The Malaysian Journal of ANALYTICAL SCIENCES ISSN 1394-2506

PHENOLIC COMPOUNDS FROM THE FRUITS OF *ORANIA SYLVICOLA*

(Sebatian Fenol daripada Buah *Orania sylvicola*)

Solihah Sukari*, Ikram M. Said

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

**Corresponding author: solihah_sukari@yahoo.com*

Abstract

Fruits of *Orania sylvicola* (Arecaceae) were extracted with methanol and partitioned with chloroform and n-butanol. Investigation of the chloroform and n-butanol partitions by repetitive chromatographic method resulted in the isolation of two known compounds, namely, 5-hydroxy-7,4'-dimethoxyflavone (1) and 4-hydroxybenzoic acid (2). Compound 2 is the major phenolic compound and has been used as a chemotaxonomic marker for the Arecaceae family. Their structures were elucidated with UV-VIS, IR, NMR (^{1}H, ^{13}C, HSQC, HMBC, COSY and NOESY) and MS.

Keywords: arecaceae, *orania sylvicola*, 4-hydroxybenzoic acid, 5-hydroxy-7,4'-dimethoxyflavone

Abstrak

Buah *Orania sylvicola* (Arecaceae) diekstrak menggunakan metanol dan dipartisikan kepada bahagian kloroform dan n-butanol. Kajian ke atas partisi kloroform dan n-butanol dengan kaedah kromatografi yang berulangan telah menemui dua sebatian yang diketahui, iaitu 5-hidroksi-7,4'-dimetoksiflavon (1) dan asid 4-hidroksibenzoik (2). Sebatian 2 ialah sebatian fenol yang utama dan bertindak sebagai penanda kemotaksonomi bagi famili Arecaceae. Struktur sebatian ini dikenal pasti melalui UV-VIS, IM, RMN (^{1}H, ^{13}C, HSQC, HMBC, COSY and NOESY) dan JM.

Kata kunci: arecaceae, o*rania sylvicola*, asid 4-hidroksibenzoik, 5-hidroksi-7,4'-dimetoksiflavon

Introduction

Orania, genus from family Arecaceae (palm tree) have been identified previously as consisting of 28 species [1]. Most of the species in genus *Orania* show a robust canopy while a few exist as a small understory palm, distributed from Malaysia to the Philippines, New Guinea, Australia, Indonesia and West Irian, but mostly it is found in New Guinea [2]. *Orania* species can be found at lowland coastal swampy heath forest about 10 m above sea level and highland tropical humid rainforest about 1220 m above sea level [1]. There is little economic use, in some species, the fruits and buds are said to be poisonous [3 - 5]. *Orania sylvicola* species or in ancient times known as *O. macrocladus*, locally called 'Ibul' is one of the poisonous fruit [6]. *O. sylvicola* is found in Malaysia and Indonesia notably in Ulu Kesial (Kelantan), Melaka, Pahang, Pulau Pangkor, Cameron Highland, Perlis, Fraser Hill and Jawa [5 - 6]. It is a solitary large palm about 60 feet tall and 18 inch diameter [5]. Fruits are globose with 4.5 - 5 cm diameter, dull green when young and yellowish green when mature [1]. There has been no work done on the chemical constituents for any species of *Orania*. In this paper, we investigate the chemical substituents of *O. sylvicola* fruits where two phenolic compounds were isolated and their structures elucidated with various spectroscopic techniques.

Materials and Methods

General Experimental Procedure

All solvents used for chromatography were of analytical reagent grade. Silica gel 60 for CC and radial chromatography were purchased from Merck (Darmstadt, Germany). ^{1}H, ^{13}C, HSQC, HMBC, COSY and NOESY NMR spectra were taken on a Bruker/ Avance 111 600 MHz Cryoprobe spectrometer in $CDCl_3$ and MeOD with TMS as internal standard. UV-VIS spectra were recorded on a Shimadzu UV-VIS Spectrophotometer- 2450. IR

spectral data were measured on a Perkin Elmer Fourier Transform Infrared 400 Attenuated Total Reflectance (ATR) and sampel used are in solid state. Mass spectra were obtained from direct injection ESI-MS Bruker MicroTOF Q with flow rate 180 µL/hour using syringe pump.

