New Chemical Constituent from the Rhizomes of Johannesteijsmannia altifrons
(Juzuk Kimia Baru daripada Rizom Johannesteijsmannia altifrons)

NOOR AZIIRAA SABRI*, W.A. YAACOB, NUR SHAFIQA ABDULLAH

ABSTRACT
A new compound namely 2-[(1′E)-3′-hydroxyl-1′-methyl-1′-propen-1′-yl]-6-methoxy-7-[(2′′-methylheptyl)oxy]-5-benzofurancarboxylic acid and three known compounds of β-sitosterol, γ-taraxasterol and stigmasterol were isolated from the n-hexane extracts of the rhizomes and fruits of Johannesteijsmannia altifrons using vacuum liquid, column and radial chromatography. The structures of the isolated compounds were determined by means of 1D and 2D NMR, FT-IR, UV-VIS spectroscopy and mass spectrometry.

Keywords: Arecaceae; β-sitosterol; Johannesteijsmannia altifrons; new compound, 2-[(1′E)-3′-hydroxyl-1′-methyl-1′-propen-1′-yl]-6-methoxy-7-[(2′′-methylheptyl)oxy]-5-benzofurancarboxylic acid; stigmasterol; γ-taraxasterol

INTRODUCTION
Johannesteijsmannia belongs to the Arecaceae family comprises of four species, J. altifrons, J. lanceolata, J. magnifica and J. perakensis. Johannesteijsmannia altifrons is also known as Daun Sang (Malay) and Joey Palm (English) can be found in various parts of Peninsular Malaysia namely in Kelantan, Perak, Terengganu, Pahang, Johor and Kedah. These fan palms are usually found growing under shade or in dappled light without a trunk and can grow up to 10-20 feet tall and 15 feet wide. In the forest, the leaves drop down to the base of the palm and form compost. The leaves are also commonly used as material for roof building by the local people (Ng 2006).

The family of Arecaceae has been reported to contain flavonoids (Muhaisen 2014), alkaloids (Dyana & Kanchana 2012), terpenoids, steroids, fatty acids and tannins (Bennmehdi et al. 2012). However, to the best of our knowledge, no chemical constituent has ever been isolated or characterized from this genus. Thus, a phytochemical investigation on J. altifrons is done to further enrich our knowledge on this species. In this study, the n-hexane extracts from the rhizomes and fruits of J. altifrons were purified and led to the discovery of known β-sitosterol (1), γ-taraxasterol (2), stigmasterol (3) and new 2-[(1′E)-3′-hydroxyl-1′-methyl-1′-propen-1′-yl]-6-methoxy-7-[(2′′-methylheptyl)oxy]-5-benzofurancarboxylic acid (4) as in Figure 1.

MATERIALS AND METHODS
GENERAL
There are different spectroscopic methods used to elucidate the structures of β-sitosterol, γ-taraxasterol, stigmasterol and 2-[(1′E)-3′-hydroxyl-1′-methyl-1′-propen-1′-yl]-6-methoxy-7-[(2′′-methylheptyl)oxy]-5-benzofurancarboxylic acid (4) as presented in Table 1.
The rhizomes and fruits of *J. altifrons* were collected at Pelagat Forest Reserve, Besut, Terengganu in February 2015. A voucher specimen (UKMB 40309) was deposited at the Herbarium of Universiti Kebangsaan Malaysia, Bangi (UKMB).

**EXTRACTION AND ISOLATION**

The air-dried, ground rhizomes (3.22 kg) and fruits (786 g) of *J. altifrons* were extracted for 3 days in methanol at room temperature, followed by filtration to give methanol solutions. They were then extracted with *n*-hexane several times, combined and reduced under pressure to afford 19.62 g (0.61%) and 4.42 g (0.56%) of greenish brown of rhizome and fruit *n*-hexane extracts. Both extracts were fractionated by VLC, eluted with increasing polarity of *n*-hexane and ethyl acetate. The fractions were combined based on their silica gel TLC profiles. For the fruit extract, the VLC gave sixteen fractions (F1–F16). Fractions F2 to F6 were combined and further fractionated using RC to produce four combined fractions (F2–F4). The third fraction (F3) contained β-sitosterol (I) (4.6 mg). Subsequently, fractions F5 to F12 were combined and purified by RC to yield seven combined fractions (F5–F12); fractions F13 and F14 contained γ-taraxasterol (2) (2.5 mg) and stigmasterol (3) (0.55 g), respectively. For the rhizome extract, the fractionation through VLC yielded eleven fractions (F1–F11). Fraction F4 contained stigmasterol (3) (2.92 g). Fractions F2 and F6 were combined and further fractionated using CC to yield five combined fractions (F2–F5). Fraction F5 was purified using RC to produce four combined fractions (F5–F8). The third fraction (F5) was identified as 2-[(1′E)-3′-hydroxy-1′-methyl-1′-propen-1′-yl]-6-methoxy-7-[(2′′-methylheptyloxy)-5-benzofurancarboxylic acid (4) (7.5 mg).

