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journal homepage: www.elsevier.com/locate/biochemsysecoChemical constituents of *Ulmus pumila* L. and their chemotaxonomic significanceDeri Hu^{a,1}, Zhenyu Liu^{b,1}, Yue Yu^{a,1}, Chenghao Wu^a, Jinze Liu^a, Dongzhou Kang^a, Junzhe Min^{a,**}, Mingshan Zheng^{a,c,*}^a School of Pharmaceutical Sciences, Yanbian University, Yanji, 133002, PR China^b Medical College of Yanbian University, Yanji, 133002, PR China^c Key Laboratory of Natural Medicines of the Changbai Mountain, Ministry of Education, Yanbian University, Yanji, 133002, PR China

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ABSTRACT

A phytochemical study of the leaves of *Ulmus pumila* L. led to the isolation of 32 compounds, including fourteen flavonoids, which include flavonols (1–6), dihydroflavones (7–11), and dihydroflavonols (12–14), five terpenoids, which include megastigmane glycosides (15–17) and triterpenoids (18–19), four sugars (20–23), one phenylpropanoid (24), two phenolic glycosides (25–26), two aromatic glycosides (27–28), one phenolic (29), one lignan glycoside (30), one steroid glycoside (31), and one fatty acid (32). The structures of these isolated compounds were determined by analysis of their NMR spectroscopic (¹H and ¹³C) data and by comparison with previously reported data. Compounds 4 and 8–10 were first reported from *U. pumila*; compound 12 was obtained from the genus *Ulmus* for the first time, while compounds 2, 6, 7, 11, 13, 15–17, 19–28, 30, and 32 have never been isolated from the Ulmaceae family. Chemotaxonomic studies have shown that the genus *Ulmus* may be closely related to the genera *Hemiptelea*, *Zelkova*, and *Holoptelea*.

1. Subject and source

The genus *Ulmus* L. is a typical member of the Ulmaceae family naturally distributed throughout the northern hemisphere in Eurasia, North America, and Northern Africa (Martin-Benito et al., 2005). According to the worldflora online/plant list 2024, 44 species are reported as accepted species, including *Ulmus pumila* L. that possesses 21 synonyms (<http://www.worldfloraonline.org>, accessed on August 30, 2024). In China, there are about 21 species of *Ulmus* plants that are widely distributed (Flora of China Editorial Committee, 2003). The fruit, bark, and leaves of *Ulmus pumila* L. can be used as medicine, and have been reported to possess antioxidant (Gu et al., 2019), anti-inflammatory (Lee et al., 2018), and anti-adipogenic activities (Ghosh et al., 2012).

In the present study, the leaves of *U. pumila* were collected in the Changbai Mountain area, Jilin Province, China, in June 2021 and were identified by Prof. Ming-shan Zheng (School of Pharmaceutical Sciences, Yanbian University, China). The voucher specimen (YSY-991106) was

deposited at Pharmacognosy Laboratory of the College of Pharmacy, Yanbian University.

2. Previous work

Previous phytochemical investigations of *U. pumila* have mainly focused on the bark of this plant, revealing the presence of flavonoids (Ma et al., 2019; Cheng et al., 2020), triterpenoids (Wang et al., 2006), sesquiterpenoids (Wang et al., 2004), monosaccharides (Lee et al., 2018), and sterols (Melek et al., 2021). To the best of our knowledge, there has been limited research on the chemical composition of the leaves of *U. pumila*.

3. Present study

The air-dried leaves of *U. pumila* (8.8 kg) were extracted with 75% ethanol (3 × 60 L) by heating under reflux. The crude extract (2.3 kg)

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was suspended with distilled water and successively partitioned into petroleum ether (PE, 3 × 1.4 L), ethyl acetate (EtOAc, 3 × 1.4 L), and *n*-butyl alcohol (*n*-BuOH, 3 × 1.4 L) sequentially, to give PE (90.0 g), EtOAc (199.0 g), *n*-BuOH (430.0 g), and aqueous fractions (930.0 g), respectively.

