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*Full Length Research Paper*

# Non-alkaloid components from *Uncaria sinensis* (Oliv.) Havil. and their chemotaxonomic significance

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**Ten non-alkaloid compounds (1-10) including one aromatic compound, one anthraquinone, two triterpenoids and five flavonoids were isolated from *Uncaria sinensis* (oliv.) Havil. for the first time, except compounds 3 and 9. The chemotaxonomic significance of these compounds was summarized.**

**Key words:** *Uncaria sinensis* (oliv.) Havil., Rubiaceae, natural products, chemotaxonomic studies.

## INTRODUCTION

The genus *Uncaria* (Rubiaceae) comprises more than 30 species widely distributed in tropical regions, including Asia, Africa, South America (Risdale, 1978). 12 species of them are distributed in China, which have been found widespread use in traditoinal medicine. *Uncaria sinensis* (Oliv.) Havil. has been used as the treatment of hypertension, headache and fever in Part I of Chinese Pharmacopoeia (2010 edition), and found growing in Province Sichuan, Guangxi, Yunnan, Hubei, Guizhou, Hunan, Shanxi, Gansu, etc., in China (Luo, 1999; State Pharmacopeia Committee of China, 2010). The isolation of alkaloids were brought into focus in previous studies of the *Uncaria* genus, the previous study also led to isolation of some flavonoids and triterpenies (Laus, 2004; Heitzman et al., 2005). However, few information is available on flavonoids, triterpenoids and anthraquinones of *U. sinensis*, and there is no information about the chemotaxonomic significance of flavonoids from the *Uncaria* genus.

## EXPERIMENTAL

### Collection and preparation of plant material

The medicinal material was collected at Province Guangxi in 2007, and identified by Dr. Jing Quan Yuan at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen has been deposited there (Voucher specimen #20070026).

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### Extraction and isolation

In our experiments, the dried and powdered bard and branch with curved hooks of the plant (8.5 kg) of *U. sinensis* were exhaustively extracted with 70% ethanol three times. The extract was filtered and concentrated on reduced pressure until only $H_2O$ remained. The remaining solution was sequentially partitioned with petroleum ether, $CHCl_3$, EtOAc and n-BuOH. The petroleum ether and $CHCl_3$ partions were subjected to column chromatography on silica gel using petroleum ether- EtOAc as the mobile phase and yield compounds 1-4 (Figure 1). The EtOAc partition was sperated by using repeated column chromatography over silica gel eluted by a step gradient of MeOH-$CHCl_3$ (0:100, 1:99, 2:98, 5:95, 10:90, 20:80, 30:70, v/v) then over Sephadex LH20 gel eluted by MeOH-$CHCl_3$ (6:4, v/v) and afforded compounds 5-9 (Figure 1). The n-BuOH partion was repeatedly subjected to column chromatography on silica gel, MCI gel, and reversed phase C18 gel, to yielde compound 10 (Figure 1). These compounds including five flavonoids (6-10), two triterpenes (4 and 5), one anthraquinone (2), and one aromatic compound (1) were identified as paeonol (1) (Yu et al., 1986), physcion (2) (Wang et al., 2010), β-sitosterol (3), oleanane acid (4) (Zhang et al., 2000), ursolic acid (5) (Zhou et al., 2003), kaempferol (6) (Lan, 2010), quercetin (7) (Fathiazad et al., 2006), linarin (8) (Zeng et al., 2006), quercetin-3-O-β-D-galactopyranside (9) (Shi et al., 2010) and quercetin-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (10) (Ma et al., 2009) by comparison of NMR and MS data with reported data. Compound 3 was identified by TLC experiment compared with standard substance of β-sitosterol. Compounds 1, 2, 4-8, 10 were isolated from *U. sinensis* for the first time. Compound 6, NMR data: $^1$HNMR (600MHz, DMSO-$d_6$),δ: 8.04 (2H, dd, J = 2.4, 9.0Hz, H-2',

