Sains Malaysiana 44(8)(2015): 1125–1128

The Chemical Constituents of *Ellipeia cuneifolia* and Their Antibacterial Activity

(Komposisi Kimia *Ellipeia cuneifolia* dan Aktiviti Antibakterianya)

HASLIZA YUSOF, LAILY B. DIN, WAN A. YAACOB*, NAZLINA IBRAHIM, BOHARI M. YAMIN & A. LATIFF

ABSTRACT

Chromatographic purification of chloroform extract of the twigs of Ellipeia cuneifolia *has led to the discovery of three compounds comprising of 2′,4′-dihydroxy-4,6′-dimethoxychalcone; tepanone; and O-methylmoschatoline. Structures of the compounds were established by interpreting their spectral data and by comparing them with those of the literature. Two of them showed antibacterial activities.*

Keywords: *Annonaceae; antibacterial activity; chalcone;* Ellipeia cuneifolia*; oxoaporphine; retrochalcone*; *twig*

ABSTRAK

Penulenan kromatografi terhadap ekstrak kloroform ranting Ellipeia cuneifolia *membawa kepada penemuan 3 sebatian iaitu 2′,4′-dihidroksi-4,6′-dimetoksikalkon; tepanon; dan O-metilmoskatolina. Struktur sebatian ini telah ditentukan dengan mentafsirkan data spektrum dan membandingkannya dengan data spektrum daripada penerbitan. Dua daripadanya menunjukkan aktiviti antibakteria.*

Kata kunci: *Aktiviti antibakteria; Annonaceae;* Ellipeia cuneifolia*; kalkon; oksoaporfina*; *ranting; retrokalkon*

INTRODUCTION

Ellipeia Hook & Thom. of the family Annonaceae is a very small genus of climbers with about merely five species and known to be distributed in Peninsular Malaysia, Borneo and Sumatra (Kessler 1993). In Peninsular Malaysia only one species of *E. cuneifolia* occurs, mostly in the north-eastern coast of peninsula (Ridley 1922; Sinclair 1955). Locally the plant is known as *tepan* and according to local herbalists, a decoction of the roots has been used for post-parturition. Previously, Colegate et al. (1992) reported the isolation of a new retrochalcone, tepanone. A reinvestigation of the plant collected from the same locality showed two chalcones and one alkaloid, besides tepanone ((2*E*)-1-phenyl-3-(2′-hydroxy-3′,4′,6′-trimethoxyphenyl) prop-2-enone). In this study, we report the results of a phytochemical investigation of the twigs. Compounds 1 showed inhibitory against *Bacillus subtilis*, *Enterobacter aerogenes* and *Escherichia coli*, whereas compound 3 exhibited very strong activities against *B. subtilis* and *Staphylococcus aureus*.

MATERIALS AND METHODS

GENERAL

The ^{1}H and ^{13}C NMR spectra were recorded with Avance III 600 MHz Bruker in $CDCl_3$ and acetone-d_6 with the chemical shifts, δ, in ppm and the values of coupling constants, *J* in Hz. LC-MSToF spectra were taken on the Dionex/Bruker Micro ToFQ, FTIR spectra were recorded on the Perkin Elmer Spectrum 400 FT-IR/FT-NIR and UV spectrophotometer Shimadzu UV-160 (200-400 nm).

PLANT MATERIAL

Twigs of *Ellipeia cuneifolia* were collected in October 2011 from the coastal area of Kota Bharu, Kelantan. A voucher specimen (ALM 3142) was deposited at the herbarium of Universiti Kebangsaan Malaysia, Bangi.

EXTRACTION AND ISOLATION

Dried ground twigs of *Ellipeia cuneifolia* (0.95 kg) were steeped three times in chloroform at room temperature over three days each to give 7.86 g (0.83%) of a dark-green extract after solvent removal by a rotary evaporator. The $CHCl_3$ extract was subjected to vacuum liquid chromatography (VLC) by using silica 7747 (Merck) eluted with increasing polarity of *n*-hexane and ethyl acetate. The eluates were combined based on their silica gel thin layer chromatography (TLC) (Merck 5554) profile to yield seven fractions (A-G). Fraction D (0.48 g) was purified by column chromatography (CC), preparative TLC and Sephadex LH-20 to give compounds 1 (3.4 mg). Fractions E (0.85 g) and G (0.56 g) were separately purified by CC to give compounds 2 (3.2 mg) and 3 (5.8 mg), respectively.