The EtOAc fraction (181.1 g) was subjected to silica gel column chromatography (CC) and eluted with PE–EtOAc (100:1–0:1, v/v) to afford seven fractions, including Fr. 1 (5.2 g), Fr. 2 (16.2 g), Fr. 3 (30.6 g), Fr. 4 (23.2 g), Fr. 5 (13.0 g), Fr. 6 (22.0 g), and Fr. 7 (52.0 g). Fr. 2 (15.2 g) was fractionated by silica gel CC by gradient elution with PE–EtOAc (50:1–0:1, v/v) to give ten subfractions, including Fr. 2–1

(1.0 g), Fr. 2–2 (1.2 g), Fr. 2–3 (1.6 g), Fr. 2–4 (1.2 g), Fr. 2–5 (1.3 g), Fr. 2–6 (1.1 g), Fr. 2–7 (1.2 g), Fr. 2–8 (1.3 g), Fr. 2–9 (1.0 g), and Fr. 2–10 (3.2 g). Fr. 2–2 (1.1 g) was purified using a Sephadex LH-20 column by eluting with CH₂Cl₂–MeOH (3:7, v/v) to afford compound 1 (20.3 mg). Fr. 2–5 (1.2 g) was subjected to reverse-phase (RP) CC by gradient elution with MeOH–H₂O (8:2–1:0, v/v) to yield compounds 3 (34.3 mg). Fr. 2–6 (1.0 g) subjected to RP CC by eluting with MeOH–H₂O (6:4–1:0, v/v) to yield compounds 2 (10.2 mg), 4 (6.6 mg), and 5 (4.3 mg), successively. Fr. 2–7 (1.1 g) was purified by recrystallization from MeOH to afford compound 18 (4.9 mg). Fr. 2–8 (1.2 g) was subjected to RP CC by gradient elution with MeOH–H₂O (7:3–1:0, v/v) to yield compound 9

