Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 3, 2013

Research Article

ISOLATION AND CHARACTERIZATION OF 3-ACETYL ALEURITOLIC ACID AND SCOPOLETIN FROM STEM BARK OF *ALEURITES MOLUCCANA* (L.) WILLD

WISNU CAHYO PRABOWO*, KOMAR RUSLAN WIRASUTISNA, MUHAMAD INSANU

School of Pharmacy, Institute Technology Bandung, Jl. Ganesha 10 Bandung 40132 Indonesia 0222508143. Email: louwishnu@yahoo.com

Received: 07 May 2013, Revised and Accepted: 20 Jun 2013

ABSTRACT

Objective: In Indonesia, stem bark of *Aleurites moluccana* is traditionally known as potential alternative medicine for diarrheoa and typhoid fever treatment. The objectives of this research were to isolate and characterize chemical compounds of the *Aleurites moluccana* stem bark. *Methods:* Extraction was done by maceration using methanol. The extract was fractionated by liquid-liquid extraction method (using n-hexane and ethyl acetate), vacuum liquid chromatography and chromatotron. *Results:* Isolates were characterized using UV-Vis spectrophotometer and NMR. Scopoletin was isolated from the methanolic extract of the stems of *Aleurites moluccana*. This compound has not been previously isolated or reported from the stems bark of this species. *Conclusion:* In this research, two compounds were isolated, pentacyclic triterpen 3-acety aleuritolic acid and coumarine scopoletin.

Keywords: *Aleurites moluccana* stem bark, 3-acetyl aleuritolic acid, Scopoletin, Traditional medicine.

INTRODUCTION

Candlenut trees (*Aleurites moluccana* (L.) Willd) can be found all over the world. These plants are used for spice of cuisine. The area of candlenut trees cultivation in Indonesia reached 212,487 hectares in 2004 amounted to 88,481 tons is not insufficient to meet the requirement. Import candlenuts reached 11 tons/year with a value of $ 21,000. This condition will certainly increase candlenut cultivation to meet the needs of the Indonesian people [1]. The candlenut stem bark is traditionally used for diarrhoea (dysentery) [2], it is also used in East Borneo to cure typhoid. They have been screened for their potential uses as alternative medicines for many infectious diseases in other countries [3]. In Japan, the stem bark was used for tumors while in malaysia, decoction of the leaves was used in treating coughs, diarrhea, pains in the chest and hernia. Boiled leaves were used for headaches, fever, ulcers, and gonorrhea. The candlenut fresh sap was used to treat thrush and candidiasis [4][5]. The fruit juice was squeezed into the mouths of new born babies to make them vomit and to clear their throats [6]. *Aleurites moluccana* seeds contain glycerides, linoleic, palmitic, stearic, myristic acid, oil, protein, vitamin B1 while the stem bark contains alkaloids, polyphenols, flavonoids, coumarins, tannins, steroids and triterpenoids [7] [8]. The aims of this study were to isolate and characterize the compounds from the stem bark of *Aleurites moluccana* which was potential for drug candidates.

MATERIALS AND METHODS

Plant collection and identification

The fresh stem barks were collected from Klaten, Central Java in October 2011. Determination was done in Herbarium School of Life Sciences, Institute Technology Bandung. The stem bark were sorted, cleaned, and dried in shade at room temperature for 5 days. They were sawed and milled to obtain coarse powder.

Extraction and isolation

Crude drugs were extracted by maceration using distilled solvents for 24 hours, maceration process was repeated for 3 times. Extract was evaporated by vacuum rotary vaporator, yielded 106.6 g of dry extract by liquid-liquid extraction using n-hexane and ethyl acetate. The ethyl acetate extract was fractionated by vacuum liquid chromatography and produced 18 fractions. Compound (1) was obtained from fraction C in the form of white crystals (49 mg). This compound showed red spots after it was sprayed by Lieberman Burchard spray reagent. Fraction J was isolated with chromatoron twice. The isolation process used organic solvent mixtures starting from ethyl acetate-hexane 1:1, and followed by mixtures of ethyl acetate- hexane with increasing polarity. Fraction J2 (40 mg) was recrystallized using ethyl acetate to obtain compound (2) the form of yellow needle crystals. It was detected by TLC under UV light at 366 nm, and confirmed by 5% KOH spray reagent.

Characterization

Isolates were characterized using UV-Vis spectrophotometer and nuclear magnetic resonance spectroscopy 1D-NMR (^{1}H-NMR, NOE, ^{13}C-NMR, and DEPT) and 2D-NMR (HSQC and HMBC).