2′,4′-Dihydroxy-4,6′-dimethoxychalcone 1 (3.4 mg): white needles; R_f = 0.78 (4:6, *n*-hexane-ethyl acetate); ESI-MS (m/z): 323.0891 $[M+Na]^+$, $C_{17}H_{16}O_5$; UV (MeOH) λ_{max}: 226, 292, 357 nm; FTIR (ATR) cm^{-1}: 3241, 2940, 1623, 1609, 1511, 1196, 1109, 789; ^{1}H NMR (acetone-d_6, 600 MHz) δ_H:

7.90 (1H, *d*, *J* = 15.3 Hz, H-α), 7.74 (1H, *d*, *J* = 15.3 Hz, H-β), 7.68 (2H, *d*, *J* = 8.7 Hz, H-2, 6), 7.00 (2H, *d*, *J* = 8.7 Hz, H-3, 5), 6.06 (1H, *d*, *J* = 2.1 Hz, H-5′), 5.98 (1H, *d*, *J* = 2.1 Hz, H-3′), 3.96 (3H, *s*, 6′-OCH_3), 3.86 (3H, *s*, 4-OCH_3); ^{13}C-APT NMR (acetone-d_6, 150 MHz) δ_C : 192.2 (C-9), 168.1 (C-2′), 165.1 (C-4′), 163.4 (C-6′), 161.7 (C-4), 142.1 (C-β), 130.2 (C-2, 6), 128.1 (C-1), 125.0 (C-α), 114.4 (C-3, 5), 105.4 (C-1′), 96.1 (C-3′), 91.3 (C-5′), 55.5 (6′-OCH_3), 54.9 (4-OCH_3).

Tepanone 2 (3.2 mg): yellow needles; R_f = 0.69 (4:6, *n*-hexane-ethyl acetate); ESI-MS (m/z): 337.1005, $C_{18}H_{18}O_5$; UV (MeOH) λ_{max} : 224, 257, 364 nm; FTIR (ATR) cm^{-1}: 3306, 2847-2998, 1639, 1551, 1466, 1337, 1110, 1203, 847, 793; ^{1}H NMR ($CDCl_3$, 600 MHz) δ_H : 8.23 (1H, *d*, *J* = 16.2 Hz, H-β), 8.04 (2H, *d*, *J* = 7.2 Hz, H-2′, 6′), 8.00 (1H, *d*, *J* = 16.2 Hz, H-α), 7.55 (1H, *t*, *J* = 7.2 Hz, H-4′), 7.48 (2H, *t*, *J* = 7.5 Hz, H-3′, 5′), 6.09 (1H, *s*, H-5), 6.61 (1H, *s*, 2-OH), 3.94 (3H, *s*, 4/6-OCH_3), 3.90 (3H, *s*, 4/6-OCH_3), 3.88 (3H, *s*, 3-OCH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ_C : 191.9 (C-9), 156.9 (C-6), 154.0 (C-4), 150.6 (C-2), 139.1 (C-1′), 135.8 (C-β), 132.2 (C-4′), 129.7 (C-3), 128.5 (C-2′, 6′), 128.4 (C-3′, 5′), 122.5 (C-α), 105.2 (C-1), 88.2 (C-5), 61.3 (3-OCH_3), 56.0 (4/6-OCH_3), 55.9 (4/6-OCH_3).

