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FLAVONOIDS FROM THE LEAVES OF MULBERRY MISTLETOE *SCURRULA PARASITICA* L. (LORANTHACEAE)

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Summary

Five flavonoids (**1–5**) were isolated from leaves of *Scurrula parasitica* L. parasitizing on the mulberry tree (*Morus alba* L.), including kaempferol (**1**), quercetin (**2**), juglanin (**3**), avicularin (**4**), and quercitrin (**5**). Their chemical structures were elucidated by spectroscopic methods (NMR and MS) and comparison with literature data. Notably, compounds **1** and **3** were isolated from *S. parasitica* L. for the first time.

Keywords: *Scurrula parasitica* L.; Loranthaceae; *Flavonoids; Avicularin; Quercitrin.*

1. Introduction

The Loranthaceae, known as the mistletoe family, is one of the most species-rich parasitic plants, containing approximately 78 genera and around 1000 species. These species are distributed across Africa, Asia, Europe, Australia, South America, and New Zealand [1]. Many species of this family have long been used in indigenous medical systems of diverse cultures. Several mistletoe species can parasitize the same host, and a single species may exhibit distinct characteristics depending on the host plant it grows on [2]. Among the different types of mistletoe, the one parasitizing the mulberry tree (*Morus alba* L.), known as Sang Ji Sheng in Chinese, benalu teh in Malay, basokisei in Japanese, and Tang ký sinh in Vietnamese, is considered as the most widely used in the traditional medicine of Asian countries [3],[4],[5]. Several mistletoe species have been reported to parasitize the mulberry tree, including *Agelanthus brunneus* (Engl.) Tiegh., *Dendrophthoe falcata* (L.f.) Ettingsh., *Tapinanthus glomeratus* Danser, *Scurrula parasitica* L., *Taxillus chinensis* (DC.) Danser (Loranthaceae), and *Viscum album* L. (Viscaceae) [6]. Among these, *S. parasitica* L. and *T. chinensis* (DC.) Danser are the two species most commonly used. In Vietnam, the medicinal material known as "**Tang ký sinh**" or "mulberry mistletoe" (*Tầm gửi cây dâu*) is sourced from *Loranthus parasiticus* (L.) Merr. or *S. parasitica* L. growing on mulberry (*M. alba* L.). It is valued in folk medicine for the treatment of anemia and bleeding in pregnant and postpartum women, as well as dysmenorrhea and chronic debilitation. It is also traditionally believed to tonify the liver and kidneys, strengthen tendons and bones, and promote meridian circulation [7],[8].

M. alba L. contains a wide range of bioactive compounds, including ascorbic acid, carotene, B vitamins, and flavonoids such as rutin, quercetin, and apigenin, together with coumarins, triterpenes, sterols, amino acids, and organic acids. One of its major constituents is 1-deoxynojirimycin (DNJ). These compounds have been associated with antidiabetic, antioxidant, anti-inflammatory, and analgesic activities [9],[10]. Numerous studies have reported that host plants may transfer characteristic constituents to the parasitic mistletoes growing on them, thereby influencing their chemical composition and medicinal quality [11],[12]. Therefore, the phytochemical profile of *M. alba* may affect the chemical composition of the mistletoe parasitizing this species. Phytochemical studies have identified flavonoids as the primary bioactive constituents of *S. parasitica*, together with sesquiterpenes and

sterols [13],[14]. The present study aims to investigate the chemical composition of mulberry mistletoe (*S. parasitica* L.).

2. Materials and methods

2.1. Plant material

Leaves of *Scurrula parasitica* L. were collected in December 2023 in Hung Yen province, Vietnam, and identified by MSc. Nguyen Quynh Nga and MSc. Phan Van Truong (Center of Medicinal Material Resources – NIMM). A voucher specimen (DV-251023) has been deposited at the Center of Medicinal Material Resources, NIMM.