Plant material

A fruit of O. sylvicola were collected from Perlis, Malaysia and was identified by Herbarium of Taman Botani, Putrajaya (voucher specimen, HTBP 2577). Mature fruits were de-shelled and dried under room temperature. It was then ground to fine meal using a grinder.

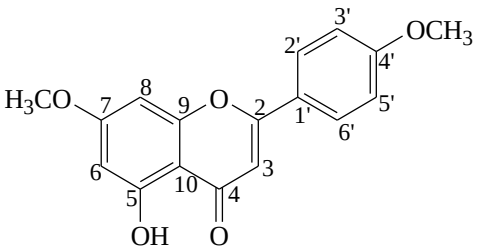
Extraction and Isolation

The fine meal (2 kg) was extracted by cold extraction method with n-hexane (3 L) followed by methanol (3 L), successively. After two days extraction, the mixture was filtered to separate the soluble and residue. The extraction was repeated three times. MeOH extracts were combined and evaporated to dryness by rotary evaporator. The dried MeOH extract (44 g) was dissolved in 80% MeOH and 20 % H_2O, and partitioned between $CHCl_3$ and n-BuOH by liquid-liquid extraction yielding chloroform fraction (1.18 g), n-butanol fraction (8.90 g) and the methanol aqueous fraction (27.85 g).

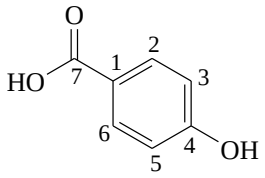
Separations on the chemical components of the chloroform fraction start with injection on column chromatography. The eluent consisting a mixture of diethyl eter: chloroform: methanol (6: 3.5: 0.5 v/v) at normal phase silica gel. By observation with thin layer chromatography (TLC), seven combination fractions were pooled. The third fraction was applied to a silica gel column chromatography eluted with hexane: chloroform: methanol (2.5: 7: 0.5, v/v), yielding compound 1 (0.5 mg), which was purified with repeated radial chromatography using 100% chloroform. For the n-butanol fraction, the crude extract was injected on silica gel column chromatography and eluted with hexane: chloroform: methanol (3.5: 6: 0.5, v/v) with increasing polarity, yielded six fractions. Purification of fraction two by repeated radial chromatography afforded compound 2 (7.9 mg).

Results and Discussion

Two known phenolic compounds were isolated where compound 1 was from chloroform fraction while compound 2 was from n-butanol fraction.



5-hydroxy-7,4'-dimethoxyflavone (**1**)



4-hydroxybenzoic acid (**2**)

Fig. 1. Structures of compound **1** & **2**

Compound **1**, a white amorphous solid having molecular formula $C_{17}H_{14}O_5$, deduced from positive ESIMS at m/z 321.0518 $[M+Na]^+$ and m/z 299.0713 $[M+H]^+$. The skeleton of 1 was further supported by the presence of 17 carbons in its ^{13}C NMR spectrum. UV absorption of 1 in MeOH at 269 nm and 326 nm is typical of a flavone structure [7]. The IR spectrum exhibited a broad vibrational band due to hydroxyl group at 3418 cm^{-1} and a carbonyl band at 1738 cm^{-1}. From ^1H NMR, two signals for two aromatic methoxy groups appeared at δ3.90 and δ3.89 were placed at C-7 and C-4', respectively as they showed ^3J correlation with these carbons at δ165.4 and δ162.6 ppm in its HMBC spectrum. Strong NOE correlations of the δ3.90 methoxy proton with H-6 and H-8, and correlation with