o-Methylmoschatoline 3 (5.8 mg): orange needles. R_f = 0.39 (4:6, *n*-hexane-ethyl acetate). ESI-MS (m/z): 322.1098 $[M+H]^+$, $C_{19}H_{15}O_4N$. FTIR (ATR) cm^{-1}: 3127, 3069, 2948, 2858, 1657, 1578, 1465, 1384, 1202, 1089, 935, 755. ^{1}H NMR ($CDCl_3$, 600 MHz) δ_H : 9.11 (1H, *d*, *J* = 8.4 Hz, H-11), 8.97 (1H, *d*, *J* = 5.4 Hz, H-5), 8.58 (1H, *dd*, *J* = 1.2, 7.8 Hz, H-8), 8.22 (1H, *d*, *J* = 5.4 Hz, H-4), 7.75 (1H, *dt*, *J* = 1.2, 8.4 Hz , H-10), 7.54 (1H, *t*, *J* = 7.8 Hz , H-9), 4.20 (3H, *s*, 3-OCH_3), 4.11 (3H, *s*, 2-OCH_3), 4.09 (3H, *s*, 1-OCH_3). ^{13}C NMR ($CDCl_3$, 150 MHz) δ_C : 182.7 (C-7), 156.5 (C-1), 148.5 (C-3), 147.4 (C-2), 145.5 (C-6a), 144.6 (C-5), 134.6 (C-11a), 134.4 (C-10), 131.4 (C-7a), 131.1 (C-11c), 129.0 (C-8), 128.2 (C-9), 127.7 (C-11), 122.8 (C-3a), 119.2 (C-4), 115.7 (C-11b), 61.8 (3-OCH_3), 61.5 (2-OCH_3), 61.0 (1-OCH_3).

BIOASSAY

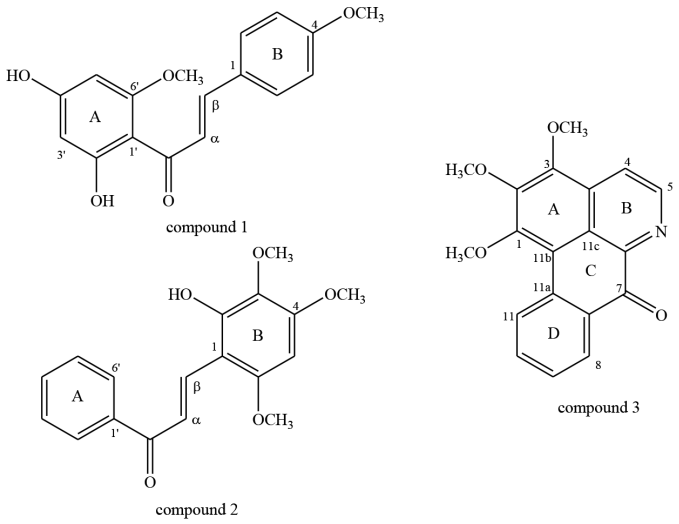
The crude extract and pure compounds were assayed for antibacterial activity against the bacteria *Bacillus subtilis* (ATCC 11774), *Staphylococcus aureus* (ATCC 11632), *Enterobacter aerogenes* (ATCC 13048) and *Escherichia coli* (ATCC 10536). The crude extract was dissolved at a concentration of 2 mg/mL and pure compounds were prepared at 1 mg/mL in DMSO. The amounts of crude extract and pure compounds on the discs were 20 and 10 µg, respectively, whereas the standard Chaloramphenicol was 30 µg.

RESULTS AND DISCUSSION

Compound 1 (3.4 mg) was isolated as white needles. The ESI-MS gave molecular ion at m/z 323.0891 $[M+Na]^+$, which corresponded to the molecular formula $C_{17}H_{16}O_5$. The UV spectrum in methanol showed absorption at 226, 292 and 357 nm. The prominent and broad absorption at 3241 cm^{-1} in the FTIR spectrum indicated the presence of hydroxyl (OH) group together with prominent band at 1623 cm^{-1} for the existence of carbonyl (C=O) group. The ^{1}H NMR spectrum showed two two-proton doublets at δ_H 6.83 (H-3, H-5) and 7.18 (H-2, H-6) corresponding to four aromatic protons of ring B. Two doublet signals at δ_H 5.95 and 6.02 were assigned to H-3′ and H-5′ of ring A. Two distinct methoxy signals at δ_H 3.75 and 3.88, which were positioned at C-4 (ring B) and C-6′ (ring A). A very downfield, signal at δ_H 13.91 was attributed to 2′-OH and another one singlet signal at δ_H 9.55 was assigned to 4′-OH. Two one-proton doublet signals at δ_H 7.74 and 7.90 (*J* = 15.3 Hz) were attributed to *trans* H-β and H-α, respectively. Through comparison of the observed data with the literature, compound 1 is identified as 2′,4′-dihydroxy-4,6′-dimethoxychalcone [10, 12]. This compound was also isolated from other genus of Annonaceae family i.e. *Goniothalamus gardneri* (Seidel et al. 2000).

