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Chemical Constituents of Artocarpus odoratissimus from Sarawak

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ABSTRACT

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Key words: Moraceae, Artocarpus odoratissimus, pinocembrin, pinostrobin, α -amyrin acetate, β -amyrin acetate. Previous studies showed that Artocarpus species are rich in phenolic compounds, including flavonoids, stilbenoids and arylbenzofurons. Furthermore, compounds from Artocarpus species exhibited diverse biological activities including antibacterial, antitubercular, antiviral, antifungal, antiplatelet, antiarthritic, tyrosinase inhibitory and cytotoxicity. However, there is no phytochemical investigation on the Artocarpus odoratissimus from Sarawak. Thus, it would be interesting to develop a chemical profile of such Sarawakian species. Various chromatographic methods, such as liquid vacuum chromatography, radial chromatography and column chromatograpy were employed to isolate the chemical constituents from the different parts, namely root, bark and leaves of Artocarpus odoratissimus. The isolation and purification using different solvents system ratio of hexane and ethyl acetate (10:0, 8:2, 6:4, 4:6, 2:8, 0:10) and ethyl acetate and methanol (8:2) have led to two flavonoids; pinocembrin (1) and pinostrobin (2), and six triterpenoids; α -amyrin acetate (3), β -amyrin acetate (4), traxateryl acetate (5), hexyl dodecanoate (6), β -sitosterol (7) and stigmasterol (8). The structures (1) – (8) were elucidated using various spectroscopic analysis, which included the Mass Spectroscopy (MS), Infrared (IR) spectroscopy, Ultra-violet (UV) spectroscopy and Nuclear Magnetic Resonance (NMR) spectroscopy, as well as by comparison with the reported data. Although the occurrence of compounds 1 and 2 were widely distributed in many of the plant species, but it was uncommon to the Artocarpus species which could be of chemotaxonomic significance to the genus Artocarpus.

INTRODUCTION

The genus *Artocarpus* belonging to the family of Moraceae consists of about 55 species. It is widely distributed throughout subtropical and tropical regions of the World from Indian subcontinent south of the Himalayas, Sri Lanka, Burma, Thailand, Indo-China, Southern China, Taiwan and Malay Peninsula (Kochummen, 2000). *Artocarpus odoratissimus* originated in Borneo. It is cultivated in the Philippines and is also found in the wild extensively in Brunei Darussalam, Sabah and Sarawak (Subhadrabandhu, 2001). Some *Artocarpus* species are used as traditional medicines. For example *A. odoratissimus* (terap) isused by local communities in Sarawak as an antidote

against centipede and scorpion stings by applying the ash from the leaves on the wounds, while for treatment of scabies, the ash is added with a little amount of coconut oil (Chai, 2006). The leaves can also be used to heal ulcers and burn. Besides, a decoction of the root is consumed for diarrhoea. Compounds from Artocarpus species exhibited diverse biological activities including antibacterial, antitubercular, antiviral, antifungal, antiplatelet, antiarthritic, tyrosinase inhibitory and cytotoxicity (Jagtap and Bapat, 2010). Previous studies showed that Artocarpus species are rich in phenolic compounds, including flavonoids, stilbenoids and arylbenzofurons. Ee et al. (2010) reported that the isolation of a new prenylated pyranoflavone derivatives, artosimmin and traxateryl acetate. Artosimmin exhibited significant cytotoxicity against cancer cell lines (HL-60 and MCF-7) and showed antioxidant properties. However, no phytochemical investigation or structural characterization on the Artocarpus odoratissimus from Sarawak has been reported to date, therefore it would be interesting to develop a chemical profile of such Sarawakian species.

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MATERIAL AND METHODS

General Experimental Procedure

Melting points were determined on Stuart model SMP10 melting points apparatus. Infrared spectra were recorded on Perkin Elmer FTIR Frontier model spectrophotometer; using potassium bromide (KBr) disc. GC-MS chromatograms were recorded using a Perkin Elmer Clarus 680 spectrometer.

