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Isolation of Flavones from The Anti--inflammatory Fraction of *Orthosiphon Stamineus* Extracts

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ABSTRACT Fraction Cf2b from the crude chloroform extract of *Orthosiphon stamineus* was found to be the most active in inhibiting carrageenan induced hind paw oedema in mice. It suggested that the most active anti-inflammatory fraction of the extract was found in the fraction Cf2b. Two compounds have been isolated and identified from this active fraction; 5,3'-dihydroxy-6,7,4'-trimethoxyflavone **1** and 5,6,7,3',4'-pentamethoxyflavone **2**. Compounds **1** and **2** are flavones which commonly known as eupatorin and sinensetin respectively. Both compounds were identified and elucidated through spectroscopic methods. Eupatorin is the major compound and sinensetin is the second major compound present in the active fraction

Keywords : *Orthosiphon stamineus*, flavones, sinensetin, eupatorin, anti-inflammatory

ABSTRAK Fraksi Cf2b daripada ekstrak kloroform mentah tumbuhan *Orthosiphon stamineus* telah didapati menunjukkan kesan yang paling aktif dalam aruhan karagenan edema pada tapak kaki mencit. Ini mencadangkan bahawa fraksi Cf2b mempunyai afiniti antiinflamasi yang paling aktif. Dua sebatian telah diasingkan daripada fraksi ini iaitu 5,3'-dihidroksi-6,7,4'-trimetoksiflavon **1** dan 5,6,7,3',4'-pentametoksiflavon **2**. Sebatian **1** dan **2** adalah flavon yang biasanya dikenali sebagai eupatorin dan sinensetin. Kedua-dua sebatian ini diidentifikasi dan dielusidasikan melalui kaedah spektroskopik. Eupatorin merupakan sebation utama manakala sinensetin merupakan sebatian kedua utama dalam fraksi aktif ini.

INTRODUCTION

Traditional medicines are widely used alongside with modern medicine in many countries of Southeast Asia and play an important role in promoting the health care system [1,2]. *Orthosiphon* (*O.*) *stamineus* Benth. [syn.: *O. aristatus* (Bl.) Miq., *O. grandiflorus* Bold., *O. spicatus* (Thumb) Bak.; Lamiaceae], locally known as misai kucing, is one of the popular traditional folk medicine extensively used in Southeast Asia for the treatment of wide range of diseases such as rheumatism, diabetes, hypertension, tonsillitis, epilepsy, anti-inflammatory, gallstone and menstrual disorder. [3,4]. Owing to its beneficial pharmaceutical properties, *O. stamineus* is consumed as a healthy Jawa tea to facilitate body detoxification [5]. Recently, in the course of our study on this medicinal plant, we found that the chloroform extract from the leaves of *O. stamineus* showed significant inhibitory activity on carrageenan induced paw oedema. Further fractionation of the chloroform extract gave the sub-fraction Cf2b which was the most active [6]. After repeated column and thin layer chromatography (TLC) on Cf2B, two flavones; eupatorin **1** and sinensetin **2,** were isolated. In this paper, we wish to report the isolation and structure elucidation of these flavones.

MATERIALS AND METHODS

General experimental procedures

^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ on Bruker AC 300 (400 MHz for ^{1}H-NMR and 300 MHz for ^{13}C-NMR). GC-MS spectra were measured on Agilent 5793N/6980 GC-MS system. All melting points were determined with Gallenkamp (England) melting point apparatus and were uncorrected. IR spectra were taken on Nexus FT-IR spectrophotometer and recorded in KBr. UV spectra was taken on Hitachi U-2000 and determined in MeOH.

Plant materials

The dried herbal leaves of *O. stamineus* were obtained from the island of Penang. The plant was identified and a voucher specimen was deposited in the herbarium of the School of Pharmaceutical Sciences, University of Science Malaysia with reference number 027.

Extraction and Isolation

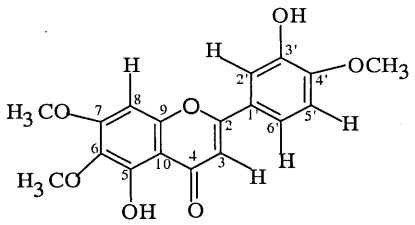
The air-dried leaves of *O. stamineus* (4kg) were finely grounded and then were exhaustively extracted with petroleum ether (40°C-60°C), followed by extraction with chloroform and lastly with methanol. Each extract was concentrated separately using rotary evaporator to give their respective crude extract [7]. The chloroform extract (196.2 g) was subjected to a dry-flash chromatography on silica gel (70g), and eluted with petroleum ether, followed by a mixture of increasing polarity of petroleum ether-chloroform and chloroform-methanol which afforded 11 fractions of 150ml each. These fractions were then combined to three fractions Cf1, Cf2 and Cf3 on the basis of the TLC profiles similarity. The most active fraction Cf2 (2.75g) was rechromatographed (silica gel, 100g) and eluted with petroleum ether:ethyl acetate (70:30) to yield two sub-fractions Cf2A and Cf2B for which only sub-fraction Cf2B (1.5g) showed the most significant anti-inflammatory activity. Combined column and TLC on sub-fraction Cf2B afforded eupatorin **1** (5.4mg) and sinensetin **2** (4.7mg).