Compound 2 (3.2 mg) appeared as yellow needles. The ESI-MS gave a molecular ion of 337.1005 $[M+Na]^+$ consistent with molecular formula of $C_{18}H_{18}O_5$. Additionally, the FTIR spectrum showed the presence of OH at 3306 cm^{-1} and C=O at 1639 cm^{-1}. The ^{1}H NMR spectrum showed distinct methoxy signals at δ_H 3.88 (C-3), 3.90 (C-4/C-6) and 3.94 (C-4/C-6) of ring B. Furthermore, two one-proton singlet signals at δ_H 6.09 and 6.61 were attributed to H-5 and 2-OH of ring B. The five aromatic signals at δ_H 7.48 (2H, *t*, *J* = 7.3 Hz, H-3′, H-5′), 7.55 (1H, *t*, *J* = 7.3 Hz, H-4′) and 8.04 (2H, *d*, *J* = 7.3 Hz, H-2′, H-6′) were corresponding to the protons of ring A. The two one-proton doublet signals (*J* = 16.2 Hz) at δ_H 8.00 and 8.23 were assigned to *trans* H-α and H-β, respectively. Through comparison of the observed data with the literature, compound 2 is identified as tepanone (Colegate et al. 1992).

Compound 3 was isolated as orange needles. Its ESI-MS gave a molecular ion of m/z 322.1098 $[M+H]^+$, which corresponds to the molecular formula $C_{19}H_{15}O_4N$. The FTIR spectrum showed the presence of lactam carbonyl group at 1657 cm^{-1}. The ^{1}H NMR spectrum confirmed the presence of three methoxy groups at δ_H 4.09, 4.11 and 4.20. The aromatic protons of ring B showed two one-proton doublets signals at δ_H 8.22 and 8.79 (*J* = 5.4 Hz) due to H-4 and H-5. The four aromatic protons at δ_H 7.54 (*t*, *J* = 7.7 Hz, H-9), 7.75 (*dt*, *J* = 1.2, 7.7 Hz, H-10), 8.58 (*dd*, *J* = 1.2, 7.7 Hz , H-8) and 9.11 (*d*, *J* = 8.7 Hz, H-11), which were ascribed to the protons of the unsubstituted D ring of the aporphine nucleus [14]. The signal of H-11 was more downfield due to the presence of the three methoxy groups at ring A, which deshielded the proton. From the X-ray crystallography data, compound 3 was comfirmed as *o*-methylmoschatoline. Figure 1 shows the structure of compound 3 by X-ray crystallography. This compound was isolated from genus *Xylopia* (Annonaceae) i.e. *X. championii* (Wijeratne et al. 1996) and *X. ferruginea* (Zawawi et al. 2012).



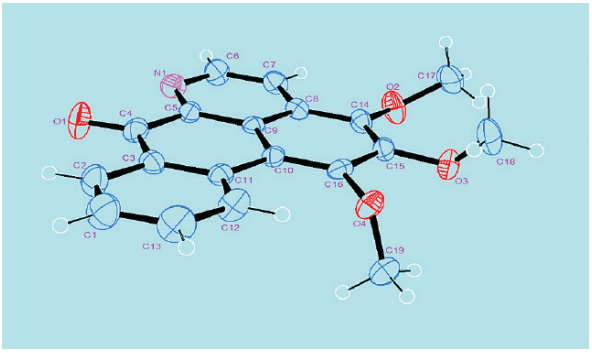


FIGURE 1. *o*-Methylmoschatoline structure by X-ray crystallography

TABLE 1. Antibacterial activity of the crude extract and compounds 1-3

Sample	Inhibitory zone (mm)			
	B. subtilis	*S. aureus*	*E. aerogenes*	*E. coli*
Crude extract	-	-	-	-
1	11.5±0.0	-	12.0±0.0	6.0±0.7
2	-	-	-	-
3	35±0.0	26.5±0.0	-	-
Chloramphenicol	23±0.0	20.5±0.2	26±0.7	26±0.3