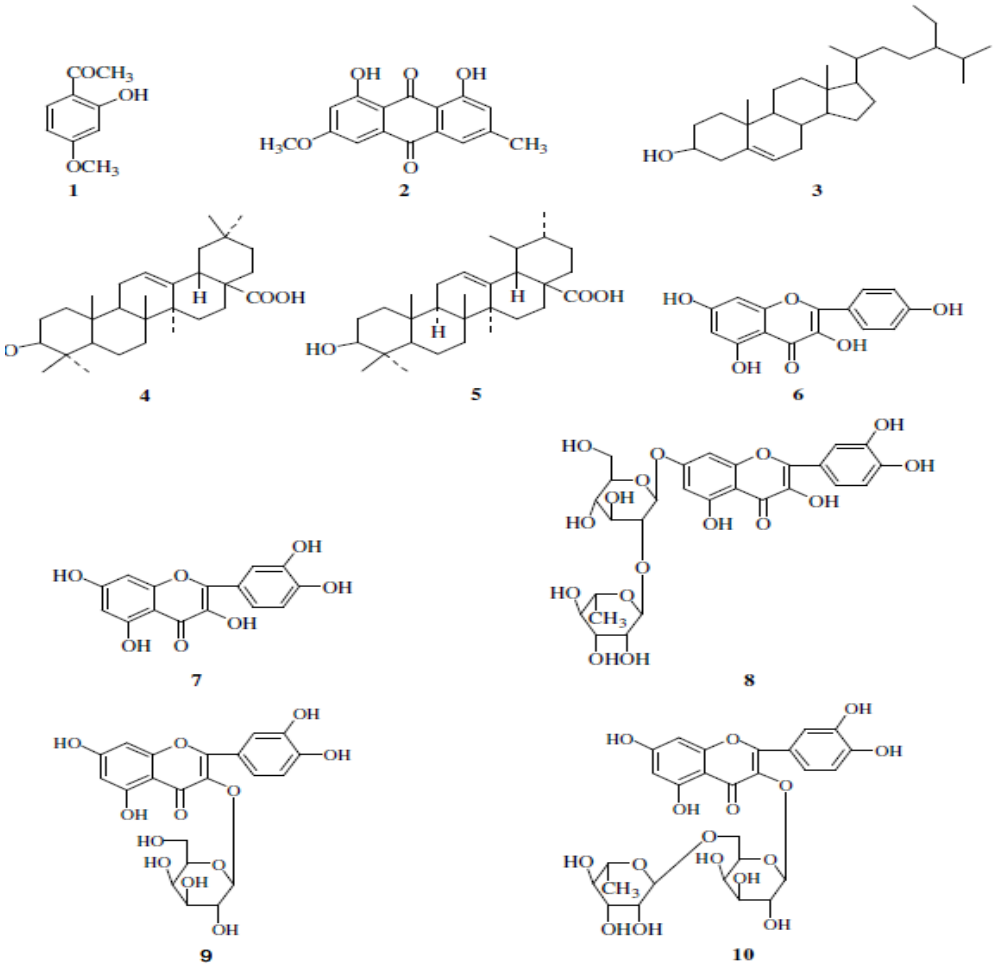


**Figure 1.** Compounds 1–10 isolated from *Uncaria sinensis*.

6'), 6.92 (2H, dd J = 2.4, 9.0Hz, H-3',5'), 6.44 (1H, d, J = 1.8Hz, H-8), 6.19 (1H, d, 1.8Hz, 6), 2.47 (1H, s, 5-OH). $^{13}$CNMR (150MHz, DMSO-$d_6$), δ: 147.3 (C-2), 136.1 (C-3), 176.4 (C-4), 161.2 (C-5), 98.7 (C-6), 159.7 (C-7), 94.0 (C-8), 156.7 (C-9), 103.5 (C-10), 122.1 (C-1'), 130.0 (C-2', C-6'), 115.9 (C-3', C-5'), 164.4 (C-4').