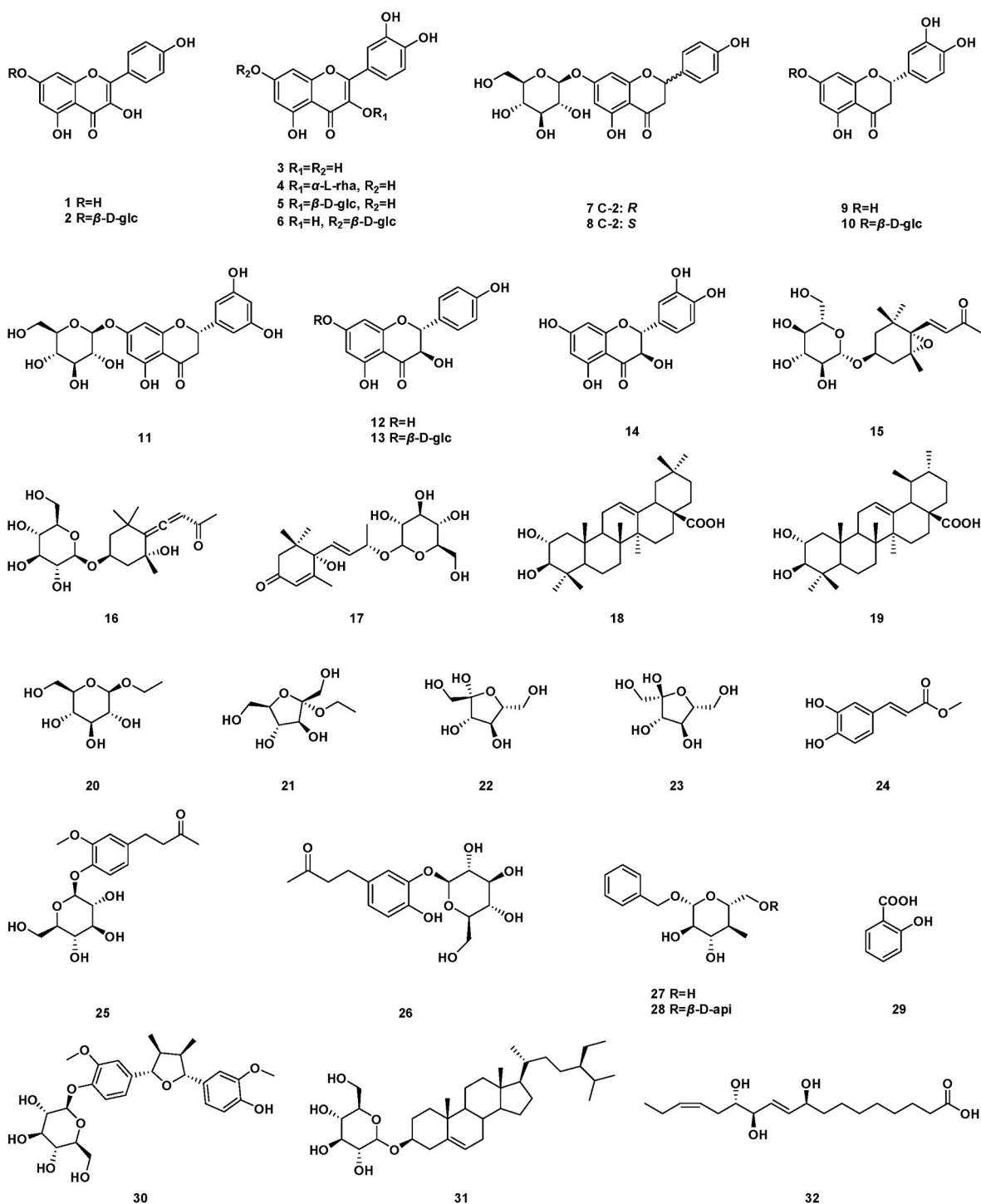


Fig. 1. Chemical structures of compounds 1–32 isolated from the leaves of *Ulmus pumila* L.

(8.1 mg). Fr. 3 (29.6 g) was fractionated by silica gel CC by gradient elution with CH₂Cl₂-MeOH (30:1-0:1, v/v) to give five subfractions, including Fr. 3-1 (1.2 g), Fr. 3-2 (5.3 g), Fr. 3-3 (5.1 g), Fr. 3-4 (6.8 g), and Fr. 3-5 (9.3 g). Fr. 3-3 (3.0 g) was subjected to RP CC by eluting with MeOH-H₂O (1:1-1:0, v/v) to yield a mixture of compounds **7** and **8** (188.3 mg). Fr. 3-4 (1.7 g) was purified by recrystallization from MeOH to afford compound **19** (4.2 mg). Fr. 4 (22.2 g) was fractionated using silica gel CC by gradient elution with CH₂Cl₂-MeOH (20:1-0:1, v/v) to give six subfractions, including Fr. 4-1 (4.2 g), Fr. 4-2 (3.3 g), Fr. 4-3 (4.1 g), Fr. 4-4 (2.8 g), Fr. 4-5 (3.2 g), and Fr. 4-6 (2.3 g). Fr. 4-1 (2.3 g) was subjected to silica gel CC using CH₂Cl₂-MeOH (30:1-2:1, v/v) to obtain compounds **29** (6.1 mg), **15** (9.2 mg), **16** (3.6 mg), and **17** (6.3 mg), successively. Fr. 4-2 (1.9 g) was fractionated using silica gel CC by gradient elution with CH₂Cl₂-MeOH (5:1-1:0, v/v) to give six subfractions, including Fr. 4-2-1 (0.1 g), Fr. 4-2-2 (0.5 g), Fr. 4-2-3 (0.1 g), Fr. 4-2-4 (0.1 g), Fr. 4-2-5 (0.1 g), and Fr. 4-2-6 (0.4 g). Fr. 4-2-2 (400.1 mg) subjected to RP CC by eluting with MeOH-H₂O (2:8-1:0, v/v) to yield compounds **10** (11.1 mg), **11** (10.6 mg), and **12** (20.9 mg), successively. Fr. 4-3 (1.7 g) was purified using a Sephadex LH-20 column by eluting with 100% MeOH to afford compounds **13** (9.9 mg) and **14** (7.1 mg). Fr. 4-4 (1.1 g) was separated successively using a Sephadex LH-20 column by eluting with CH₂Cl₂-MeOH (2:8, v/v) and an RP column by eluting with MeOH-H₂O (10:1-1:0, v/v) to give compounds **30** (6.3 mg) and **32** (4.1 mg). Fr. 4-5 (2.2 g) was subjected to RP CC by eluting with MeOH-H₂O (1:9-1:0, v/v) to yield compounds **25** (18.8 mg), **26** (7.5 mg), and **27** (16.9 mg), successively. Fr. 5 (12.0 g) was subjected to silica gel CC using CH₂Cl₂-MeOH (10:1-0:1, v/v) as the mobile phase to give nine subfractions, including Fr. 5-1 (1.0 g), Fr. 5-2 (1.9 g), Fr. 5-3 (1.0 g), Fr. 5-4 (1.0 g), Fr. 5-5 (1.2 g), Fr. 5-6 (1.3 g), Fr. 5-7 (1.9 g), Fr. 5-8 (1.2 g), and Fr. 5-9 (1.0 g). Fr. B5-2 (1.0 g) was subjected to RP CC by gradient elution with MeOH-H₂O (1:9-1:0, v/v) to yield compounds **28** (8.0 mg) and **31** (38.6 mg). Fr. 5-7 (1.8 g) was subjected to RP CC by gradient elution with MeOH-H₂O (1:9-1:0, v/v) to yield a mixture of compounds **20** and **21** (7.3 mg), **22** (13.3 mg), and **23** (5.9 mg), successively. Fr. 5-8 (1.0 g) was purified using a Sephadex LH-20 column by eluting with 100% MeOH to afford compounds **6** (20.1 mg) and **24** (3.1 mg).

