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Chemical constituents of the aerial parts of *Etlingera brevilabrum* (Zingiberaceae)

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ABSTRACT

To isolate the chemical constituents from the leaves and stems of Etlingera brevilabrum, the plant parts were firstly extracted by n-hexane. Then the extracts were purified using different chromatography methods such as vacuum liquid chromatography, column chromatography, and preparative thin layer chromatography. Finally tow compounds were isolated from the extracts. To elucidate the structures, the spectroscopic data of the compounds were compared with the literature values. According to the analysis the pure compounds were identified as β-sitosterol and stigmasterol.

Keywords: *Etlingera brevilabrum*; Zingiberaceae; chemical constituents; β-sitosterol; stigmasterol.

INTRODUCTION

Zingiberaceae with 53 genera and over 1200 species is known as the largest family of the order *Zingiberales*. It is widely distributed in the tropics especially in Southeast Asia [1]. The family is a famous natural resource that provides people around the world with many products used for food, spices, medicines, dyes, perfumes, and aesthetics [2]. Zingiberaceae is divided into four subfamilies including *Hedychieae*, *Zingibereae*, *Alpineae*, and *Globbeae* with the *Etlingera* genus belonging to the *Alpineae* tribe [3]. *Etlingera* is an Indo-Pacific genus that includes more than 100 species. *Etlingera* consists of terrestrial and evergreen herb. The plant grows from sea level to the altitude of 2500 m [4]. *Etlingera brevilabrum* (Valeton) R.M.Sm is characterized with the presence of many purple spots on the upper and under sides of its leaves especially on the new ones [5] (Figure 1). The plant has been known to have several uses in traditional medicine in Sarawak, Malaysia. The leaves are used medicinally on children suffering from long-lasting fever by rubbing the body with the roasted leaves; the base is used to relieve stomach-aches; the pounded leaves are used to treat dry skin on legs; the smoke from burned leaves are used to treat itchy skin; the juice of young stem used to cure eye ailments. The stem can be rubbed on skin infected by insect bites and general itching. The plant also has medicinal use against cholera. The fruits are edible and is enjoyed by the local people, as well as animals such as squirrels, porcupines, and forest rats [4]. In this study we isolated and elucidated the constituents of the stems and leaves extracts from *E. brevilabrum*. So far, there has been no investigation on chemical constituent of the plant parts in the literature. However in our previous studies we reported the chemical compositions of the essential oils [6], antioxidant [7] and antimicrobial activity [8] of the different parts of *E. brevilabrum* species.

Figure 1 Leaves of *Etlingera brevilabrum* (Source [9])

MATERIALS AND METHODS

Plant material

The studied plant parts were the stems and leaves of *E. brevilabrum*. They were collected from their natural habitat in Sabah, Malaysia. The voucher specimens of WYA 500, was deposited at the Universiti Kebangsaan Malaysia Herbarium (UKMB).

Plant extract

The air- dried parts of *E. brevilabrum* including (150 g from each other) were ground and macerated in n-hexane at room temperature for 72 h. After filtration, the solvent was evaporated under reduced pressure using Heidolph evaporator (Laborota 4000 eco). The procedure was repeated for several times to produce 4.50 g leaves extract (HL*b*), and 3.61 g stems extract (HS*b*).

Isolation

Different chromatographic techniques which include vacuum liquid chromatography (VLC), column chromatography (CC), and preparative thin layer chromatography (PTLC) were used to isolate the constituent of the stems and leaves extracts of *E. brevilabrum*. The chromatographic methods were carried out using various suitable solvents, which were assessed from the TLC profiles of the extracts or fractions, to afford the pure compounds. Silica gel 60 GF_{254} (Merck 7747) for VLC and silica gel 60 GF_{254} (Merck 7730) for PTLC were used. The CC was carried out using silica gel 0.063-0.2 mm (70-230 mesh) of Merck 7734.