Compound 7, NMR data: $^{1}$HNMR (600MHz, DMSO-$d_6$),δ: 7.67 (1H, d, J = 2.4Hz, H-2'), 7.53 (1H, dd, J = 2.4, 8.4Hz, H-6'), 6.88 (1H, d, J = 8.4Hz, H-5'), 6.40 (1H, d, J = 1.8Hz, H-8), 6.18 (1H, d, J = 1.2Hz, H-1'''), 3.87 (3H, s, —$OCH_3$), 1.09 (3H, d, J = 6.0Hz, Rha—$CH_3$). $^{13}$CNMR (150MHz, DMSO-$d_6$), δ: 163.9 (C-2), 103.8 (C-3), 182.0 (C-4), 161.1 (C-5), 99.9 (C-6), 162.9 (C-7), 94.8 (C-8), 157.0 (C-9), 105.4 (C-10), 122.7 (C-1'), 128.4 (C-2', C-6'), 114.7 (C-3', C-5'), 162.4 (C-4'), 99.6 (C-1''), 73.0 (C-2''), 76.2 (C-3''), 70.7 (C-4''), 75.6 (C-5''), 66.0 (C-6''), 100.5 (C-1'''), 70.3 (C-2'''), 69.6 (C-3'''), 72.0 (C-4'''), 68.3 (C-5'''), 17.8 (C-6''').

Compound 9, NMR data: $^{1}$HNMR (600MHz, DMSO-$d_6$),δ: 7.52 (1H, d, J = 1.8Hz, H-2'), 7.66 (1H, dd, J = 1.8, 8.4Hz, H-6'), 6.81 (1H, d, J = 8.4Hz, H-5'), 6.40 (1H, d, J = 1.8Hz, H-8), 6.19 (1H, d, J = 1.8Hz, H-6), 5.36 (1H, d, J = 7.8Hz, H-1''). $^{13}$CNMR (150MHz, DMSO-$d_6$), δ: 156.2 (C-2), 133.5 (C-3), 177.4 (C-4), 161.2 (C-5), 98.7 (C-6), 164.2 (C-7), 93.5 (C-8), 156.3 (C-9), 103.8 (C-10), 121.1 (C-1'), 115.2 (C-2'), 144.8 (C-3'), 148.5 (C-4'), 115.9 (C-5'), 122.0 (C-6'), 101.8 (C-1''), 71.2 (C-2''), 73.2 (C-3''), 67.9 (C-4''), 75.8 (C-5''), 60.1 (C-6''). Compound 10, NMR data: $^{1}$HNMR (600MHz, DMSO-$d_6$),δ: 7.63 (1H, dd, J = 8.4, 1.8Hz, H-6'), 7.53 (1H, d, J = 1.8Hz, H-2'), 6.81 (1H, d, J = 8.4Hz, H-5'), 6.38 (1H, d, J = 1.8Hz, = 1.8Hz, H-6), 12.48 (1H, s, 5-OH). $^{13}$CNMR (150MHz, DMSO-$d_6$), δ: 146.8 (C-2), 135.7 (C-3), 175.8 (C-4), 160.7 (C-5), 98.1 (C-6), 163.8 (C-7), 93.3 (C-8), 156.1 (C-9), 103.0 (C-10), 121.9 (C-1'), 115.6 (C-2'), 145.0 (C-3'), 147.7 (C-4'), 115.0 (C-5'), 119.9 (C-6').

Compound 8, NMR data: $^{1}$HNMR (600MHz, DMSO-$d_6$),δ: 12.92 (1H, s, 5-OH), 8.06 (2H, d, J = 9.0Hz, H-2', 6'), 7.16 (2H, d, J = 9.0Hz, H-3', 5'), 6.96 (1H, s, H-3), 6.80 (1H, d, J = 2.4Hz, H-8), 6.47 (1H, d, J = 2.4Hz, H-6), 5.07 (1H, d, J = 7.8Hz, H-1''), 4.56 (1H, d, J H-8), 6.17 (1H, d, J = 1.8Hz, H-6), 5.31 (1H, d, J = 7.8Hz, H-1''), 4.41 (1H, d, J = 1.2Hz, H-1'''), 1.06 (1H, d, J = 6.0Hz, H-6'''). $^{13}$CNMR (150MHz, DMSO-$d_6$), δ: 156.3 (C-2), 133.4 (C-3), 177.3 (C-4), 161.2 (C-5), 98.9 (C-6), 164.8 (C-7), 93.7 (C-8), 156.4 (C-9), 103.7 (C-10), 121.0 (C-1'), 115.2 (C-2'), 144.9 (C-3'), 148.7 (C-4'), 116.0 (C-5'), 121.8 (C-6'), 102.1 (C-1''), 71.1 (C-2''), 73.1 (C-3''), 68.2 (C-4''), 73.6 (C-5''), 65.2 (C-6''), 100.0 (C-1'''), 70.4 (C-2'''), 70.6 (C-3'''), 71.9 (C-4'''), 68.1 (C-5'''), 17.9 (C-6''').