The extract and compounds isolated from *E. cuneifolia* were evaluated for antibacterial activity. The crude extract did not have antibacterial activity. Compounds 1 showed inhibitory against *Bacillus subtilis*, *Enterobacter aerogenes* and *Escherichia coli*, whereas compound 3 exhibited very strong activities against *B. subtilis* and *Staphylococcus aureus*. The inhibitory values are shown in Table 1.

CONCLUSION

Chromatographic separation of chloroform extract of the twigs of *Ellipeia cuneifolia* yielded three compounds as 2′,4′-dihydroxy-4,6′-dimethoxychalcone, tepanone and *O*-methylmoschatoline. In addition, tepanone could be considered as a chemical marker for this species.

ACKNOWLEDGEMENTS

We would like to thank the Ministry of Education, Malaysia for the financial support under UKM-DLP-2012-033 and UKM-DLP-2013-018 grants and postgraduates scholarship under MyBrain15. Special thanks to School of Chemical Sciences and Food Technology, Faculty of Science and Technology, UKM and Centre for Research and Innovation Management (CRIM) UKM for the technical support on NMR, LC-MSToF, UV and FTIR instrumentation.

REFERENCES

Colegate, S.M., Din, L.B., Ghisalberti, E.L. & Latiff, A. 1992. Tepanone, a retrochalcone from *Ellipeia cuneifolia*. *Phytochemistry* 31: 2123-2126.

Kessler, P.J.A. 1993. Annonaceae. In *The Families and Genera of Vascular Plants*. *Volume II*. *Flowering Plants*. *Dicotyledons: MagnoliidHamamelid and Caryophyllid Families,* edited by Kubitzki, K., Rohwer, J.G. & Bittrich, V. Berlin: Springer-Verlag. pp. 93-129.

Ridley, H.N. 1922. *The flora of the Malay Peninsula*. Vol II. Reprinted 1967. London: L. Reeve & Co.

Seidel, V., Bailleul, F. & Waterman, P.G. 2000. (Rel)-1β,2α-di-(2,4-dihydroxy-6-methoxybenzoyl)-3β,4α-di-(4-methoxyphenyl)-cyclobutane and other flavonoids from the aerial parts of *Goniothalamus gardneri* and *Goniothalamus thwaitesii*. *Phytochemistry* 55: 439-446.

Sinclair, J. 1955. A revision of the Malayan Annonaceae. *Gardens' Bulletin Singapore* 14(2): 149-516.

Wijeratne, E.M.K., Hatanaka, Y., Kikuchi, T., Tezuka, Y. & Gunatilaka, A.A.L. 1996. A dioxoapophine and other alkaloids of two Annonaceous plants of Sri Lanka. *Phytochemistry* 42: 1703-1706.

Zawawi, N.K.N.A., Ahmat, N., Ahmad, R., Jaafar, F.M. & Ghani, N.A. 2012. Oxoaporphine alkaloids and flavonols from *Xylopia ferruginea* (Annonaceae). *Biochemical Systematics and Ecology* 43: 7-9.

Hasliza Yusof, Laily B. Din, Wan A. Yaacob* & Bohari M. Yamin
School of Chemical Sciences and Food Technology
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
43600 Bangi, Selangor D.E.
Malaysia

Nazlina Ibrahim
School of Biosciences and Biotechnology
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
43600 Bangi, Selangor D.E.
Malaysia

A. Latiff
School of Environmental and Natural Resource Sciences
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
43600 Bangi, Selangor D.E.
Malaysia

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

Received: 6 October 2014
Accepted: 9 April 2015