The ¹H NMR spectra were recorded using Bruker 400 spectrometers which run at approximately 400 MHz while ¹³C NMR were measured using the same instrument and condition which run at approximately 100 MHz. Chemical shifts are given in δ (ppm) values relative to that of the solvents used. The column chromatography was performed using silica gel Merck Kieselgel 60 Art. No. 1.09385.1000 of particle size 0.040 – 0.063 mm (230-400 mesh), silica gel 60 GF₂₅₄ (MERCK 1.007730) and silica gel 60 PF254 (MERCK 1.007749) were used for liquid vacuum and radial chromatography respectively.

Plant Materials

The leaves, bark and root of *A. odoratissimus* were collected from Kampung Mambong and Bau, Sarawak. Voucher specimen of the plant is kept in the School of Chemistry and Environmental Studies, Faculty of Applied Sciences at the University Teknologi MARA (UiTM) Sarawak, Samarahan Campus 2, Sarawak, Malaysia.

Extraction and Isolation

The air-dried leaves (500.00 g), stem bark (1097.06 g) and root (722.14 g) of A. odoratissimus were extracted by a cold extraction method for 24 hours and repeated thrice using n-hexane, ethyl acetate and methanol as solvents. The removal of solvents under reduced pressure gave different crude extracts, such as the nhexane, ethyl acetate and methanol crude extracts, respectively. The crude extracts were fractionated using silica gel vacuum liquid chromatography (VLC) with a stepwise gradient polarity solvents system such as hexane, hexane-ethyl acetate, ethyl acetatemethanol and methanol, followed by column chromatography to give major fractions. Fractions with the same R_f values were combined and rechromatographed in appropriate solvent systems until pure isolates were obtained. The isolation and purification of crude ethyl acetate root extract using different solvents system ratio of hexane and ethyl acetate (10:0, 8:2, 6:4, 4:6, 2:8,0:10) and ethyl acetate and methanol (8:2) have led to two flavonoids; had afforded pinocembrin (1), pinostrobin (2) α -amyrin acetate (3) and β -amyrin acetate (4) while the crude hexane bark extract had afforded traxateryl acetate (5) and hexyl dodecanoate (6). In addition, the leaves methanol extract had yielded β -sitosterol (7) and stigmasterol (8) (Figure 1)



Fig. 1: structure of compounds (1-8) isolated from A. odoratissimus.

int.); 256 (100) [M⁺ C₁₅H₁₂O₄], 238 (14), 179 (70), 152 (80), 124 (45), 104 (18), 77 (20), 69 (20) and 51(9); ¹H NMR (400 MHz; CDCl₃): δ 2.86 (3-cis), 3.10 (3-trans), 5.43 (2H, t, J=6.7), 6.03 (H-6), 6.03 (H-8), 7.41-7.49 (H-2'-H6'), 12.07 (C-5-OH). ¹³C NMR (100 MHz; CDCl₃): δ 43.37 (C-3), 79.18 (C-2), 95.56 (C-8), 96.81 (C-6), 102.97 (C-4a), 126.16 (C-2', C-6'), 128.87 (C-3', C-4', C-5'), 138.42 (C-1'), 163.11 (C-8a), 164.34 (C-5), 195.65 (C-4).

Pinostrobin (2)

Colorless crystal; mp 100-102^oC; IR (KBr) v_{max} cm⁻¹: 3467 (Ar-H stretching), 1741 (C=O ester), 1497, 1578 (C=C Aromatic), 1538, 1360 (C-O), 968 (=C-H) cm⁻¹; EI-MS m/z (% rel. int.); 270 (90) [M⁺ C₁₆H₁₄O₄], 269 (50), 252 (8), 193 (100), 166 (80), 138 (50), 110 (18), 95 (35), 78 (15) and 51 (20);. ¹H NMR (400 MHz; CDCl₃): δ 2.81 (3-cis), 3.86 (C-7-OMe), 3.10 (3-trans), 5.43 (2H, t, J=6.7), 6.03 (H-8), 6.04 (H-6), 7.28-7.49 (H-2'-H6'), 12.07 (C-5-OH). ¹³C NMR (100 MHz; CDCl₃): δ 43.36 (C-3), 55.60 (C-7-OMe), 79.25 (C-2), 95.49 (C-6, C-8), 103.20 (C-4a), 126.17 (C-2', C-6'), 128.92 (C-3', C-4', C-5'), 138.30 (C-1'), 163.16 (C-5), 164.36 (C-7), 164.63 (C-8a), 195.79 (C-4).