H-3' and H-5' for δ3.89 methoxy protons were observed at ring-B in its NOESY spectrum. One proton singlet at δ6.59 was assigned to H-3 which showed correlation with H-6' in the NOESY spectrum. Two *ortho*-coupled doublets at δ7.85 and δ7.02 and two meta-coupled doublets at δ6.38 and δ6.49 were assigned to H-2' and H-6', and H-3' and H-5', and H-6 and H-8, respectively on the basis of HSQC and HMBC studies. From the COSY spectrum, there were correlations between protons at H-2' and H-3' or H-5' and H-6'. The singlet at δ12.82 corresponded to a chelated hydroxy group at H-5. Thus from the foregoing spectral features, the structure of 1 was established as 5-hydroxy-7,4'-dimetoxyflavone (Table 1, Fig. 1 and Fig. 2). This analysis was also consistent with other reports in the literature for 1 [8 - 10].

Compound **2** was obtained as a white amorphous solid and its molecular formula suggested as $C_7H_6O_3$, deduced from negative ESIMS at m/z 137.0235 $[M+H]^+$. The IR absorption bands of 2 showed signals for hydroxyl group (3462 cm^{-1}) and carbonyl group (1654 cm^{-1}). Broad absorption band at frequency from 3400 cm^{-1} to 2400 cm^{-1} showed the presence of carboxylic acid. ^1H NMR spectrum for 2 in $CDCl_3$ showed two signals for proton resonance at aromatic ring. Two *ortho*-coupled doublet of triplet at δ 7.89 and δ 6.84, each integrating for two protons, H-2 and H-6, and H-3 and H-5 respectively. The proton NMR spectrum was in agreement with the literature [13] while ^{13}C NMR spectrum (Table 1), exhibited seven carbons resonance at five different signals. Signals at δ131.6 and δ114.7 ppm, each corresponding to two carbons, C-3 and C-5, and C-2 and C-6. The signal at δ 168.8, is correlated to carbon at C-7 or COOH. The above spectral analysis strongly indicates that the structure of compound 2 is 4-hydroxybenzoic acid or *p*- hydroxybenzoic acid (Table 1 and Fig. 1). From the literature, compound 2 was used as a chemotaxonomic marker for the family Arecaceae [12]. Besides, compound 2 also has been reported in *Euterpeoleracea martius* (acai) and *Calamus quiquesetinervius* Burret [13, 14].

Table 1: 1H & 13C (600 MHz) NMR data for compound **1** (MeOD) and **2** ($CDCl_3$)

Position	**1**			**2**	
	δ_H (mult, *J* in Hz)	**δ_C**	**HMBC (δ ^{1}H)**	**δ_H (mult, *J* in Hz)**	**δ_C**
1					121.3
2		164.1	H-3; H-2', H-6'	7.89 (dt, *J* =2.4, 9)	131.6
3	6.59 (s)	104.4		6.84 (dt, *J* =2.4, 9)	114.7
4		182.5	H-3		161.9
5				6.84 (dt, *J* =2.4, 9)	114.7
6	6.38 (d, *J* =2.4)	98.1		7.89 (dt, *J* =2.4, 9)	131.6
7		165.4	OCH_3-7; H-6; H-8		168.8
8	6.49 (d, *J* =2.4)	92.7			
9		157.7	H-8		
10		105.6	H-3; H-6; H-8; 5-OH		
1'		123.6	H-3'; H-5'		
2'	7.85 (d, *J* =9)	128.1			
3'	7.02 (d, *J* =9)	114.5			
4'		162.6	OCH_3-4'; H-2'; H-3'; H-5'; H-6'		
5'	7.02 (d, *J* =9)	114.5			
6'	7.85 (d, *J* =9)	128.1			
5-OH	12.82 (s)	162.2	H-6		
7-OMe	3.90 (s)	55.8			
4'-OMe	3.89 (s)	55.6			

