INTRODUCTION

Cananga is a small genus, belonging to the family Annonaceae and consists of only two species viz. Cananga odorata and Cananga latifolia that are distributed throughout the tropical and subtropical regions of the world\textsuperscript{1,2}. Cananga odorata (Lam) Hook.F. & Thomson (Annonaceae) is an evergreen tree that can grow up to 100 feet. It is widespread in the lowland forests in Malaysia and has been grown as a garden tree particularly for its attractive fragrant flowers. In Malaysia, C. odorata is locally known as kenanga, chenanga and ylang-ylang. Traditionally, the plant parts have been used for the treatment of malaria, asthma and scabies\textsuperscript{1,3}. Previous phytochemical studies have been carried out on the leaves and seeds of C. odorata, resulting to the isolation of (\textpm)-usinamine, \textpm)-nornuciferine, (\textpm)-axinagoreine, (\textpm)-cychinamine, \textpm)-trans-cinnamic acid, \textpm)-isophonodin, \textpm)-acetylnornuciferine, (\textpm)-ushinsunine, (\textpm)-nornuciferine, (\textpm)-cleistopholine, liriodenine, (\textpm)-anonaine, (\textpm)-nornuciferine, (\textpm)-consolideine and increasing percentages of ethyl acetate and methanol. Every 25 ml eluents were collected and these were combined according to their TLC pattern to afford six major combined fractions. Further isolation was performed on fraction 1 by column chromatography, eluted with solvent mixtures of hexane and ethyl acetate to yield \textpm)-sitosterol (1, 10.0 mg) and stigmasterol (2, 10.0 mg). Fraction 3 was further separated by column chromatography and eluted with mixtures of dichloromethane and chloroform with gradual increase in ethyl acetate and methanol to give 30 fractions of 25 ml each. Then, fractions 10-13 were combined and further separated with column chromatography using n-hexane-\textpm)-EtOAc (7:3), yielding liriodenine (3, 7.8 mg). Fraction 4 from the flash chromatography was rechromatographed on a column chromatography (sephadex LH20 using CHCl\textsubscript{3}-MeOH 9:1) to afford lyciscamine (4, 2.8 mg) The spectral data of 1-4 were in agreement with those reported in the literature.

\textbf{\textpm)-Sitosterol (1)}

The compound was obtained as colorless needle-shaped crystals with R\textsubscript{s} value of 0.75 (30\% ethyl acetate:n-hexane). IR (KBr) \textnu\textsubscript{max} cm\textsuperscript{-1}: 3433, 2960, 2866, 1464, 1381, 1053; EIMS m/z (relative intensity \%): 412 ([M]+, 6), 396 (11), 329 (15), 303 (6), 273 (11), 255 (21), 171 (22), 159 (37), 145 (46), 143 (39), 105 (65), 91 (100), 79 (62); \textnu\textsuperscript{1}H-NMR and \textnu\textsuperscript{13}C-NMR spectra were recorded using a Varian SCDNR VXR 500, operating at 500 and 125 MHz, respectively. The NMR spectra were obtained in CDCl\textsubscript{3} with chemical shifts expressed in \textdelta and coupling constant (J) in Hz with tetramethylsiline (TMS) as an internal standard.

\textbf{Stigmasterol (2)}

The compound was obtained as colorless needle-shaped crystals with R\textsubscript{s} value of 0.75 (30\% ethyl acetate:n-hexane). IR (KBr) \textnu\textsubscript{max} cm\textsuperscript{-1}: 3433, 2960, 2866, 1464, 1381, 1053; EIMS m/z (relative intensity \%): 412 ([M]+, 6), 396 (11), 329 (15), 303 (6), 273 (11), 255 (21), 171 (22), 159 (37), 145 (46), 143 (39), 105 (65), 91 (100), 79 (62); \textnu\textsuperscript{1}H-NMR (500 MHz, CDCl\textsubscript{3}) \textdelta: 0.69 (3H, s, H-6); 1.18 (1H, br s, H-4); 1.48 (1H, br s, H-5).

ABSTRACT

A phytochemical study of the leaves of Cananga odorata (Lam) Hook.F. & Thomson (Annonaceae) yielded four compounds, comprising of two sterols, \textpm)-sitosterol (1) and stigmasterol (2), and two oxoaporphine alkaloids: liriodenine (3) and lycisamine (4). The compounds were isolated using various chromatographic methods and structural elucidation was accomplished by spectroscopic techniques (i.e. NMR, MS, UV and IR) and comparison with literature values. The structure elucidation of the alkaloids using high field NMR (500 MHz) is reported for the first time.