a-Amyrin Acetate (3)

White needles; mp 210-215 °C; IR (KBr) v_{max} cm⁻¹: 2924, 2853 (C-H stretching), 1734 (C=O ester), 1449, 1378 (CH₃ bending), and (C-O) 1243 cm⁻¹; EI-MS m/z (% rel. int.); 468 (1) [M⁺, C₃₂H₅₂O₂], 408 (10), 365 (15), 219 (18), 218 (100), 203 (75), 189 (38), 175 (28); ¹H NMR (400 MHz; CDCl₃): δ 0.80 (3H, s, H-28), 0.87 (12H, s, H-23, H-24, H-25, H-26), 0.98 (3H, s, H-30), 1.01 (3H, s, H-29), 1.07 (3H, s, H-27), 2.05 (3H, s, CH₃-CO), 4.50 (1H, dd, *J* = 6,10, H-3), 5.13 (1H, t, *J* = 3.6, H-12); ¹³C NMR (100 MHz; CDCl₃): δ 15.7 (C-25), 16.7 (C-26), 16.9 (C-24), 17.5 (C-29), 18.3 (C-6), 21.3 (CH₃-CO), 21.4 (C-30), 23.2 (C-27), 23.4 (C-11), 23.7 (C-2), 26.6 (C-16), 28.0 (C-23), 28.0 (C-28), 28.8(C-15), 31.3 (C-21), 32.9 (C-7), 33.8 (C-17), 36.8 (C-10), 37.7 (C-4), 38.5 (C-1), 39.6 (C-19), 39.7 (C-20), 40.0 (C-8), 41.5 (C-22), 42.1 (C-14), 47.6 (C-9), 55.3 (C-5), 59.1 (C-18), 80.9 (C-3), 124.3 (C-12), 139.6 (C-13), 171.0 (CH₃-<u>C</u>O).

β-Amyrin Acetate (4)

Colorless solid; mp 238-241 °C; IR (KBr) v_{max} cm⁻¹: 2918, 2849 (C-H stretching), 1735 (C=O ester), 1452, 1377 (CH₃ bending), 1245 (C-O) cm⁻¹; EI-MS m/z (% rel. int.); 468 (10) [M⁺, C₃₂H₅₂O₂], 453 (2), 408 (1), 219 (18), 218 (100), 203 (25), 189 (11) and 175 (5); ¹H NMR (400 MHz; CDCl₃): δ 0.83 (3H, s, H-28), 0.87 (12H, s, H-23, H-24, H-29, H-30), 0.97 (6H, s, H-25, H-26), 1.13 (3H, s, H-27), 2.05 (3H, s, CH₃-CO), 4.50 (1H, m, H-3), 5.18 (1H, t, *J* = 3.68, H-12); ¹³C NMR (100 MHz; CDCl₃): δ 15.6 (C-25), 16.7 (C-26), 16.8 (C-24), 18.3 (C-6), 21.3 (<u>CH₃-CO</u>), 23.2 (C-11), 23.6 (C-2), 23.7 (C-30), 26.0 (C-27), 26.1 (C-15), 26.9 (C-16), 28.0 (C-23), 28.4 (C-28), 31.1 (C-20), 32.5 (C-17), 32.6 (C-7), 33.3 (C-29), 34.7 (C-21), 36.8 (C-10), 37.1 (C-22), 37.7 (C-4), 38.2 (C-1), 39.8 (C-8), 41.7 (C-14), 46.8 (C-19), 47.2 (C-18), 47.6

(C-9), 55.2 (C-5), 80.9 (C-3), 121.6 (C-12), 145.2 (C-13), 171.0 (CH₃-<u>C</u>O).