The leaves extract isolation procedure

A 4 g of the leaves hexane extract (HL*b*) was fractionated using VLC by eluting with increased polarity of *n*-hexane:EtOAc from 100% n-hexane to *n*-hexane-EtOAc mixtures with 5% increment of EtOAc to 100% EtOAc. After solvent evaporation, the fractions with similar TLC profiles were combined to give 8 fractions of VHL*b*1-8. Fraction VHL*b*5 (0.6 g) was further purified using CC by eluting with *n*-hexane:EtOAc of 100:0 to 20:80. After combining similar fractions based on their TLC profiles, 12 fractions of CVHL*b*5.1-5.12 were obtained. The fraction CVHL*b*5.4 (8 mg) showed one spot on TLC(β-sitosterol). The fraction CVHL*b*5.6 (60 mg) was developed using PTLC with 95:5 *n*-hexane:EtOAc as an eluent to yield compound PCVHL*b*5.6 (6 mg)(stigmasterol). The isolation procedure for the *n*-hexane leaves extract from *Etlingera brevilabrum* is summarized in Figure 2.

The stems extract isolation procedure

VLC was applied to 3.1 g of HS*b* with n-hexane:EtOAc as the eluent at different ratios of 100:0 to 0:100 to increase the polarity. A total of 20 fractions were collected. They were concentrated *via* evaporation by leaving to stand under a fume-hood. The fractions were then developed on TLC. The ones that showed similarity in their profiles were combined to give 7 fractions of VHS*b*1-7. Fraction VHS*b*4 (0.42 g) was further purified using CC with the eluent n-hexane:$CHCl_3$ from 100:0 to 40:60. In this step the similar fractions were again combined to produce 10 fractions of CVHS*b*4.1 to CVHS*b*4.10. In the final isolation attempt to obtain a pure compound from the stem extract of *E. brevilabrum*, fraction CVHS*b*4.3 (54 mg) was subjected to PTLC eluting with 90:10 *n*-hexane:$CHCl_3$.

PCVHS*b*4.3 (4 mg) as a pure compound was obtained (stigmasterol). The isolation procedure of the stem extract from *E. brevilabrum* is summarized in Figure 2.

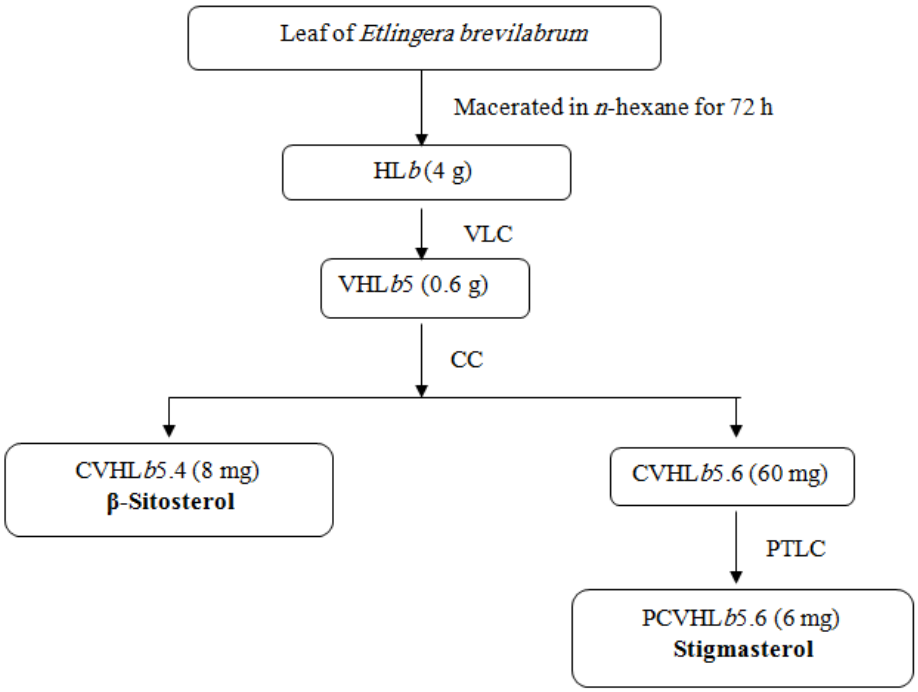


Figure 2: Schematic isolation procedure of the hexane extract from the leaves of *Etlingera brevilabrum*