Keywords: Cananga odorata, Annonaceae, Leaves, Steroids, Alkaloids.
The compound was obtained as yellow needle-shaped crystals with Rf value of 0.50 (50% ethyl acetate:chloroform). IR (KBr) v max cm⁻¹: 1737. UV max nm (log ε), MeOH: 260, 280, 338 nm. EIMS 70 eV, m/z (rel. int.): 291 ([M⁺]+, 100), 266 (62), 248 (96), 235 (22), 177 (30), 149 (32). 1H-NMR (CDCl₃, 500 MHz): 9.19 (1H, s, H-9), 7.24 (1H, s, H-3), 4.10 (3H, s) and 4.02 (3H, s) ppm (1H, J = 5.6, 0.7 Hz, 11), 8.61 (1H, J = 7.5 Hz, H-3), 7.60 (1H, J = 8.4 Hz, 1-H, H-10), 7.60 (1H, J = 8.4 Hz, 1-H, H-10), 7.72 (1H, J = 14 Hz, H-9), 4.61 (1H, d, J = 8 Hz, H-11) ppm. A typical AB quartet of the two doublets at 8.92 and 7.84 ppm (1H, J = 8.0 Hz) were assigned to H-8, H-10 and H-11 protons, respectively. A typical AB quartet of the two doublets at 7.76 ppm (1H, J = 8.0 Hz) showed the presence of H-10 and H-11 protons, respectively. In the aromatic region, the three doublets at 7.84 ppm (1H, J = 7.5 Hz) and 8.67 (1H, J = 5.6 Hz) ppm were assigned to H-8, H-9 and H-11 protons, respectively, while the triplet at 7.75 ppm (1H, J = 7.5 Hz, H-9) was due to H-9. The 13C-NMR spectrum of (3) indicated the presence of one methylene, seven methines and eight quartenary carbons. These spectroscopic properties were similar to those of the known compound liriodenine.

In the 1H-NMR spectrum of (3), the two singlets at 7.20 and 6.38 were assigned to H-3 and the methylene protons of a methyleneoxy group (O-CH₂-O), respectively, and a doublet at δ 8.90 ppm (J=5.5 Hz) showed the presence of H-5. In the aromatic region, the three doublets at δ 8.60 (J=8.0 Hz), 7.76 (J=7.5 Hz) and 8.67 (J=5.6 Hz) ppm were assigned to H-8, H-10 and H-11 protons, respectively, while the triplet at δ 7.75 ppm (1H, J=7.5 Hz, H-9) was due to H-9. The 13C-NMR spectrum of (3) indicated the presence of one methylene, seven methines and eight quartenary carbons. These spectroscopic properties were similar to those of the known compound liriodenine.

The 1H spectrum of (4) revealed a total of seven aromatic protons and two aromatic methylene groups at δ 6.10 (3H, s) and 4.02 (3H, s) ppm. A typical AB quartet of the two doublets at δ 0.92 and 7.84 ppm that had a coupling constant J=5.6 Hz were assigned to H-5 and H-4 of o xoaporphine skeleton. In the HMBC spectrum also showed appropriate three-bond correlation between H-4 (δ 7.84) and the carbon at 106.6 (C-3) and 122.3 (C-11c) ppm, while the H-5 (δ 8.92) showed a three-bond correlation to the carbon at 135.5 (C-3a) and 145.4 (C-6a) ppm. Another four signals of aromatic proton centered at δ 9.19 (dd, J=5.6, 0.7 Hz, H-11), 8.61 (dd, J=5.6, 0.7 Hz, H-8), 7.78 (dd, J=5.5, 0.7 Hz, H-9) ppm.

**RESULTS AND DISCUSSION**

The crude ethyl extract from the leaves of *Cananga odorata* was fractionated using a column chromatography on silica gel to afford several fractions. Purifications were carried out on these fractions by repeated column chromatography, yielding four pure compounds. The compounds were identified by spectroscopic methods, i.e. NMR, MS, UV and IR, and compared with literature data.