RESULT

From the isolation process, two compounds were obtained. They were characterized using UV-Visible and NMR. Compound (1) showed maximum absorption of $\lambda_{(MeOH)}$ 204 nm. It indicated that the compound did not contain chromophore [9]. ^{13}C-NMR spectrum ($CDCl_3$) of compound (1) displayed 32 carbon resonances, they can be analyzed as 5 quaternary carbons (C) at 29.28, 37.67, 37.92, 38.98 and 37.35 ppm, 3 metin groups (CH) at 55.62, 49.08, and 41.87 ppm, 10 methylene groups (CH_2) at 37.33, 23.45, 18.69, 40.87, 17.55, 33.69, 31.55, 35.43, 33.37, and 30.91 ppm, and 7 methyl group (CH_3) at 27.95, 16.56, 15.56, 26.00, 22.36, 31.99 and 28.71 ppm. These resonances show a pentacyclic triterpene skeleton. The acetoxy group was seen at 171.50 ppm. Position of acetoxy group (CH3C(=O)O) was determined from HSQC spectrum, which revealed that protons were found to be related with C-3 and it can be recognized from HMBC spectrum of quartenary carbon at acetoxy group. Carboxylic acid group was found at 182.40 ppm. Position of carboxylic acid group (COOH) was determined from HMBC spectrum, which revealed that carbon on carbonil groups and C-17 were found to be related with protons at C-16 and C-22. Double bond (C=C) showed deshielding condition within at 160.43 and 116.78 ppm. Position of this double bond was clearly determined from the HMBC spectrum, which revealed that H-15 was found to be related C-15 and recognized with near carbon C-14. The structure of compound (1) was thus determined to be 3-acetyl aleuritolic acid skeleton. Compound (2) showed maximum absorption of UV-Visible spectrum at $\lambda_{(MeOH)}$ 229, 252, 298, and 348 nm. The UV-Visible spectrum indicated the presence of coumarine skeleton and confirmed by 5% KOH spray reagent. The spot showed an increasing in the intensity of blue colour on UV 366 nm [10]. ^{13}C-NMR spectrum (Acetone-d6) of compound (2) displayed 10 carbon resonances. They showed carbonyl group (C=O) at 161.33 ppm, and methoxy group (OCH_3) at 56.73 ppm. Position of methoxy was clearly determined from the NOE spectrum. Which revealed that the protons on metoxy by HMQC spectrum were found to be related with proton that C-5. Non-oxygenated aromatic carbons were detected at 112.09, 109.98, 103.73 and 113.30 ppm. The rest of oxygenated aromatic carbons appeared at 144.67, 145.98, 151.14, and 151.86 ppm.

DISCUSSION

Based on UV-Vis and NMR spectra, compound (1) is 3-acetyl aleuritolic acid. It was first reported by Silva of dichloromethane

extract of stem bark *Aleurites moluccana* [5]. This compound was reported to inhibit lymphocytic leukemia tumor [11], inhibition of DNA topoisomerase II, lung carcinoma and cytotoxic activity [12]. It was active against *Vibrio cholera, Escherichia coli, Shigella dysentery, Staphylococcus aureus*, *Salmonella typhimurium* [13] [14] and significant antifilarial against *Onchocerca gutturosa* [15]. Compound (2) is scopoletin. Although this compound has been isolated from genus *Scopolia* Jacq [16] and reported marker in the identification of Morinda fruits [17], but our finding is the first report from the genus of *Aleurites*. Scopoletin was reported for its interesting pharmacological activities which were antithyroid, antioxidative, antihyperglycemic [18], immunomodulatory effect on tumoral and normal lymphocytes [19], antiproliferation, antioxidant [20], vasorelaxant [21], antipyretic [22], antiplatelet aggregation [23], antidiabetes, applications in cardiovascular disease [24] neuroprotective [25], hypotensive [26], antimicrobial [27], antiinflammatory [28], inhibition the release of PGE2 and suppressed the expression of COX-2 [29]. Based on previous researches and pharmacological reports, this plant will be potential for medicinal applications.