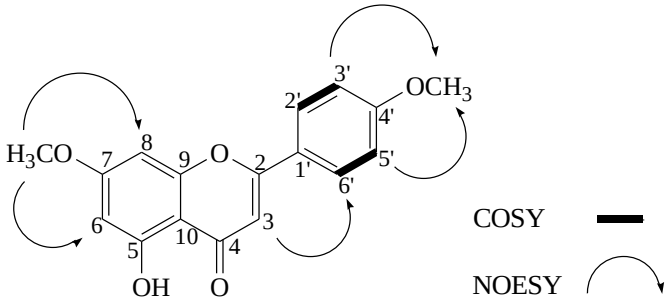


Fig. 2. 1H-1H COSY and NOESY correlation for **1.**

Conclusion

In the studies of chemical components from *Orania sylvicola* fruits, two known phenolic compounds, 5-hydroxy-7,4'dimetoxyflavone (1) and 4-hydroxybenzoic acid (2) were isolated and successfully identified. 4-hydroxybenzoic acid has been used as a chemotaxonomic marker of Arecaceae family. While, 5-hydroxy-7,4'dimetoxyflavone has not been reported yet from this family. Particularly, both compounds is now reported for the first time from the fruits of the species *O. sylvicola*.

Acknowledgement

The research was partially supported by the grant UKM-PNI-NBD0009-2007 and gratefully acknowledged.

References

1. Keim, A. P & Dransfield, J. (2012). A monograph of the genus Orania (Arecaceae: Oranieae). *The Board of Trustees of the Royal Botanic Gardens, Kew Bulettin*, 67: 127-190.
2. Essig, F. B. (1980). The genus *Orania Zipp* (Arecaceae) in New Guinea. *Lyonia*, 1 (5): 211-233.
3. McCurrach, J. C. (1960). *Palms of the World*. Harper and Brothers, New York.
4. Ridley, H. N. (1925). *Flora of the Malay Peninsula*. V. L. Reeve & Co., Ltd., London.
5. Whitmore, T. C. (1973). *Palms of Malaya*. Oxford Univ. Press, London.
6. Gimlette, J. D. (1915). *Malay Poison and Charm Cures*. Oxford University Press. Kuala Lumpur.
7. Mabry, T.J., Markham, K.R., Thomas, M.B. (1970). *The Systematic Identification of Flavonoids*. Springer Verlag, New York.
8. Herrera, J. C., Romero, A. J. R., Crescente, O. E., Acosta, M. & Pekerar, S. (1996). Analysis of 5-hydroxy-7-methoxyflavones by normal-phase high performance liquid chromatography. *Journal of Chromatography A*, 740: 201-206.
9. Kolak, U., Hacibekiroglu, I., Ozturk, M., Ozgokce, F., Topcu, G. & Ulubelen, A. (2009). Antioxidant and anticholinesterase constituents of *Salvia poculata*. *Turk J Chem*, 33: 813-823.
10. Rossi, M. H., Yossida, M. & Maia, J. G. S. (1997). Neolignans, Strylpyrones and Flavonoids from an Aniba Species. *Phytochemistry*, 45(6): 1263-1269.
11. Tan, J., Bednarek, P., Liu, J., Schneider, B., Svatos, A. & Hahlbrock, K. (2004). Universally occurring phenylpropanoid and species-specific indolic metabolites in infected and uninfected *Arabidopsis thaliana* roots and leaves. *Phytochemistry*, 65: 691–699.
12. Chakraborty, M., Das, K., Dey, G. & Mitra, A. (2006). Unusually high quantity of 4-hydroxybenzoic acid accumulation in cell wall of palm mesocarps. *Biochemical Systematics and Ecology*, 34: 509 - 513.

13. Chang, C. L., Wang, G. J., Zhang L. J., Tsai, W. J., Chen, R. Y., Wua, Y.C. & Kuo, Y. H. (2010). Cardiovascular protective flavonolignans and flavonoids from *Calamus quiquesetinervius*. *Phytochemistry*, 71: 271 - 279.
14. Pacheco-Palencia, L. A., Duncan, Ch. E. & Talcot, St. T. (2009). Phytochemical composition and thermal stability of two commercial acai species, *Euterpe oleracea* and *Euterpe precatoria*. *Food Chem*, 115: 1199–1205.