2.2. General experimental procedures

NMR spectra were recorded on a Bruker AM 600 FT-NMR spectrometer (Karlsruhe, Germany) using TMS as the internal standard. MS data were analyzed with an LC-MS/MS system (Shimadzu, Japan). *Silica gel* (Merck, 63–200 mm) and RP-C_{18} (Fuji Silysia Chemical Ltd, 75 µm) were used for column chromatography (CC). Thin-layer chromatography (TLC) was carried out with *silica gel* $60F_{254}$ (Merck, 0.25 mm) and RP-$18F_{254}$ (Merck, 0.25 mm) plates. Spots were detected under UV light at 254 and 365 nm or by spraying with 10% sulfuric acid in 96% ethanol (EtOH) and heating.

2.3. Extraction and isolation

The dried leaves of *S. parasitica* L. (2.2 kg) were coarsely powdered and extracted with 90% ethanol (25 L, 3 × 24 h, room temperature). The combined extracts were concentrated under reduced pressure to yield 179.4 g of a crude semi-solid ethanol extract. A portion of this extract (176.5 g) was suspended in hot water (1.0 L) and successively partitioned with organic solvents to afford *n*-hexane (35.3 g), dichloromethane (4.7 g), ethyl acetate (51.7 g), and aqueous (84.0 g) extracts. The ethyl acetate extract (41.7 g) was subjected to *silica gel* CC and eluted with a gradient of dichloromethane–methanol (90:1, 60:1, 40:1, 20:1, 15:1, 10:1, 7:1, 5:1, 3:1, 1:1, v/v) to obtain nine fractions (E1–E9). Compound **1** (13 mg) was purified from fraction E1 (1.6 g) by recrystallization from acetone. Fraction E2 (1.3 g) yielded crystals, which were washed with acetone to afford compound **2** (20 mg). Fraction E4 (2.3 g) was further chromatographed on *silica gel* CC and eluted with dichloromethane–methanol (8:1, 5:1, 3:1, 1:1, 0:1, v/v) to obtain compounds **3** (9.2 mg), **4** (60.2 mg), and **5** (200 mg).

Kaempferol (**1**): Yellow amorphous powder; ESI-MS: *m/z* 285.15 $[M–H]^-$; ^{1}H-NMR (600 MHz, DMSO-d_6) and ^{13}C-NMR (150 MHz, DMSO-d_6): Table 1.

Quercetin (**2**): Yellow amorphous powder; ESI-MS: *m/z* 301.15 $[M–H]^-$; ^{1}H-NMR (600 MHz, DMSO-d_6) and ^{13}C-NMR (150 MHz, DMSO-d_6): Table 1.

Juglanin (**3**): Yellow amorphous powder; ESI-MS: *m/z* 417.2 $[M–H]^-$; ^{1}H-NMR (600 MHz, DMSO-d_6) and ^{13}C-NMR (150 MHz, DMSO-d_6): Table 1.

Avicularin (**4**): Yellow amorphous powder; ESI-MS: *m/z* 433.15 $[M–H]^-$; ^{1}H-NMR (600 MHz, DMSO-d_6) and ^{13}C-NMR (150 MHz, DMSO-d_6): Table 1.

Quercitrin (**5**): Yellow amorphous powder; ESI-MS: *m/z* 447.20 $[M–H]^-$; ^{1}H-NMR (600 MHz, DMSO-d_6) and ^{13}C-NMR (150 MHz, DMSO-d_6): Table 1.

3. Result and discussion

Five flavonoids (**1–5**) were isolated as yellow amorphous powders from the EtOAc fraction of mulberry mistletoe leaves by chromatographic techniques. Their structures were elucidated on the basis of NMR and MS data, in comparison with literature values (Fig. 1).

