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Recommended Citation
Suttisri, Rutt; Homhaun, Atthachai; Srangpol, Amornrat; Yamchamuang, Pornpimol; Pengsuparp, Thitima; and Saifah, Ekarin (1999) "Chemical Constituents of the Leaves of Harpullia cupanioides Roxb. (องค์ประกอบทางเคมีของใบหงอนไก่ดง)," The Thai Journal of Pharmaceutical Sciences: Vol. 23: Iss. 1, Article 4.
Available at: https://digital.car.chula.ac.th/tjps/vol23/iss1/4

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Chemical Constituents of the Leaves of Harpullia cupanioides Roxb. (องค์ประกอบทางเคมีของใบหงอนไก่ดง)

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องค์ประกอบทางเคมีของใบหงอนไก่ดง

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ผู้เขียนที่สามารถติดต่อได้

บทคัดย่อ: ฟลาโวนอยด์ 2 ชนิด คือ 3-hydroxy-4′,5,7-trimethoxyflavone กับ flavokavain A และสเตียรอยด์ 1 ชนิด คือ stigmasterol ได้ถูกแยกออกจากฝักใบและผลงานของใบหงอนไก่ดง พบว่าผลลัพธ์โครงสร้างของสารสำคัญนี้ได้รับการตรวจสอบและเปรียบเทียบกับค่าที่มีการรายงานไว้แล้ว

กุญแจคำ: ฟลักและ, Sapindaceae, 3-hydroxy-4′,5,7-trimethoxyflavone, flavokavain A, stigmasterol.
Chemical Constituents of the Leaves of *Harpullia cupanioides* Roxb.

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**ABSTRACT:** Two flavonoids, 3-hydroxy-4',5,7-trimethoxyflavone and flavokavain A, and a steroid, stigmasterol, have been isolated from the hexane-soluble fraction of the leaves of *Harpullia cupanioides* Roxb. The structures of these compounds were determined by spectroscopic analysis and comparison with reported values.

**Key words:** *Harpullia cupanioides*, Sapindaceae, 3-hydroxy-4',5,7-trimethoxyflavone, flavokavain A, stigmasterol.

**INTRODUCTION**

*Harpullia cupanioides* Roxb. (Thai name, Ngon-kai-dong) is a tree, 10-20 m in height, belonging to the family Sapindaceae. The plant occurs throughout Thailand and is one of the mega species of the genus *Harpullia* that can appear to have wide variation within the species (1). In Sri Lanka, the fruits of *H. cupanioides* have been used for washing (2). The family Sapindaceae is well-known for its saponin contents and a number of reports on triterpenoid saponins and sapogenins from this plant and other *Harpullia* species have appeared (3-5). The methanol extract of the leaves of *H. cupanioides*, subjected to antiviral screening test, displayed activity against both herpes simplex virus types 1 and 2. The hexane-soluble fraction of this extract also exhibited cytotoxicity against KB and BC cell lines with the ED₅₀ of 5.9 and 5.0 μg/ml, respectively. In this paper we report the isolation and characterization of two flavonoids, 3-hydroxy-4',5,7-trimethoxyflavone (1) and flavokavain A (2), and a steroid, stigmasterol (3), from the hexane-soluble fraction of the leaves of this plant.

**MATERIALS AND METHODS**

**Plant material**

The leaves of *Harpullia cupanioides* Roxb. were collected in March, 1997 at Khao Ang Rue Nai Wildlife Sanctuary, Chachoengsao Province, Thailand and identified by comparison with herbarium specimens in the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Co-operatives, Bangkok. A voucher specimen of the plant is deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

**General**

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. UV spectra were obtained on a Milton Roy Spectronic 3000 spectrophotometer, using methanol as the solvent. IR spectra were acquired on a FT-IR Perkin Elmer Spectrum 2000 spectrophotometer in KBr. EI-MS spectra were determined on a Micromass (Platform II) mass spectrometer at an ionizing voltage of
70 eV. $^1$H- and $^13$C-NMR spectra were recorded on a Bruker Avance DPX-300 NMR spectrometer at 300 MHz and 75 MHz, respectively, in CDCl$_3$. CC was carried out on Merck silica gel 60 and Sephadex LH-20. TLC was performed with precoated silica gel 60 F254 (0.25 mm) plates. The spots were visualized by spraying with 10% H$_2$SO$_4$ in methanol and then heating at 110° for 3 min.

**Extraction and isolation**

The dried and ground plant material (1.9 kg) was extracted with MeOH (3 × 8 liters) at room temperature for 1 week. The combined MeOH extract was concentrated to about 1 liter under reduced pressure, diluted with an equal volume of H$_2$O, and partitioned successively with hexane and CHCl$_3$. Evaporation of the hexane portion (10.0 g) was chromatographed over a silica gel plate. The spots were visualized by spraying with 10% $^3$H$_2$SO$_4$ in methanol and then heating at 110° for 3 min.