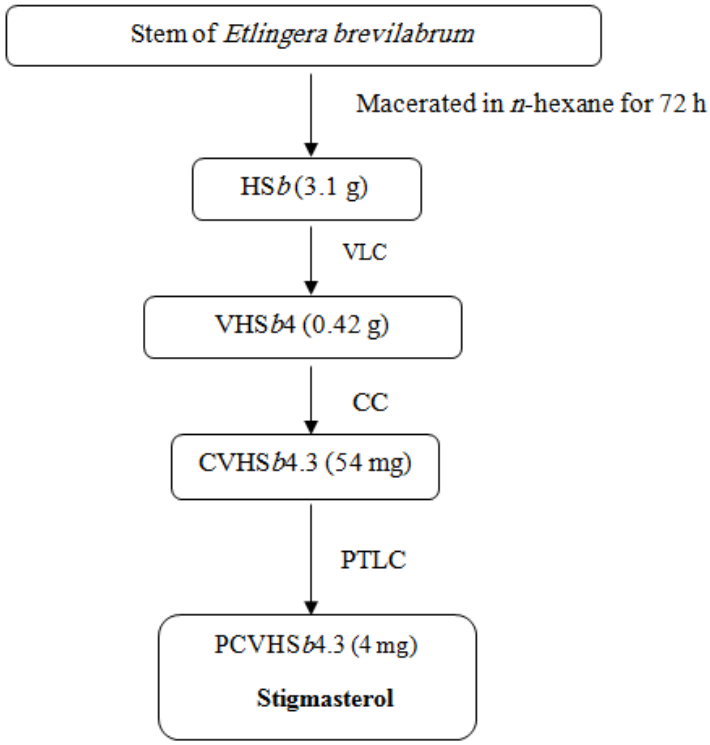


Figure 3: Schematic isolation procedure of the hexane extract from the stems of *Etlingera brevilabrum*

Physical, Chemical, and Spectroscopic Data of the Pure Compounds

The melting points were measured using Electrotermal 9100 apparatus and are uncorrected. FT-IR spectra were obtained using Perkin-Elmer. The mass spectra were recorded using Perkin Elmer Clarus 600 spectrometer. The NMR spectra were recorded using Bruker 600 MHz. Chemical shifts in ppm were referenced to internal $CDCl_3$.

β-Sitosterol (1): White needles. Molecular formula: $C_{29}H_{50}O$. Molecular weight: 414. Melting point: 143-145 °C. FT-IR ν_{max} cm^{-1} (KBr): 3430, 2938, 1646, 1459, 1381, 1054. EI-MS m/z: $[M]^+$ = 414. ^1H NMR ($CDCl_3$, 600 MHz) δ (ppm): 0.69 (3H, s), 0.83 (3H, *d*, *J* = 7 Hz), 0.85 (3H, *d*, *J* = 7 Hz), 0.87 (3H, *d*, *J* = 7 Hz), 0.93 (3H, *d*, 6.6Hz), 1.03 (3H, *d*, *J* = 6 Hz), 1.99 (2H, *m*), 2.28 (2H, *m*), 3.53 (1H, *m*), 5.36 (1H, *t*, 3.0 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 11.9, 12.1, 18.8, 19.0, 19.4, 19.8, 21.1, 23.1, 24.3, 26.1, 28.2, 29.1, 31.7, 31.8, 31.9, 34.0, 36.2, 36.5, 37.3, 39.8, 42.2, 42.3, 45.9, 50.1, 56.1, 56.8, 71.8, 121.7, 140.8.