Traxateryl Acetate (5)

White needles; mp 239 0 C; IR (KBr) v_{max} cm⁻¹: 2924, 2852 (C-H stretching), 1733 (C=O ester), 1449, 1366 (CH₃ bending), and (C-O) 1243 cm⁻¹; EI-MS m/z (% rel. int.); 468 (1) [M⁺, C₃₂H₅₂O₂], 408 (10), 365 (15), 219 (18), 203 (75), 189 (38) and 175 (28); ¹H NMR (400 MHz; CDCl₃): δ 0.81 (6H, s, H-25 & 28), 0.88 (3H, s, H-24), 0.89 (3H, s, H-30), 0.93 (3H, s, H-29), 0.99 (3H, s, H-27), 1.02 (3H, s, H-23), 1.08 (3H, s, H-26), 4.52 (1H, m, H-3), 5.13 (1H, t, J = 3.68 Hz); ¹³C NMR (100 MHz; CDCl₃): δ 15.8 (C-27), 16.8 (C-24), 16.9 (C-25), 17.5 (C-28), 18.2 (C-6), 21.3 (<u>CH₃-CO</u>), 21.4 (C-29), 23.2 (C-26), 23.4 (C-12), 23.6 (C-2), 26.6 (C-15), 28.1 (C-11), 28.1 (C-30), 28.7 (C-23), 31.2 (C-7), 32.8 (C-16), 33.7 (C-17), 36.8 (C-10), 37.7 (C-4), 38.4 (C-1), 39.6 (C-13), 39.6 (C-19), 40.0 (C-14), 41.5 (C-22), 42.0 (C-8), 47.6 (C-9), 55.2 (C-5), 59.0 (C-18), 80.9 (C-3), 124.3 (C-21), 139.6 (C-20), 170.9 (CH3-CO).

Hexyl Dodecanoate (6)

White solid; mp 61-62 ^oC, IR (KBr) v_{max} cm⁻¹: 2924 (C-H stretching), 1735 (C=O ester), 1456, 1369 (CH₃ bending), and (C-O) 1242 cm⁻¹; EI-MS m/z (% rel. int.); 284 (15) [M⁺ C₁₈H₃₆O₂.], 241 (10), 157 (15), 101 (35), 88 (100), 73 (18) and 55 (28); ¹H NMR (400 MHz; CDCl₃): δ 0.88 (3H, t, *J* = 6.6 Hz), 0.91 (3H, t, *J* = 6.8), 1.27-1.45 (22H, m), 1.57-1.65 (4H, m), 2.31 (2H, t, *J*=7.4), 4.06 (2H, t, J=6.7); ¹³C NMR (100 MHz; CDCl₃): δ 14.1 (C-12), 14.1 (C-18), 22.7 (C-11), 22.7 (C-17), 25.0 (C-3), 25.6 (C-15), 28.6 (C-14), 29.2 (C-4), 29.3 (C-5), 29.3 (C-9), 29.5 (C-7), 29.6 (C-6), 29.6 (C-8), 31.9 (C-10), 31.9 (C-16), 34.4(C-2), 64.4 (C-13), 174.1 (C-1).