**Cytotoxicity test**

The method used was previously described by Skehan et al. (8). The ED$_{50}$ values are defined as the maximum concentration of the test substance which causes cytotoxic effects in 50% of the cultured cells. These values were calculated using non-linear regression analysis (percent survival versus concentration). Ellipticine was used as positive control in all experiments.

**Determination of antiviral activity**

Anti-herpes simplex virus (HSV) activity was assayed according to the modified procedure described previously (8,9). All substances were tested at concentrations lower than those causing cytotoxicity to the host cell (Vero cell line). Acyclovir was employed as positive control.

**RESULTS AND DISCUSSION**

Column chromatography of the hexane extract of H. cupanoides leaves led to the isolation of compounds 1-3. Compound 3 was identified as stigmasterol, a common plant sterol, by reference to reported spectral data. Spectroscopic examination of 1 and 2 revealed both compounds to be flavonoids of different types. Compound 1 is a flavonol with three methoxyl substituents which appeared as three-proton singlets at 8 3.94, 3.88 and 3.82 ppm in the $^1$H-NMR spectrum. Three-proton singlets at 8 3.90 (3H, OMe), 3.84 (3H, OMe), 3.82 (3H, OMe); $^1$C-NMR (CDCl$_3$, 75 MHz) : 8 192.3 (s, C=O), 168.1 (s, C-4), 165.8 (s, C-6), 162.2 (s, C-2'), 161.1 (s, C-4), 142.3 (d, C-5), 130.0 (d, C-2 and C-6), 128.2 (s, C-1), 125.0 (d, C-3), 114.2 (d, C-3 and C-5), 106.3 (s, C-1'), 93.7 (d, C-3), 91.2 (d, C-5), 59.9 (q, OMe), 55.6 (q, OMe), 55.4 (q, OMe).

Stigmasterol (3) Stigmasterol was identified by comparison with the $^1$H- and $^13$C-NMR data of this compound reported in the literature (6,7).

**Flavokavain A** (2) Yellow rhombic crystals, mp 104-107° (acetone). UV $\lambda_{max}$ (MeOH) nm (log $e$) : 363 (4.50); $\lambda_{max}$ (acetone) nm (log $e$) : 314 (89), 299 (32), 297 (70), 286 (49), 283 (24), 271 (38), 207 (89), 192 (27), 180 (90), 161 (70), 152 (83), 143 (98), 121 (83); $^1$H-NMR (CDCl$_3$, 300 MHz) : 8 7.78 (2H, s, H-$\alpha$ and H-$\beta$), 7.55 (2H, d, $J$ = 8.7 Hz, H-3 and H-5), 6.91 (2H, d, $J$ = 8.7 Hz, H-2 and H-6), 6.09 (1H, d, $J$ = 2.0 Hz, H-3'), 5.95 (1H, d, $J$ = 2.0 Hz, H-5'), 3.90 (3H, s, OMe), 3.84 (3H, s, OMe), 3.82 (3H, s, OMe); $^1$C-NMR (CDCl$_3$, 75 MHz) : 8 192.3 (s, C=O), 168.1 (s, C-4), 165.8 (s, C-6), 162.2 (s, C-2'), 161.1 (s, C-4), 142.3 (d, C-5), 130.0 (d, C-2 and C-6), 128.2 (s, C-1), 125.0 (d, C-3), 114.2 (d, C-3 and C-5), 106.3 (s, C-1'), 93.7 (d, C-3), 91.2 (d, C-5), 59.9 (q, OMe), 55.6 (q, OMe), 55.4 (q, OMe).
(J = 8.9 Hz) between the signals of H-2/H-6' at δ 8.14 ppm and H-3/H-5' at δ 7.00 ppm indicated the position of the third methoxyl group as at C-4'. Therefore, 1 is 3-hydroxy-4',5,7-trimethoxyflavone, a flavonoid compound reportedly synthesized in order to prove the structure of a flavonol glucoside from Calystegia japonica Chois. (10). However, this is the first report of its occurrence in nature.

Compound 2 was shown to be a chalcone with ring B similar to that of 1. The 13C-NMR spectrum of 2 displayed the carbonyl, α and β carbon signals at δ 192.3, 125.0 and 142.3 ppm, respectively. Comparison with reported NMR data (11, 12) facilitated in the assignments of a hydroxyl group to position 2' and two methoxyl groups to positions 4' and 6' of the ring A of 2. Hence, 2 was identified as the chalcone flavokavain A (2'-hydroxy-4',6'-trimethoxychalcone), formerly reported as a constituent of Boesenbergia pandurata rhizomes (13) and kava (Piper methysticum) roots (14).

Both flavonoids exhibited no activity when subjected to anti-HSV assay. However, it is interesting to note that the chemical structure of 2 is highly similar to that of Ro-090410 (4), a chalcone derivative which has been shown to possess specific anti-rhinovirus activity at IC50 value of lower than 1.0 µM (15).

![Structure of compounds 1 and 2](image-url)

**REFERENCES**