## RESULTS AND DISCUSSION

Flavonoids are the major phyto-constituents isolated from the *Uncaria* genus besides alkaloids and triterpenes. 26 flavonoids have been isolated from the *Uncaria* genus to date based on the references (Table 1 and Figure 2).

**Table 1.** Occurrence of flavonoids in some species of *Uncaria* genus

| Compound | Source (Reference) |
|---|---|
| Kaempferol（6） | *Uncaria sinensis* |
| Quercetin（7） | *Uncaria sinensis*<br>*Uncaria rhynchophylla* (Zhu et al.,1997) |
| Linarin（8） | *Uncaria sinensis* |
| Hyperin（9） | *Uncaria sinensis*<br>*Uncaria rhynchophylla* (Ma et al., 2009; Aimi et al., 1982; Han et al., 2000) |
| Quercetin-3-O-α-L- rhamnopyranosyl-(1→6)-β-D-galactopyranoside（10） | *Uncaria sinensis*<br>*Uncaria rhynchophylla* (Ma et al., 2009; Aimi et Al., 1982; Han et al., 2000) |
| Gambiriin A1（11） | *Uncaria gambir* (Nonaka and Nishioka, 1980; Taniguchi et al., 2007) |
| Gambiriin A2（12） | *Uncaria gambir* (Nonaka and Nishioka, 1980; Taniguchi et al., 2007) |
| Gambiriin A3（13） | *Uncaria gambir* (Nonaka and Nishioka, 1980) |
| Gambiriin B1（14） | *Uncaria gambir* (Nonaka and Nishioka, 1980; Taniguchi et al., 2007) |
| Gambiriin B2（15） | *Uncaria gambir* (Nonaka and Nishioka, 1980; Taniguchi et al., 2007) |
| Gambiriin B3（16） | *Uncaria gambir* (Nonaka and Nishioka, 1980) |
| Gambiriin C (Procyanidin B-1,17) | *Uncaria gambir* (Nonaka and Nishioka, 1980)<br>*Uncaria sinensis* (Shimada et al., 2001) |
| Gambirflavan D3（18） | *Uncaria gambir* (Taniguchi et al., 2008) |
| Gambirflavan D4（19） | *Uncaria gambir* (Taniguchi et al., 2008) |
| Gambirflavan D5（20） | *Uncaria gambir* (Taniguchi et al., 2008) |
| Epicatechin（21） | *Uncaria gambir* (Nonaka and Nishioka, 1980)<br>*Uncaria macrophylla* (Yang et al., 2000)<br>*Uncaria sinensis* (Shimada et al., 2001) |
| Catechin（22） | *Uncaria gambir* (Nonaka and Nishioka, 1980)<br>*Uncaria sinensis* (Shimada et al., 2001) |
| Procyanidin B-2（23） | *Uncaria sinensis* (Shimada et al., 2001) |
| Cinchonain Ia（24） | *Uncaria tomentosa* (Wirth and Wagner, 1997) |
| Cinchonain Ib（25） | *Uncaria tomentosa* (Wirth and Wagner, 1997) |
| Afzelin（26） | *Uncaria hirsuta* (Wu and Chan, 1994) |

**Table 1.** Contd.

| | |
|---|---|
| Trifolin（27） | *Uncaria rhynchophylla* (Ma et al., 2009; Aimi et al., 1982) |
| Kaempferol -α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside（28） | *Uncaria rhynchophylla* (Ma et al., 2009; Aimi et al., 1982; Han et al., 2000) |
| Quercitrin（29） | *Uncaria hirsuta* (Wu and Chan, 1994) |
| Rutin（30） | *Uncaria elliptica* (Law and Das, 1987)<br>*Uncaria rhynchophylla* (Ma et al., 2009)<br>*Uncaria hirsuta* (Wu and Chan, 1994) |
| Neohesperdin（31） | *Uncaria hirsuta* (Wu and Chan, 1994) |