Table 1: Spectrum of ^{1}H-NMR, ^{13}C-NMR, DEPT, HMQC and HMBC compound (1)

Position	δC	DEPT	HMBC
1	37.33	CH_2	C2
2	23.45	CH_2	
3	81.24	CH	C2
4	37.67	C	C5; C23; C24
5	55.62	CH	
6	18.69	CH_2	C5
7	40.87	CH_2	
8	38.98	C	C7; C26
9	49.08	CH	C11
10	37.92	C	C25; C9
11	17.55	CH_2	
12	33.69	CH_2	C11
13	37.35	C	C27
14	160.43	C	C26
15	116.78	CH	
16	31.55	CH_2	C15
17	51.21	C	C16;C22
18	41.87	CH	C13
19	35.43	CH_2	C18
20	29.28	C	C29
21	33.37	CH_2	C22
22	30.91	CH_2	C20
23	27.95	CH_3	
24	16.56	CH_3	C23
25	15.56	CH_3	C1; C9
26	26.00	CH_3	C7
27	22.36	CH_3	C12
28	182.40	C	C16;C22
29	31.99	CH_3	C30
30	28.71	CH_3	C20; C21
	21.27	CH_3	CO
	171.50	CO	C3

Table 2: Spectrum of ^{1}H-NMR, ^{13}C-NMR, HSQC, ^{1}H-NOE 1D compound(2)

Position	δC	HSQC	NOE
2	161.33		
3	113.30	6.16(d)	
4	144.67	7.82(d)	CH_3;C3;C5
5	109.98	7.17(s)	CH_3;C4
6	145.98		
7	151.14		
8	103.73	6.78(s)	
9	112.09		
10	151.86		
	56.73	3.80 (s)	

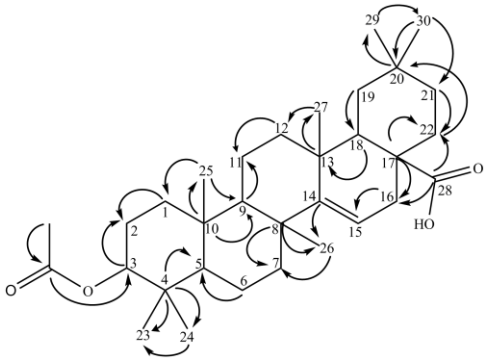

Fig.1:H-C HMQC and H-C HMBC (→) correlations of compound (1)

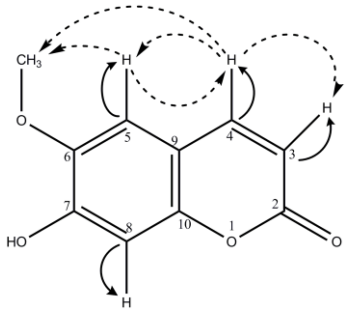

Fig. 2: H-C HSQC (→) and H-H NOE (---›) interactions of compound (2)

CONCLUSION

The phytochemical research of stem bark *Aleurites moluccana* has led to isolate and identify two known natural products, which are 3-acetyl aleuritolic acid and coumarine scopoletin.

ACKNOWLEDGMENT

This research was supported by Institute Technology Bandung (ITB).