**RESULTS AND DISCUSSION**

A new compound namely 2-[(1′E)-3′-hydroxy-1′-methyl-1′-propen-1′-yl]-6-methoxy-7-[(2′′-methylheptyloxy)-5-benzofurancarboxylic acid (7.5 mg) was isolated from the rhizomes of *J. altifrons* as a yellow powder. On TLC plate, the compound gave dark shades of purple color when viewed under UV light. Its molecular formula C30H48O6 was derived from ESI-MS which gave pseudomolecular ion at m/z 413.2677 [M+Na]+. The UV-VIS spectrum in chloroform which gave absorbance peak at 317 nm indicates the occurrence of parent benzoferan ring in the molecule. The IR spectrum displayed a broad absorbance peak at 3211 cm−1 which represents hydroxyl group. Absorption band which appeared at 1607 cm−1 indicated the presence of carbonyl group and peak at 1460 cm−1 represents benzoferan aromatic ring. Absorption peaks at 1162 and 1280 cm−1 represent C–O stretches of the hydroxyl, methoxy and ester groups.

The 1H NMR spectrum of 2-[(1′E)-3′-hydroxy-1′-methyl-1′-propen-1′-yl]-6-methoxy-7-[(2′′-methylheptyloxy)-5-benzofurancarboxylic acid showed two highly-deshielded signals at δn 6.84 and 6.31 ppm which indicated the presence of respective protons H-3 and H-4 that were attached to benzene and furan rings of the benzoferan (Table 1). A deshielded doublet signal at δn 4.10 represents the methylene protons located next to the oxygen of phenyl ether (H-1′). A multiplet signal at δn 5.30 indicated the existence of vinylic proton (H-1′). A singlet signal at δn 3.82 indicated the existence of phenyl methoxy group. The appearance of highly overlapped proton signals at δn 3.29 indicated the presence of four terminal methylenes in the n-heptyl chain of the benzoferan ring (H-3′, H-4′, H-5′ and H-6′).

The 13C NMR spectrum showed that 2-[(1′E)-3′-hydroxy-1′-methyl-1′-propen-1′-yl]-6-methoxy-7-[(2′′-methylheptyloxy)-5-benzofurancarboxylic acid contained 22 different carbons consisting of three methyls, six methylenes, four methines, one methoxy and eight quaternary carbons (Table 1). One most deshielded signal at δc 182.1 indicated the presence of carbonyl group and signals at δc 121.5 and 125.1 represent carbon-carbon double bond. Besides these, eight highly-deshielded signals of δc between 101.6 and 155.1 ppm show the presence of benzoferan ring. One signal at δc 62.1 indicated the presence of one methoxy group on benzene ring. Signals at δc 31.6, 29.7, 26.6, and 21.5 showed the presence of four terminal methylenes in the n-heptyl chain. Correlations of 1H–1H in COSY and 1H–13C in HMBC for the compound 4 are tabulated in Table 1 and shown in Figure 2.

A new compound (4) was isolated as benzofuran derivative was isolated for the first time from the Arecaceae family. Stigmasterol and β-sitosterol were found in many species of Arecaceae family, but were isolated for the first time from *J. altifrons*. Garcia et al. (1981) isolated stigmasterol and β-sitosterol from the leaves of *Phoenix canariensis*. Besides, β-sitosterol was the major sterol found in *Phoenix theophrasti*, *Phoenix dactylifera* (Malhi...
et al. 2014) and Corypha taliera (Shoeb et al. 2013). On the other hand, γ-taraxasterol was also isolated for the first time from J. altifrons. However, this compound has never been reported to be isolated from Arecaceae family. Benzofurancarboxylic acid (4), along with three known compounds of β-sitosterol (1), γ-taraxasterol (2) and stigmasterol (3). Compound 4 is the first benzofuran found in the Arecaceae family. The skeleton of benzofuran has been reported to possess many medical use. For example anti-cancer, anti-ulcer, anti-alzheimers, anti-viral and anti-inflammatory (Reshma et al. 2015).

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School of Chemical Sciences and Food Technology
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
43600 UKM Bangi, Selangor, Darul Ehsan
Malaysia

*Corresponding author; email: wanyaa@ukm.edu.my
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