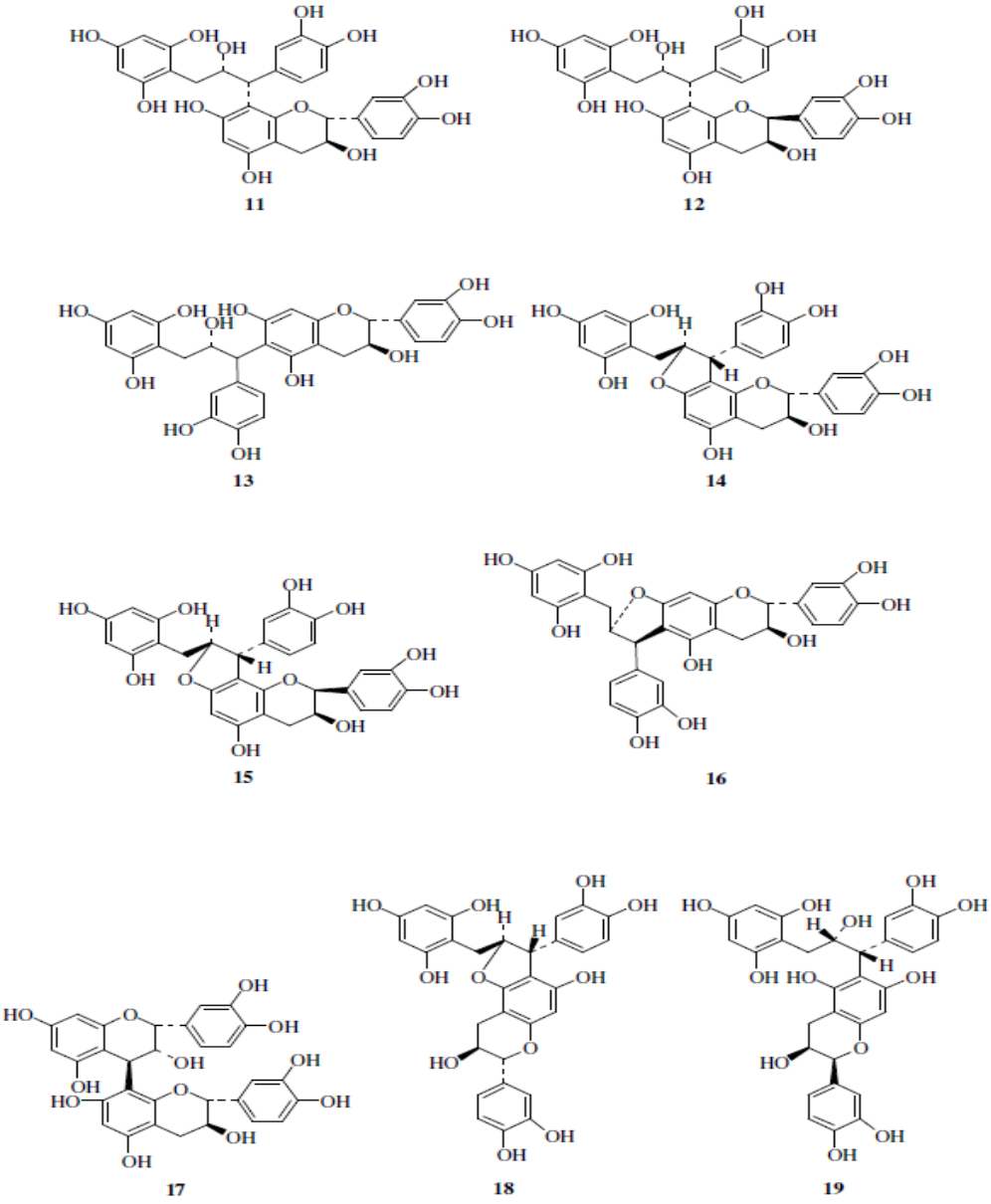
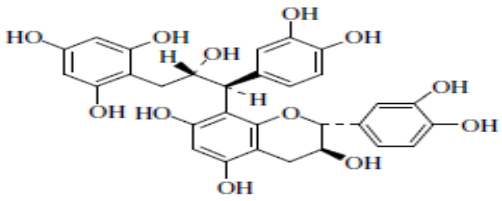