β -Sitosterol (7)

White needles; mp 145-147 °C; IR (KBr) v_{max} cm⁻¹: 3436 (OH), 2937 (C-H), 2867, 1063 (C=C), and (-CH₃) 1454, 1382, cm⁻¹; EI-MS m/z (% rel. int.); 414 (55) [M⁺, C₂₉H₅₀O], 394 (24), 381 (17), 354 (5), 329 (34), 303 (23), 273 (10), 255 (23), 231 (15), 213 (27), 199 (12), 187 (10), 173 (15), 159 (28), 145 (34), 133 (29), 119 (29), 107 (48), 95 (48), 81 (56), 57 (59), 43 (100), 41 (56); ¹H NMR (400 MHz; CDCl₃): δ 0.68-1.05 (18H, s, 6 X CH₃), 1.05-2.36 (22 H, m, 11 x CH₂), 3.53 (1H, m, H-3), 5.35 (1H, d, J = 5.1Hz, H-6). ¹³C NMR (100 MHz; CDCl₃): δ 11.8 (C-18), 12.0 (C-29), 18.8 (C-21), 19.0 (C-27), 19.4 (C-19),19.8 (C-26), 21.1 (C-11), 23.0 (C-28), 24.3 (C-15), 26.0 (C-23), 28.2 (C-16), 29.1 (C-25), 31.6 (C-2), 31.9 (C-7), 31.9 (C-8), 33.9 (C-22), 36.1 (C-20), 36.5 (C-10), 37.2 (C-1), 39.7 (C-12), 42.3 (C-4), 42.3 (C-13), 45.8 (C-24), 50.1 (C-9), 56.0 (C-17), 56.7 (C-14), 71.8 (C-3), 121.7 (C-6) and 140.7 (C-5).

Stigmasterol (8)

White needles;mp145-147 0 C; IR (KBr) ν_{max} cm⁻¹: 3436 (OH), 2937 (C-H), 1708 (C=C), and (-CH₃) 1454, 1382, cm⁻¹; EI-MS m/z (% rel. int.);412 [M⁺, C₂₉H₄₈O], 271, 255, 159, 133, 81

and 55; ¹H NMR (400 MHz; CDCl₃): δ 0.72 (3H, s, Me-18), 0.83 (3H, *t*, *J*=7.2 Hz, Me-29), 0.85 (3H, *d*, *J*=6.9 Hz, Me-27), 0.88 (3H, *d*, *J*=6.9 Hz, Me-26), 1.03 (3H, *s*, Me-19), 1.03 (3H, *d*, *J*=6.9 Hz, Me-21), 3.54 (1H, *m*, H-3), 5.03(1H, *dd*, *J*= 8.4, 15.0 Hz, H-21), 5.17 (1H, *dd*, *J*= 8.4, 15.0 Hz, H-21), 5.46 (1H, *br d*, H-6); ¹³C NMR (100 MHz; CDCl₃): δ 12.0 (C-18), 12.2 (C-29), 19.0 (C-27), 19.4 (C-26), 21.0 (C-19), 21.1 (C-11), 21.2 (C-21), 24.4 (C-15), 25.4 (C-28), 28.9 (C-16), 31.7 (C-2), 31.7 (C-7), 31.9 (C-8), 31.9 (C-25), 36.5 (C-10), 37.3 (C-1), 39.7 (C-12), 40.5 (C-20), 42.2 (C-13), 42.3 (C-4), 50.2 (C-9), 51.3 (C-24), 56.0 (C-17), 56.9 (C-14), 71.8 (C-3), 121.7 (C-6), 129.3 (C-23), 138.3 (C-22) and 140.2 (C-5).

RESULTS AND DISCUSSION

The extraction and isolation from different parts of Artocarpus odoratissimus have led to eight compounds (Figure 1). The identification of pure compounds was carried out using spectroscopic methods: Infrared (IR) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy and Gas Chromatography Mass Spectroscopy (GC-MS) and a comparison with the reported data. From the root extract, afforded two flavonoids, which were identified and characterized as pinocembrin (1) and pinostrobin (2) based on data comparison literature. As for the two triterpenoids isolated, identified as α -amyrin acetate (3) and β -amyrin acetate (4). Compound (3) and (4) were deduced based on the spectral evidence and by comparison of spectral data with literature values (Ogihara et al., 2000). Two compounds isolated and elucidated from the bark are traxateryl acetate (5) and hexyl dodecanoate (6). Compound (5) have been found and was report before by Ee et al., (2010). The structural elucidation of (6) was based on the spectroscopic data and by comparison of the data with the literature (Shimizu et al., 2012). A chloroform-soluble fraction from a methanol extract of leaves was subjected to column chromatography on silica gel to give β -Sitosterol (7) and stigmasterol (8). The structural elucidations of these compounds were based on their spectroscopic data and by comparison of these data with the literature (Ragasa et al., 2014). Based on the literature, these compounds have revealed diverse bioactivities: Pinocembrin (1) is one of the primary flavonoids isolated from the variety of plants, mainly from Pinus heartwood, Eucalyptus, Populus, Euphorbia, and Sparattosperma leucanthum, in the diverse flora and purified by various chromatographic techniques.