RESULTS AND DISCUSSION

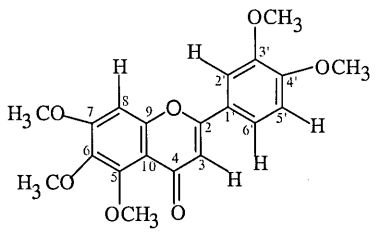
Isolation of the anti-inflammatory carrageenan induced-paw oedema of *O. stamineus* by extensive chromatographic techniques resulted in the isolation of two flavones. Their structures were established by spectral methods.

Compound **1** was obtained as a yellow powder with the melting point of 197-199°C, literature 196-198°C [8]; IR (KBr) 3430cm^{-1} (OH), 2945cm^{-1} (CH), 1653cm^{-1} (C=O), 1497-1566cm^{-1} (C=C aromatic), 1605 (C=C stretch); molecular formula $C_{18}H_{16}O_7$, m/z 344. Compound **1** on the TLC gave positive test on flavone which showed dark blue colouration after being sprayed with NP/PEG and was observed under UV 365 nm. The UV spectrum of **1**, showed maxima at 243 (1.06) and 343 (1.36), confirmed the presence of a flavone skeleton. On the basis of the data obtained from IR, UV, ^{1}H-NMR, ^{13}C-NMR, GC-MS and comparison with the literature values, compound **1** was identified as 5,3'-dihydroxy-6,7,4'-trimethoxyflavone (eupatorin) [9].

Compound **2** was obtained as colourless solid; melting point of 174-176°C; IR (KBr) 2996cm^{-1} (CH), 1633cm^{-1} (C=O), 1450-1546 cm^{-1} (C=C aromatic); molecular formula $C_{20}H_{20}O_7$, m/z 372. The UV spectrum of **2**, showed maxima at 307 nm (1.05) and 334 nm (1.32), thus suggesting that compound **2** is a flavone. Comparison of the obtained spectral data; IR, UV, ^{1}H-NMR, ^{13}C-NMR, GC-MS and the literature values, confirmed that compound **2** is indeed 5,6,7,3',4'-pentamethoxyflavone (sinensetin) [10]. Table 1-3 list the proton and carbon chemical shift values of compound **1** and **2**.



5,3'-dihydroxy-6,7,4'-trimethoxyflavone (**1**)



5,6,7,3',4'-pentamethoxyflavone (**2**)

Table 1 : ^{1}H NMR data for **1** and **2** ($CDCl_3$, δ (ppm)

	1	**2**
H-3	6.56 (s)	6.61 (s)
H-8	6.59 (s)	6.81 (s)
H-2'	7.46 (m)	7.35 (s)
H-5'	6.97 (d, J= 8.08 Hz)	6.99 (d, J = 8.30 Hz)
H-6'	7.49 (d, J= 8.70 Hz)	7.52 (d, J= 8.14 Hz)
5-OH	12.76 (s)	-
3'-OH	5.78 (s)	-
7-OMe	4.00 (s)	4.01 (s)
3'-OMe	-	4.00 (s)
4'-OMe	3.98 (s)	3.98 (s)
6-OMe	3.94 (s)	3.94 (s)
5-OMe	-	3.89 (s)

Table 2 : ^{13}C NMR data for **1** and **2** ($CDCl_3$, δ (ppm), 300MHz)

C-atom	**1**	**2**
C2	164.2	161.5
C3	104.8	107.8
C4	183.0	177.5
C5	153.6	152.0
C6	133.0	140.5
C7	159.1	157.9
C8	90.9	96.9
C9	153.4	155.0
C10	106.5	108.5
C1'	124.9	121.2
C2'	111.1	109.2
C3'	146.4	149.8
C4'	150.0	151.9
C5'	112.7	111.6
C6'	119.5	119.9
OMe	61.2*	63.2*
OMe	56.7*	62.5*
OMe	56.5*	57.3*
OMe	-	57.2*
OMe	-	57.1*

* These assignments may be interchanged

Table 3 : DEPT 135 and DEPT 90 for **1** and **2**

C-atom	1		2	
	DEPT 135	DEPT 90	DEPT 135	DEPT 90
C3	104.9	104.9	107.8	107.8
C8	90.9	90.9	96.6	96.6
C2'	111.1	111.1	109.0	109.0
C5'	112.7	112.7	111.5	111.5
C6'	119.5	119.5	1199	119.9
OMe	61.2*	-	63.2*	-
OMe	56.7*	-	62.6*	-
OMe	56.5*	-	56.7*	-
OMe	-	-	56.5*	-
OMe	-	-	56.4*	-

* These assignments may be interchanged

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