**Figure 2.** Favonoids in some species of *Uncaria* genus.



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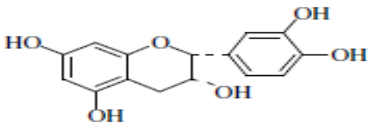

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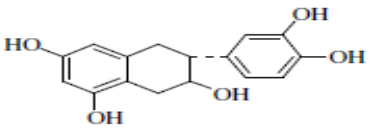

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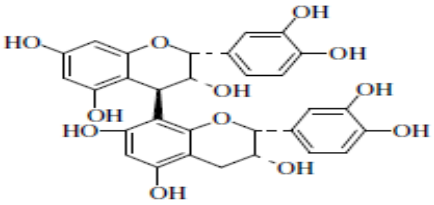

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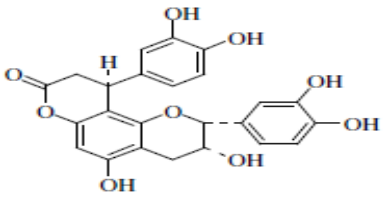

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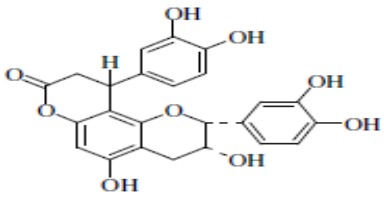

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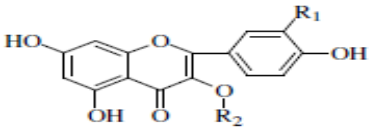

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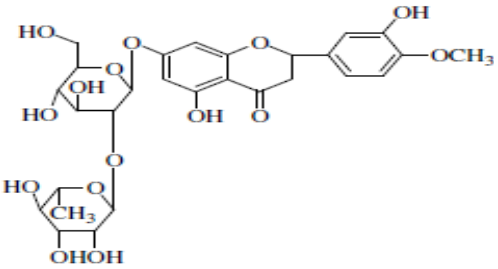

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| | $R_1$ | $R_2$ |
|---|---|---|
| **26** | H | α-L-rhamnopyranoside |
| **27** | H | β-D-galactopyranoside |
| **28** | OH | α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside |
| **29** | OH | α-L-rhamnopyranoside |
| **30** | OH | α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside |

**Figure 2.** Contd.

This report describes systematically the occurrence of flavonoids (6-31) in the *Uncaria* genus. The flavonoids identified in the *Uncaria* genus are flavan-3-ols and their dimers (11-25), flavonols and frequently as their 3-glycosides or 7-glycosides (6-10, 26-30), and 7-glycosides of flavanones (31). Flavon-3-ols and their dimers, flavonols and their 3-glycosides constitute the most common flavonoids in the *Uncaria* genus, and the sugar moieties of these flavonoid glycosides are commonly made up of glucose, galactose and rhamnose (Figures 1, 2 and Table 1). *U. sinensis* is however somewhat different since it has a greater variety of

flavonoids compared to other species of the *Uncaria* genus. Compounds 6 and 8, a flavonol and its 7-glycoside, have only been found in *U. sinensis* of the *Uncaria* genus, while the two compounds have not been found in the other species of the *Uncaria* genus to date. Flavon-3-ols and their dimers may be considered as a chemotaxonomic marker for the *Uncaria* genus as they occur in *U. gambir*, *U. sinensis*, *U. macrophylla*, *U. tomentosa*, but more research is needed to confirm this. The presence of compounds 6 and 8 as the major components may possibly be utilized for identification of *U. sinensis* which is a significant chemotaxonomic finding. The isolation and identification of ten compounds from *U. sinensis* represents a most significant phytochemical analysis of the components of the plant and may be used as a foundation for further chemotaxonomic studies on the *Uncaria* genus.

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