REFERENCES

1. Rosman R, Djauhariya E. Status Teknologi Budidaya Kemiri. Research Institute for Medicinal and Aromatic Plants 2006; 2: 55–66.
2. Wiart C, Medicinal Plants of The Asia Pasific, Drugs for The Future. World Scientific Publishing 2006; 337.
3. Acharyya S, Dash GK, Mondal S, Dash SK. Antioxidative and Antimicrobial Study Of *Spondias Mangifera* Willd Root. International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2 (4): 68.
4. Krisnawati H. Ecology, silviculture and productivity *Aleurites moluccana* (L.)Willd. Center for International Forest Research 2011; 3-4.
5. Scott S, Craig T. Poisonous Plants of Paradise (First Air and Medical Treatment of Injuries from Hawai's Plants). University of Hawaii Press 2000; 1.
6. Han ST. Medical Plants In The South Pacific. World Health Organization (WHO) Regional Publication, Manila Philippines 1998; (19): 7.
7. Silva CM, Mora TC, Santos A, Soares R. A Triterpene and A Flavonoid C-Glycoside from *Aleurites moluccana* L Willd (Euphorbiaceae). Acta Farmaceutica Bonaerense 1997; 3: 169-172.
8. Samah OA, Razar RM. Antibacterial Activity of *Aleurites moluccana* (Euphorbiaceae) Against Some Clinical Isolat. Research Journal of Biotechnology 2010; 5 (3): 1.
9. Pretsch E, Buhlmann P, Badertscher M. Structure Determination of Organic Compounds, Table of Spectral Data. Springer 2008; 4: 403.
10. Murray RD, Mendez J and Brown SA. The Natural Coumarins: Occurrence, Chemistry and Biochemistry. John Wiley and Sons 1982, 25-32.
11. Torrance SJ, Wiedhopf RM, Cole JR. Antitumor agents from *Jatropha macrorhiza* (Euphorbiaceae). 3-Acetyl Aleuritolic Acid. Journal of Pharmaceutical Sciences 1977 66: 1348-1349.
12. Wada S, Tanaka R. Isolation DNA topoisomerase-II inhibition, and cytotoxicity of three new terpenoids from the bark of *Macaranga tanarius*. Chemistry and Biodiversity 2006; 3: 473-479.
13. Mathabe MC, Hussein AA, Nikolova RV, Basson AE, Meyer JJ, Lall N. Antibacterial activities and cytotoxicity of terpenoids isolated from *Spirostachys africana*. Journal Ethnopharmacol 2008; 116: 194-197.
14. Peres MT, Delle MF, Cruz AB, Pizzolatti MG, Yunes RA. Chemical composition and antimicrobial activity of *Croton urucurana* Baillon (Euphorbiaceae). Journal Ethnopharmacol 1997; 56: 223-226.
15. Nyasse B, Ngantchou I, Nono JJ, Schneider B. Antifilarial activity in vitro of polycarpol and 3-O-Acetyl Aleuritolic Acid from Cameroonian Medicinal Plants Against *Onchocerca gutturosa.* Natural Product Research 2006; 20: 391-397.
16. Slawomira N, Tadeusz HD, Edward S, Maria W. Quantitative Determination of Coumarins, Flavonoids and Chloro Acid in The Leaves and Underground Parts of Some Species of Genus *Scopolia* Jacq. Acta Poloniae Pharmaceutica Drug Research 2002; 59 (4): 259-263.
17. Smita N, Sushma M. Preliminary Physicochemical and Phytochemical Evaluation of *Morinda Citrifolia* Fruit Extractives. International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2 (4) : 149-154.
18. Panda S, Kar A. Evaluation of The Antithyroid, Antioxidative and Antihyperglycemic Activity of Scopoletin from *Aegle marmelos* Leaves In Hyperthyroid Rats. Phytotherapy Research 2006; 20 (12): 1103-1105.
19. Manuele MG, Ferraro G, Barreiro AM, Lopez P, Cremaschi G, Anesini C. Comparative Immunomodulatory Effect of Scopoletin on Tumoral and Normal Lymphocytes. *Life Science Journal* 2006; 79: 2043-2048.
20. Lemos TL, Machado LL, Souza JS, Fonseca AM, Maia JL, Antioxidant, Icthyotoxicity and Brine Shrimp Lethality Tests of *Magonia glabrata*. Fitoterapia 2006; 77: 443-445.
21. Iizuka T, Nagumo S, Yotsumoto H, Moriyama H, Nagai M. Vasorelaxant Effects of Acer Nikoense Extract and Isolated Coumarinolignans on Rat Aortic Rings. Biological and Pharmaceutical Bulletin 2007; 30: 1164-1166.
22. Delporte C, Backhouse N, Negrete R, Salinas P, Rivas P, Cassels BK, San Feliciano A. Antipyretic, Hypothermic and Antiinflammatory Activities and Metabolites from *Solanum ligustrinum* Lood. Phytotherapy Research 1998; 12: 118-122.
23. Okada Y, Miyauchi N, Suzuki K, Kobayashi T, Tsutsui C, Mayuzumi K, Nishibe S, Okuyama T. Search for Naturally Occurring Substances to Prevent The Complications of Diabetes. Chemical and Pharmaceutical Bulletin 1995; 43: 1385-1387.
24. Dai Y, Wang Z, Ding Z, Application Of Scopoletin In Manufacture of Medicine for Treating Hyperuricaemia. *Chemical Abstracts* 2005; 144.
25. Son D, Lee P, Lee J, Lee S, Choi SY, Lee JW, Kim SY. Neuroprotective Effect of Scopoletin from Angelica Dahurica on Oxygen and Glucose Deprivation Exposed Rat Organotypic Hippocampal Slice Culture. *Food Science* and *Biotechnology* 2007; 16: 632-635.
26. Guantai AN, Addae M. Cardiovascular Effect of Artemisia Afra and Its Constituents. *Pharmaceutical Biology.* 1999; (937): 351-356.
27. Bonilla RP, Lock UO, Jurupe CH. Chemical Biological Study of *Werneria dactilophylla*. Boletin de la Sociedad Química 1991; (57): 182-188.
28. Ding Z, Dai Y, Hao H, Pan R, Yao X, Wang Z. Anti Inflammatory Effects of Scopoletin and Underlying Mechanisms. *Pharmaceutical Biology* 2009; 46 (12): 854-860.
29. Ali SM, Naveed A, Akram. Scopoletin Suppresses Pro-inflammatory Cytokines and PGE2 from LPS Stimulated Cell Line. Fitoterapia 2004; 75: 261–266.