β-sitosterol (1) and stigmasterol (2) are two steroidal compounds reported to occur in many plants in the world. Liriodenine (3) is ubiquitous in most Annonaceous plants especially in *Cananga* species. The other compound has been identified as lysicamine (4). The alkaloids belong to the oxoaporphine skeleton whose structures are known but their high field NMR (500 MHz) spectral data are reported here for the first time.

Compounds (3) and (4) exhibited intense yellow fluorescent coloration in solution, indicating the characteristic of an oxoaporphine chromophore. Compound (3) gave UV spectrum that displayed the typical maxima at 260, 280, 338 nm, while compound (4) absorbed at 238 and 268 nm, suggesting the presence of highly conjugated aromatic system. The IR spectrum of compounds (3) and (4) showed the presence of carbonyl groups based on the strong broad band absorption at 1737 and 1658 cm⁻¹, respectively.

The EIMS of compounds (3) and (4) showed molecular ions at m/z 275 and m/z 291, corresponding to the formulae C₁₈H₁₃NO₃ and C₁₈H₁₃NO₅, respectively. Based on the 1H and 13C-NMR spectra, compounds (3) and (4) were very similar to each other except for the presence of a methyleneoxy group in compound (3) and two methoxy moieties in compound (4).

In the 1H-NMR spectrum of (3), the two singlets at δ 7.20 and 6.38 were assigned to H-3 and the methylene protons of a methyleneoxy group (O-CH₂-O), respectively, and a doublet at δ 8.90 ppm (J=5.5 Hz) showed the presence of H-5. In the aromatic region, the three doublets at δ 8.60 (J=8.0 Hz), 7.76 (J=7.5 Hz) and 8.67 (J=5.6 Hz) ppm were assigned to H-8, H-10 and H-11 protons, respectively, while the triplet at δ 7.75 ppm (1H, J=7.5 Hz, H-9) was due to H-9. The 13C-NMR spectrum of (3) indicated the presence of one methylene, seven methines and eight quartenary carbons. These spectroscopic properties were similar to those of the known compound liriodenine.

The 1H spectrum of (4) revealed a total of seven aromatic protons and two aromatic methylene groups at δ 6.10 (3H, s) and 4.02 (3H, s) ppm. A typical AB quartet of the two doublets at δ 0.92 and 7.84 ppm that had a coupling constant J=5.6 Hz were assigned to H-5 and H-4 of oxoaporphine skeleton. In the HMBC spectrum also showed appropriate three-bond correlation between H-4 (δ 7.84) and the carbon at 106.6 (C-3) and 122.3 (C-11c) ppm, while the H-5 (δ 8.92) showed a three-bond correlation to the carbon at 135.5 (C-3a) and 145.4 (C-6a) ppm. Another four signals of aromatic proton centered at δ 9.19 (dd, J=5.6, 0.7 Hz, H-11), 8.61 (dd, J=5.6, 0.7 Hz, H-8), 7.78 (dd, J=5.5, 0.7 Hz, H-9) ppm.

![Fig. 1: Structures of isolated compounds](image-url)
(ddd, J=8.4, 8.4, 1.4 Hz, H-10) and 7.60 (ddd, J=8.4, 8.4, 1.4 Hz, H-9) ppm were assigned as an ABMX system characteristic of 1,2-disubstituted benzene nucleus of ring D. The remaining signals of (4) are also indicated for one aromatic proton, resonating as a singlet at δ 7.24 ppm (H-3) and two sets of three proton singlets (3H) for an aromatic methoxyl at δ 4.10 and 4.02 ppm. The presence of this singlet aromatic proton (δ 7.24) showed J correlate with an oxquartenary carbon at δ 152.8 ppm (C-2) and J correlate with two quartenary carbon at δ 122.3 (C-11c) and δ 157.5 (C-1) ppm, which then defined the position of two methoxyl groups at C-2 and C-1, respectively. Meanwhile, a connection between C-6a (δ 145.4 ppm) and C-11b (δ 120.0 ppm) was consistent with deshielding effect of the carbonyl group at C-7 (δ 182.9 ppm) on the doublet proton at δ 8.93 ppm (H-5) and one of methoxyl proton at δ 4.10 ppm (1-OCH₃). Therefore, compound (4) is an oxoaporphine-type of alkaloid with two methoxyl groups which are located in the A ring at C-1 and C-2, respectively. Considering all these spectral data the structure lysicamine (4) was assigned to this compound.

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REFERENCES