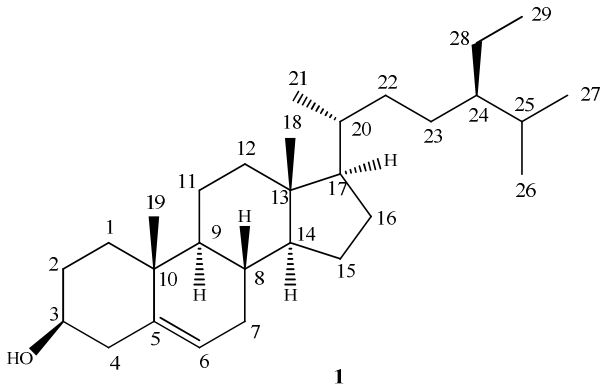
Stigmasterol (2): White needles. Molecular formula: $C_{29}H_{48}O$. Molecular weight: 412. Melting point: 164-166 °C. FT-IR ν_{max} cm^{-1} (KBr): 3432, 2938, 1639, 1459, 1381, 1053 cm^{-1}. EI-MS m/z: $[M]^+$ = 412. ^1H NMR ($CDCl_3$, 600 MHz) δ (ppm): 0.71 (3H, *s*), 0.80 (3H, *d*, *J* = 6.6), 0.82 (3H, *d*, *J* = 6), 0.85 (3H, *d*, *J* = 6.6), 0.96 (3H, *d*, *J* = 6), 1.05 (3H, *s*), 1.54 (1H, *m*), 1.98 (2H, m), 2.07 (1H, *m*), 2.29 (2H, *m*), 3.53 (1H, *m*), 5.03 (1H, *dd*, J_1 = 15.0, J_2 = 8.4 Hz), 5.18 (1H, *dd*, J_1 = 15.0, J_2 = 8.4 Hz), 5.35 (1H, *t*, *J*= 3.0 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 12.0, 12.2, 19.1, 19.4, 21.0, 21.1, 21.2, 24.4, 25.4, 28.9, 31.7, 31.9, 36.5, 37.3, 39.7, 40.5, 42.2, 42.3, 50.2, 51.2, 56.0, 56.9, 71.8, 121.7, 129.3, 138.3, 140.8.

RESULTS AND DISCUSSION

β-Sitosterol was obtained as white needles with melting point 143-145 °C [10]. A molecular ion peak at *m/z* 414 in its mass spectrum showed that the molecular formula for the compound is $C_{29}H_{50}O$. According to the IR spectrum, a broad-strong peak at 3430 cm^{-1} and a peak at 1646 cm^{-1} indicated the presence of a hydroxyl group and a carbon-carbon double bond respectively.

The ^1H-NMR spectrum showed the presence of six methyl signals at δ 0.69 (H-18), 0.83 (H-27), 0.85 (H-26), 0.87 (H-29), 0.93 (H-21), and 1.03 (H-19). A triplet peak at δ 5.36 (1H, *t*, *J* = 3.0 Hz) belonged to an olefinic hydrogen (H-6). A multiplet peak at δ 3.53 indicated the presence of a hydroxymethine group (H-3). According to the ^1H-^1H COSY spectrum, there was a correlation between peaks at δ of 5.36 (H-6) and 1.99 (H-7). The other correlation was observed between peaks at δ of 3.53 (H-3) and 2.28 (H-4).

The ^{13}C-NMR and DEPT 135 spectra of the compound gave a total of 29 peaks for all 29 different carbons. Two downfield signals observed at δ 140.8 and 121.7 belong to the endocyclic carbon-carbon double bond C-5 and C-6 respectively. A significant peak at δ 71.8 represents the C-3 that is bonded to the hydroxyl group. According to the DEPT spectra, the peaks at δ 140.8, 36.5, and 42.3 belong to the quaternary carbons of C-5, C-10, and C-13 respectively and the peaks at δ 42.3, 39.8, 37.3, 34.0, 31.9, 31.7, 29.1, 28.2, 26.1, 24.3, and 21.1 represented the methylene groups of C-4, C-12, C-1, C-22, C-7, C-2, C-28, C-16, C-23, C-15, and C-11 respectively. Based on spectroscopic data and comparison with the published data [11], the compound was characterized as β-sitosterol (**1**).



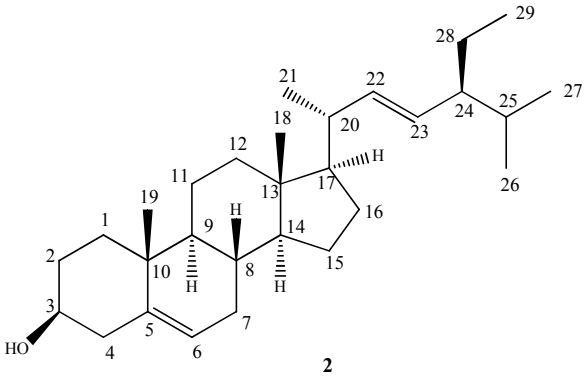
1

Yahya et al. at 2011 reported the isolation of β-sitosterol from rhizome of *Etlingera sphaerocephala* var. *grandiflora* [12]. The compound was also isolated from many species of Zingiberacea such as *Aframomum giganteum* [13], *Alpinia blepharocalyx* [14] *Alpinia galanga* [15], *Alpinia pinnanensis* [16], *Hedychium coronarium* [17], and *Kaempferia angustifolia* [18].

stigmasterol was obtained as white needles with melting point 164-166 °C [19]. EIMS spectrum gave a molecular ion peak at *m/z* 412 corresponding to a molecular of C29H48O. The IR spectrum of the compound showed a strong peak at 3432 cm-1 and a peak at 1639 cm-1 indicated a hydroxyl group and a carbon-carbon double bond.