Table 1. NMR data of compounds **1–5**

Pos.	1		2		3		4		5	
	δ_H[a,b]	δ_C[a,c]	δ_H[a,b]	δ_C[a,c]	δ_H[a,b]	δ_C[a,c]	δ_H[a,b]	δ_C[a,c]	δ_H[a,b]	δ_C[a,c]
2	-	146.8	-	146.7	-	156.3	-	156.4	-	156.4
3	-	135.7	-	135.7	-	133.3	-	133.3	-	134.2
4	-	175.9	-	175.8	-	177.6	-	177.6	-	177.7
5	-	160.7	-	160.7	-	161.2	-	161.2	-	161.2
6	6.19 (s)	98.2	6.18 (d, 1.8)	98.2	6.19 (s)	98.8	6.19 (s)	98.8	6.20 (d, 1.8)	98.7

Pos.	1		2		3		4		5	
	δ_H[a,b]	δ_C[a,c]	δ_H[a,b]	δ_C[a,c]	δ_H[a,b]	δ_C[a,c]	δ_H[a,b]	δ_C[a,c]	δ_H[a,b]	δ_C[a,c]
7	-	163.9	-	164.0	-	164.2	-	164.7	-	164.2
8	6.44 (s)	93.5	6.40 (d, 1.8)	93.3	6.43 (s)	93.7	6.39 (s)	93.6	6.39 (d, 1.8)	93.6
9	-	156.2	-	156.1	-	156.7	-	156.8	-	157.2
10	-	103.2	-	102.9	-	103.8	-	103.8	-	104.0
1′	-	121.7	-	121.9	-	120.7	-	120.9	-	120.7
2′	8.04 (d, 8.4)	129.7	7.67 (d, 1.8)	115.0	8.02 (d, 8.4)	130.7	7.48 (s)	115.5	7.30 (d, 1.8)	115.6
3′	6.92 (d, 8.4)	115.4	-	145.0	6.89 (d, 8.4)	115.4	-	145.1	-	145.2
4′	-	159.2	-	147.7	-	159.9	-	148.5	-	148.4
5′	6.92 (d, 8.4)	115.4	6.88 (d, 8.4)	115.6	6.89 (d, 8.4)	115.4	6.85 (d, 8.4)	115.5	6.86 (d, 8.4)	115.4
6′	8.04 (d, 8.4)	129.7	7.53 (dd, 1.8, 8.4)	119.9	8.02 (d, 8.4)	130.7	7.56 (d, 8.4)	121.7	7.25 (dd, 1.8, 8.4)	121.1
5-OH	12.47 (s)	-	12.47 (s)	-	12.62 (s)	-	12.63 (s)	-	12.66 (s)	-
1″					5.62 (s)	108.0	5.58 (s)	107.8	5.25 (d, 1.2)	101.8
2″					4.15 (d, 1.2)	82.1	4.16 (d, 2.4)	82.1	3.97 (br s)	70.0
3″					3.72 (m)	77.1	3.72 (m)	77.0	3.21 (m)	70.5
4″					3.53 (dd, 4.8, 10.2)	86.3	3.58 (d, 4.2)	85.8	3.14 (t, 9.6)	71.2
5″					3.26 (dd, 4.8, 12.0) 3.33 (dd, 4.2, 12.0)	60.8	3.28 (dd, 4.8, 12.0) 3.33 (dd, 3.6, 12.0)	60.8	3.50 (m)	70.3
6″									0.81 (d, 6.6)	17.4

[a] DMSO-d_6, [b] 600 MHz, [c] 150 MHz.