Pinocembrin is a major flavonoid molecule incorporated as multifunctional in the pharmaceutical industry. Its vast range of pharmacological activities has been well researched including antimicrobial, anti-inflammatory, antioxidant, and anticancer activities. In addition, pinocembrin can be used as neuroprotective against cerebral ischemic injury with a wide therapeutic time window, which may be attributed to its antiexcitotoxic effects (Rasul *et al.*, 2013). Pinostrobin (2) was isolated from *Polygonum lapathifolium nodosum* quickly penetrates through cytoplasm to the cellular nucleus of the cultured cells, and gives intensive apoptotic response in stimulating leukemic cells in vitro (Smolarz *et al.*, 2006). With increasing concentration, pinostrobin caused a gradual leakage, also contributing to breakage of the envelope and virus inactivation. Treatment effect of oral pinostrobin *in vivo* showed that pinostrobin possesses definite therapeutic effect in the development of lesion score (Wu *et al.*, 2011).

Previous studies indicated that α -amyrin acetate (3) not only improves glucose tolerance in normal rats significantly, but also lowers the blood glucose profile in STZ-induced diabetic rats and db/db mice. It also improves atherogenic lipid profiles and increases HDL-C levels significantly. These results suggest that compound (3) can be used as an effective antidiabetic cum lipid lowering agent for type 2 diabetes mellitus patients (Singh *et al.*, 2009). β -amyrin acetate (4) isolated from the *Alstonia boonei* stem bark exhibited profound anti-inflammatory activity (Okoye *et al.*, 2014).

Triterpenes (3) and (4) were also reported to exhibit sedative, anxiolytic and anticonvulsant properties (Aragao et al., 2009). β -Sitosterol (7) was observed to have growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells (Awad et al., 2007). Stigmasterol (8) isolated from B. monosperma could reduce the levels of serum triiodothyronine (T_3) and/or thyroxin (T_4) in mice. Its administration at 2.6 mg/kg/d for 20 days reduced serum triiodothyronine (T_3) , thyroxin (T_4) and glucose concentrations as well as the activity of hepatic glucose-6-phophatase (G-6-Pase) with a concomitant increase in insulin indicating its thyroid inhibiting and hypoglycemic properties. A decrease in the hepatic lipid peroxidation (LPO) and an increase in the activities of catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) suggested its antioxidative potential. The highest concentration tested (5.2 mg/kg) evoked pro-oxidative activity (Panda et al., 2009).

CONCLUSIONS

Phytochemical study on *A. odoratissimus* had successfully isolated eight pure compounds; pinocembrin (1), pinostrobin (2), α -amyrin acetate (3) and β -amyrin acetate (4) were isolated from the root of *A. odoratissimus*, traxateryl acetate (5) and hexyl dodecanoate (6) from the bark of *A. odoratissimus* whereas β -sitosterol (7) and stigmasterol (8) from the leaves. In addition, compounds 1- 4 and 6 from *A. odoratissimus* were reported for the first time.

Although the occurrence of compounds **1** and **2** were widely distributed in many of the plant species, but it was uncommon to the *Artocarpus* species which could be of chemotaxonomic significance to the genus *Artocarpus* in particular, and also to the family Moraceae.

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