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Isolation and Characterization of Flavonoids from The Leaves of Local *Orthosiphon Stamineus*

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Abstract Six flavonoid compounds were isolated from the leaves of local medicinal plant *Orthosiphon stamineus*. On the basis of chemical and spectral analyses their structures were elucidated as equpatorin **1** , sinensetin **2**, 5-hydroxy-6, 7, 3', 4'-tetramethoxyflavone **3**, salvigenin **4**, 6-hydroxy-5, 7, 3'-trimethoxyflavone **5** and 5, 6, 7, 3'-tetramethoxy-4'-hydroxy-8-C-prenylflavone **6.** Latter compound was isolated from this plant for the first time. Identification of known compounds were established through comparison of spectral data (UV, IR, MS and NMR) with literature values.

Key Words: *Orthosiphon stamineus*; Lamiaceae; local medicinal plant; flavonoids.

INTRODUCTION

Orthosiphon stamineus, Benth, known locally as 'Misai kucing' belongs to the Lamiaceae family. Malaysia and Indonesia have a tropical climate with high temperature and rainfall all year, which have enabled the plant to flourish extensively. The leaves of *Orthosiphon stamineus,* Benth are commonly used as a herbal tea for diuresis, to treat rheumatism, diabetes, urinary lithiasis, edema, eruptive fever, influenza, hepatitis, jaundice, biliary lithiasis, and hypertension [1-3].

The recent surge of interest in chemistry of this plant has led to the isolation of more than 50 components with different biological acitivies. In our recent research, a methanol extract of the leaves were seperated by series of chromatograph to give six flavonoids compounds. On the basis of chemical and spectral analyses their structures were elucidated as equpatorin **1** [4], sinensetin **2** [5], 5-hydroxy-6,7,3',4'-tetramethoxyflavone **3** [6], salvigenin **4** [7], 6-hydroxy-5,7,3'-trimethoxyflavone **5** [7] and 5,6,7,3'-tetramethoxy-4'-hydroxy-8-C-prenylflavone **6** [8].

EXPERIMENTAL

IR spectra were recorded (KBr discs) on a FT-IR spectrophotometer, validation (ν_{max} in cm^{-1}). ^1H-NMR spectra were recorded on a Bruker R-32 (300 MHz) instrument in $CDCl_3$ with TMS as an internal standard (chemical shifts in δ, ppm). UV spectra were recorded on HATACHI, U-2000 spectrophotometer Ultrospeck in methanol (λ_{max} in nm). TLC was performed with silica gel GF_{254}. All solvents were analytical reagent grade.

Plant Material

Orthosiphon stamineus Benth (Lamiaceae) leaves were collected from the Island of Penang. The plant was identified and voucher specimen was deposited in the herbarium of the School of Biology, University Sains Malaysia.

Extraction

Dried leaves of the plant (1 kg) were milled into powder and then extracted with direct methanol (10 l) in a soxhlet extractor for 36 hours. The extract was evaporated in a rotatory evaporator and dried by vacum pump. The methanolic extract (50 g) was suspended on water and extracted successively with hexane, chloroform, ethyl acetate and butanol to yield hexane (5.5 g), chloroform (11.5 g), ethyl acetate (9.3 g) and BuOH-soluble (5.2g) fractions, respectively. Chloroform soluble fraction (10 g) was subjected to chromatography on silica gel (60-120 mesh, Merck) eluted with ethyl acetate-hexane (7:3) solvent system. By repeated chromatography to give five major fractions (fraction 1, 0.176 g; fraction 2, 0.216 g; fraction 3, 1.00 g; fraction 4, 0.074 g; fraction 5, 0.143 g and fraction 6, 0.0103 g).

Further purification of fraction 1 using preparative TLC afforded Equpatorin **1** [4] as yellow crystals (ethyl acetate- hexane; 3:2).

Sinensetin **2** [5] was isolated from fraction 2 as white powder (preparative TLC; ethyl acetate-hexane; 3:2, afforded 6 mg).

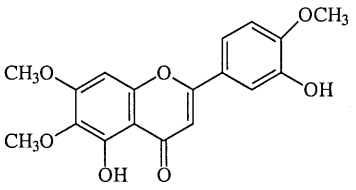
5-hydroxy-6,7,3',4'-tetramethoxyflavone **3** [6] was isolated from fraction 3 as orange needles (preparative TLC; benzene- acetone-ethyl acetate; 7:5:1, 9 mg).

Salvigenin **4** [7] was isolated from fraction 4 as yellow needles (preparative TLC; ethyl acetate-hexane; 7:3.5, afforded 5 mg).

Two compounds were afforded from fraction 5 *i.e.* 6-hydroxy-5, 7, 3'-trimethoxyflavone *5* [7] isolated as yellow needles (6 mg) and 5,6,7,3'-tetramethoxy-4'-hydroxy-8-C-prenylflavone **6** [8] (4 mg) isolated as orange needles (preparative TLC; ethyl acetate- hexane; 7:3.5).

RESULTS AND DISCUSSION

The methanol extract of the leaves was extracted with *n*-hexane, chloroform, ethyl acetate, and n-butanol. The chloroform fraction was purified and six flavonoids were obtained. By means of spectroscopic analysis, they were identified as equpatorin **1**, sinensetin **2**, 5-hydroxy-6,7,3',4'-tetramethoxyflavone **3**, salvigenin **4**, 6-hydroxy-5,7,4'-trimethoxyflavone **5** and 5,6,7,3'-tetramethoxy-4'-hydroxy-8-C-prenylflavone **6**. The compound **6** was isolated from this plant for the first time.



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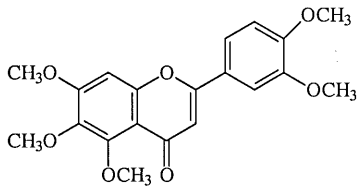

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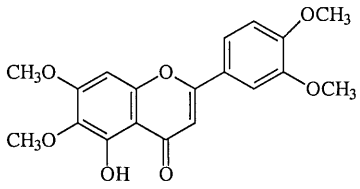

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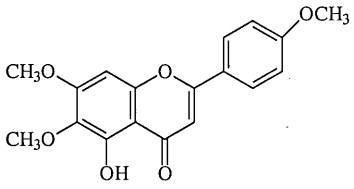

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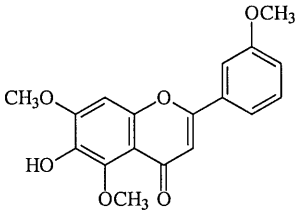

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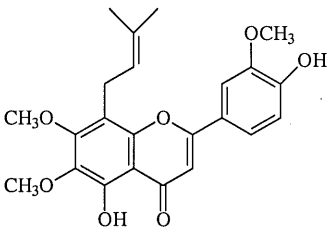

6

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