Compound **1** showed a deprotonated molecular ion peak at *m/z* 285.15 $[M–H]^-$ in the ESI-MS spectrum, corresponding to the molecular formula $C_{15}H_{10}O_6$. The ^{1}H-NMR spectrum of **1** displayed typical signals of a flavonol, including two singlets at δ_H 6.19 (1H, s, H-6) and δ_H 6.44 (1H, s, H-8) of the *meta*-substituted A ring, an AA′BB′ system at δ_H 8.04 (2H, d, J = 8.4 Hz, H-2′, H-6′) and δ_H 6.92 (2H, d, J = 8.4 Hz, H-3′, H-5′), along with a downfield singlet at δ_H 12.47 (1H, s, 5-OH). The ^{13}C-NMR spectrum exhibited 15 signals consistent with a flavonol skeleton, including a carbonyl carbon at δ_C 175.9 (C-4), seven oxygenated aromatic carbons at δ_C 146.7 (C-2), 135.7 (C-3), 160.7 (C-5), 163.9 (C-7), 156.2 (C-9), and 159.2 (C-4′), two aromatic methines of A ring at δ_C 98.2 (C-6) and 93.5 (C-8), four methines of B ring at δ_C 129.7 (C-2′, C-6′), and 115.4 (C-3′, C-5′), and two quaternary carbons at δ_C 103.2 (C-10) and 121.7 (C-1′). Based on these spectroscopic data, together with literature values [15], **1** was identified as kaempferol.

The ESI-MS spectrum of **2** displayed a deprotonated molecular ion peak at *m/z* 301.15 $[M–H]^-$, establishing its molecular formula as $C_{15}H_{10}O_7$. The NMR data of **2** were similar to those of kaempferol (**1**), showing two *meta*-coupled protons of the A ring at δ_H 6.18 (1H, d, J = 1.8 Hz, H-6) and 6.40 (1H, d, J = 1.8 Hz, H-8), together with a downfield singlet at δ_H 12.47 (1H, s, 5-OH). However, the B ring resonances differed, displaying an ABX system at δ_H 7.67 (1H, d, J = 1.8 Hz, H-2′), 6.88 (1H, d, J = 8.4 Hz, H-5′), and 7.53 (1H, dd, J = 1.8, 8.4 Hz, H-6′), along with an additional oxygenated carbon at δ_C 145.0 (C-3′) in the ^{13}C-NMR spectrum. These features indicated the presence of an extra hydroxyl group at C-3′ compared with **1**. Taken together with literature data [16], compound **2** was identified as quercetin.

The molecular formula of **3** was established as $C_{20}H_{18}O_{10}$ by a negative ESI-MS ion peak at *m/z* 417.2 $[M–H]^-$. The NMR spectra of **3** resembled

those of **1**, except for an additional proton signals at δ_H 5.62 (1H, s, H-1″), 4.15 (1H, d, J = 1.2 Hz, H-2″), 3.72 (1H, m, H-3″), 3.53 (1H, dd, J = 4.8, 10.2 Hz, H-4″), 3.26 (1H, dd, J = 4.8, 12.0 Hz, H-5″), 3.33 (1H, dd, J = 4.2, 12.0 Hz, H-5″) and five carbon signals at δ_C 108.0 (C-1″), 82.1 (C-2″), 77.1 (C-3″), 86.3 (C-4″), and 60.8 (C-5″), characteristic of an α-L-arabinofuranosyl unit. The attachment of the sugar at C-3 was confirmed by the HMBC correlation between H-1″ (δ_H 5.62) and C-3 (δ_C 133.3). So, the structure of **3** was determined to be kaempferol 3-*O*-α-L-arabinofuranoside, also known as juglanin, by its NMR data and comparison with the reported data [17].

The molecular formula of **4** was suggested as $C_{20}H_{18}O_{11}$ based on a deprotonated molecular ion peak at *m/z* 433.15 $[M–H]^-$. The NMR data of **4** closely resembled those of quercetin (**2**), except for the additional signals of an α-L-arabinofuranosyl moiety [δ_H 5.58 (1H, s, H-1″), 4.16 (1H, d, J = 2.4 Hz, H-2″), 3.72 (1H, m, H-3″), 3.58 (1H, d, J = 4.2 Hz, H-4″), 3.28 (1H, dd, J = 4.8, 12.0 Hz, H-5″), and 3.33 (1H, dd, J = 3.6, 12.0 Hz, H-5″); δ_C 107.8 (C-1″), 82.1 (C-2″), 77.0 (C-3″), 85.8 (C-4″), and 60.8 (C-5″)]. The HMBC correlation between H-1″ (δ_H 5.58) and C-3 (δ_C 133.3) confirmed the sugar attachment at C-3. Thus, **4** was identified as quercetin 3-*O*-α-L-arabinofuranoside (avicularin) by comparison with literature data [18].

