a phenolic glycoside in this research, and showed high activity on lymphocyte proliferation, it could be suggested that phenolic compound plays an important role in the plant's immune stimulatory effect.

A close related substance has been reported from another important Ant Nest plant species, *Hydnophytum formicarum* Jack. Prachayasittikul *et al* have reported the presence of a protocatechualdehyde (Fig. 9)[14]. NMR spectra of this compound showed aromatic protons resonances which were shifted downfield due to the presence of an aldehyde moiety. Therefore isolate in this research might not be the same compound with Prachayasittikul's research[14].

On the other hand, a phenolic glycosyde having an aliphatic side chain (Fig. 10) has been reported responsible to support the active compound from plant[15]. This compound (Figure 10) showed a comparable ^{1}H-NMR data with the isolate. To clarify the result, further analyses such as isolate purification,

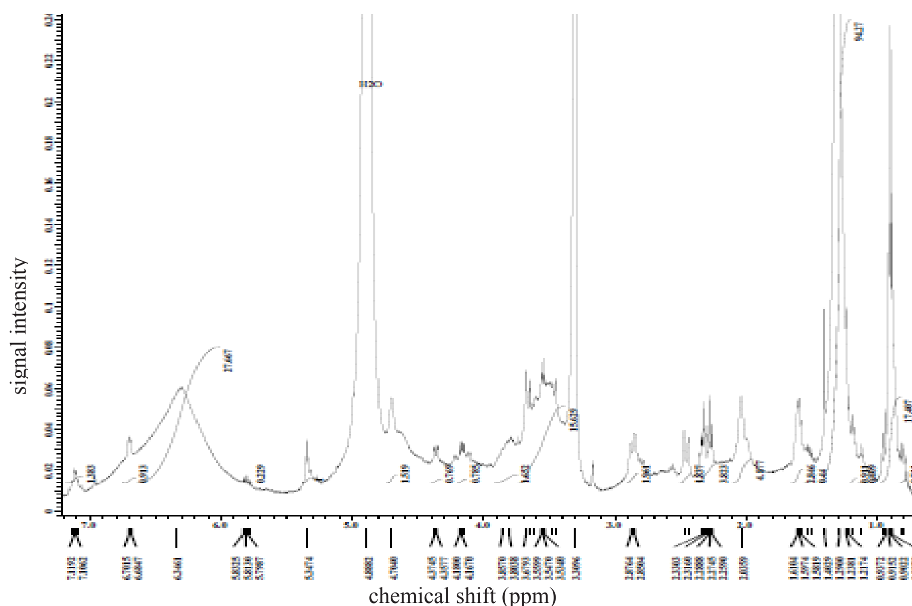

Figure 8. ^{1}H-NMR spectra of isolate.

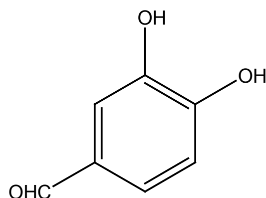

Figure 9. Protocatechualdehyde[15].

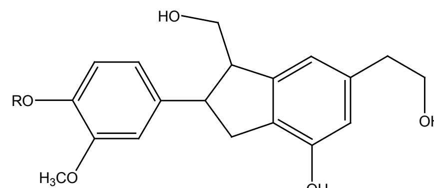

Figure 10. Phenolic glycoside with a liphatic moiety[14] R = sugar moiety.

hydrolysis as well as analyses in 2D and ^{13}C NMR spectrometry are needed. 2D-NMR data will assist in assuring the correlation between resonances especially correlation between the aliphatic and aromatic ring as well as the sugar moiety. LCMS with acidic eluent and negative mode measurement should be carried out in order to estimate the compound's molecular weight.

CONCLUSION

The result shows that *M. pendens* subfractions are potential to increase mice Balb/c lymphocyte proliferation by *in vitro* method. There is no observed significant correlation between total phenolic content and biological activity. However, phenolic compound might contribute to the activity considering the chemical characteristic of bioactive isolate. Structure characterization of the active consituent suggested a glycosylated phenolic having aliphatic side chain as one of the active constituent. Further analyses are necessary to determine the exact chemical structure of the isolate.

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DAFTAR PUSTAKA

1. Abbas KA, Lichtman AH, Pillai S. Celluler and moleculer immunology. 6th Ed. Philadelphia: Saunders Elseveier; 2007.
2. Sheehan C. Clinical immunology principles and laboratory diagnosis. 2nd Ed. Philadelphia: Lippincott Company; 1997. 7.
3. Baratawidjaja KE. Imunologi dasar. Edisi V. Jakarta: Balai Penerbit Fakultas Kedokteran Universitas Indonesia; 2002.
4. Katz P. Immunology III. Oxford: Blackwell Publising; 1985.
5. Ma'at S. Imunomodulator manfaat dan bahayanya, di seminar nasional farmasi 2010. Semarang: Stipar ''Yayasan Farmasi''; 2010. 26.
6. Soeksmanto A, Subroto MA, Wijaya H, Simanjuntak P. Anticancer activity for extracts of sarang semut plant (*Myrmecodia pendens*) to HeLa and MCM-B2 cells, Pakistan J Biol Sci. 2010. 13(3):148-51.
7. Blatrix R, Bouamer S, Morand S, Selosse MA. Ant-pant mutualisms should be viewed as symbiotic communities. Plant Signal. Behav. 2009. 4: 554-6.
8. Hertiani T, Sasmito E, Sumardi, Ulfah M. Preliminary study on immunomodulatory effect of sarang-semut tubers *Myrmecodia tuberose* and *Myrmecodia pendens*. Online Journal of Biological Sciences. 2010. 10(3):136-41.
9. Singleton VL, Orthofer R., Raventos RML. Analysis of total phenol and other oxidative substrates and antioxidants by means of folin-ciocalteu reagent, in methods in enzymology. American Journal of Enology and Viticulture. 1974. 299:152.
10. Sasmito E, Mulyaningsih S, Untari KE, Widyaningrum R. Immunostimulant activity of soybean milk against immunoglobulin (lgG, IgA) and lymphocyte cells proliferation of Balb/c mice induced hepatitis A. Indonesian J Pharm. 2006. 17(3):156-61.
11. Field, LD, Sternhell S, Kalman JR. Organic structures from spectra. 4th Ed. New Jersey: John Wiley and Sons; 2008.
12. Tejasari, Zakaria FR, Sajuthi D. Aktivitas stimulasi komponen bioaktif rimpang Jahe (*Zingiber officinale* Roscoe) pada sel limfosit B manusia secara *in vitro*. J Teknol and Industri Pangan. 2002. 13(1):47-53.
13. Triyono T, Supargiyono, Sismindari. Pengaruh polifenol teh hijau (*Camelia sinensis*) terhadap perubahan respon imun selluler mencit selama infeksi *Plasmodium berghei*. Sains Kesehatan. 2002. 15(2).
14. Prachayasittikul S, Buraparuangsang P, Worachartcheewan A, Isarankuranaayudhya C, Ruchirawat S, Prachayasittikul V. Antimicrobial and antioxidative activities of bioactive constituensts from *Hidnophytum formicarum* Jack. Molecules. 2008. 13:904-21.
15. Popoff T, Theander O. The constituents of conider needles. VI: Phenolic glicosides from *Pinus sylvestris.* Acta Chemica Scandinavica. 1977. 31(4):329-37.