The ^{1}H-NMR spectrum showed, two doublet of doublet peaks at δ 5.18 (1H, *dd*, J_1 = 15.0, J_2 = 8.4 Hz) 5.03 (1H, *dd*, J_1 = 15.0, J_2 = 8.4 Hz) were assigned to H-22 and H-23 respectively. Meanwhile two multiplet peaks at δ 5.35 (1H, *m*) and 3.53 (1H, *m*) were assigned to H-6 and H-3 respectively. The spectrum also showed the presence of six methyl signals at δ 0.71 (H-18), 1.05 (H-19), 0.96 (H-21) 0.80 (H-26), 0.85 (H-27), and 0.82 (H-29). The ^{1}H-^{1}H COSY spectrum of the compound illustrated some correlations between proton and proton such as 3.53 and 2.29 (H-3/H-4); 5.35 and 1.98 (H-6/H-7); 5.18 and 2.29 (H-22/H-20); 5.18 and 5.03 (H-22/H-23); and 5.03 and 1.54 (H-23/H-24).

The ^{13}C-NMR spectrum confirmed a total of 29 carbons. Four downfield signals at δ 140.8, 138.3, 129.3, and 121.7 were recorded for olefinic carbons which were assigned to C-5, C-22, C-23, and C-6 respectively. Besides, the peak at δ 71.8 showed the presence of a hydroxmethine carbon in the compound (C-3). According to the DEPT 135 spectrum, the peaks at 140.8, 42.2, and 36.5 for quaternary carbons of C-5, C-13, and C-10 were not recorded. Meanwhile the peaks at δ 42.2, 39.7, 37.3, 31.9, 31.7, 28.9, 25.4, 24.4, and 21.1 were assigned to methylene groups of C-4, C-12, C-1, C-7, C-2, C-16, C-28, C-15, and C-11. Based on the spectroscopic data and comparison with the published data [20], the compound was confirmed as stigmasterol (**2**).



2

The presence of stigmasterol in *Etlingera sphaerocephala* var. *grandiflora* has been reported by Yahya et al. [12]. The compound was also isolated from other Zingiberaceae genera such as *Alpinia pinnanensis* [16], *Alpinia gagnepainii* [21], *Hedychium coronarium* [17], and *Curcuma oligantha Trimen* [22].

β-Sitosterol and stigmasterol are the major phytosterols which has been found in a great variety of plants. Their structures are similar to that of cholesterol except for the presence of a side chain of ethyl group at carbon 24. According to the previous studies, the compounds have owned various useful biological activities for human health. β-Sitosterol is a suitable curative for hyper-cholesterolemia and inflammation, cancer treatment, and modulation of immunity. Moreover the compound has revealed antimutagenic effect, antigenotoxic capacity and lymphocyte potential [23]. The other study expressed anti-hyperglycemic effects of the compound. Plasma β-sitosterol concentration was able to reduce type 2 diabetes patient, and also effective in lowering the blood glucose level [24]. Stigmasterol is an anti-inflammatory, anti-osteoarthritis, and anti-catabolic potential agent [25]. Stigmasterol suppresses hepatic cholesterol synthesis more than β-sitosterol [26]. Anti-diabetic, anti-peroxidative, and anti-amnesic properties of stigmasterol were also reported [27,28].

CONCLUSION

In this study we reported the isolation of two phytosterols namely β-sitosterol and stigmasterol from hexane extracts of leaves and stems of *E.brevilabrum*. In our previous studies we measured and reported the antioxidant [7] and antimicrobial activities [8] of the extracts from different parts of *E.brevilabrum*. Since the biological activities of a plant depends on it's chemical composition, it can be concluded the biological activities of *E.brevilabrum* may be due to the presence of β-sitosterol, stigmasterol and also their synergistic effects with the other chemical constituents which exist in the plant.

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