The ESI-MS spectrum of **5** showed a deprotonated molecular ion peak at *m/z* 447.2 $[M–H]^-$, corresponding to the molecular formula $C_{21}H_{20}O_{11}$. The NMR spectra of **5** were similar to those of **2**, except for the presence of an α-L-rhampyranosyl unit [δ_H 5.25 (1H, s, H-1″), 3.97 (1H, br s, H-2″), 3.21 (1H, m, H-3″), 3.14 (1H, t, J = 9.6 Hz, H-4″), 3.50 (1H, m, H-5″), and 0.81 (3H, d, J = 6.6 Hz, H-6″), and δ_C 101.8 (C-1″), 70.0 (C-2″), 70.5 (C-3″), 71.2 (C-4″), 70.3 (C-5″), and 17.4 (C-6″)]. The HMBC cross-peak of H-1″ with C-3 identified the position of the sugar unit at C-3. Thus, the structure of **5** was determined to be quercetin 3-*O*-α-L-rhamnoside, also known as quercitrin, by comparing its NMR spectra with the reference [19].

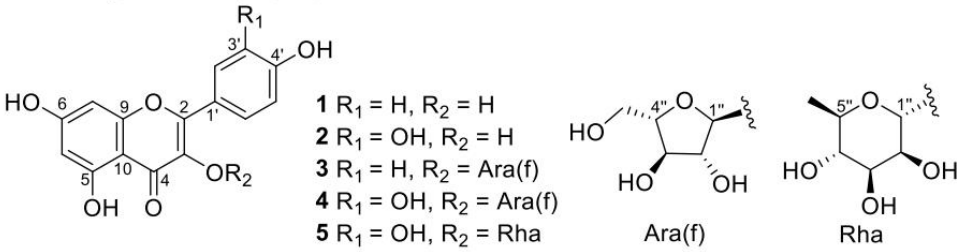

Fig. 1. Chemical structures of compounds **1–5**

Compounds kaempferol (**1**) and juglanin (**3**) were reported for the first time from *S. parasitica* L. parasitizing on the mulberry tree (*M. alba* L.), also known as Tang ký sinh. The results indicate that flavonoids are the major constituents of Tang ký sinh, with avicularin (**4**) and quercitrin (**5**) identified as the most abundant. In addition, all isolated compounds were found to be glycosides of either kaempferol or quercetin. Previous studies have shown that mulberry leaves are also rich in flavonoids, including glycosides of kaempferol and quercetin [10],[20]. This finding supports the notion that the phytochemical composition of mistletoes is strongly influenced by their host plants. All isolated compounds have demonstrated antioxidant, anti-inflammatory, and various other biological activities. Kaempferol (**1**) and quercetin (**2**) are two important flavonoids with potent biological effects on human health, including antioxidant, anti-inflammatory, antibacterial, antifungal, and anticancer activities [21],[22]. Juglanin (**3**) has also attracted increasing attention and exhibited anti-inflammatory, antioxidant, anti-fibrotic, and anti-apoptotic effects [23]. Avicularin (**4**) is known for its anti-inflammatory, anti-allergic, antioxidant, anti-tumor, and hepatoprotective activities [24], whereas quercitrin (**5**) possesses antioxidant, anti-inflammatory, antibacterial, analgesic, wound-healing, vasodilatory, and immunomodulatory properties [25].

4. Conclusion

From the 90% EtOH extract of the leaves of the mulberry mistletoe *S. parasitica* L., five compounds were isolated, including kaempferol (**1**), quercetin (**2**), juglanin (**3**), avicularin (**4**), and quercitrin (**5**). Notably, compounds **1** and **3** were isolated for the first time from this species.

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