Phytochemical study of the EtOAc extract of *U. pumila* led to the isolation of 32 compounds (**1**-**32**, Fig. 1). By comparing their NMR spectroscopic data with those reported in the literature, the isolated compounds were identified as kaempferol (**1**) (Yu et al., 2024), kaempferol 7-*O*-glucoside (**2**) (Wang et al., 2023), quercetin (**3**) (Yu et al., 2024), quercitrin (**4**) (Yu et al., 2024), quercetin 3-*O*-glucoside (**5**) (Yu et al., 2024), quercetin 7-*O*-glucoside (**6**) (Wang et al., 2024), (2*R*)-naringenin 7-*O*-β-*D*-glucopyranoside (**7**) (Xu et al., 2017), (2*S*)-naringenin 7-*O*-β-*D*-glucopyranoside (**8**) (Xu et al., 2017), eriodictyol (**9**) (Schilbert et al., 2024), eriodictyol 7-*O*-β-*D*-glucopyranoside (**10**) (Schilbert et al., 2024), 3',5',5'-trihydroxyflavanone 7-*O*-β-*D*-glucopyranoside (**11**) (Wang et al., 2023), (2*R*,3*R*)-dihydrokaempferol (**12**) (Chang et al., 2004), (2*R*,3*R*)-dihydrokaempferol 7-*O*-glucoside (**13**) (Iwashina et al., 2022), taxifolin (**14**) (Kim et al., 2000), icariside B2 (**15**) (Vu et al., 2022), staphylionoside D (**16**) (Tian et al., 2024), roseoside (**17**) (Kaweetripob et al., 2023), maslinic acid (**18**) (Wang et al., 2006), corosolic acid (**19**) (Yu et al., 2021), ethyl β-*D*-glucopyranoside (**20**) (Dai et al., 2018), ethyl β-*D*-fructofuranoside (**21**) (Dai et al., 2018), β-*D*-fructofuranose (**22**) (Yu et al., 2023), α-*D*-fructofuranose (**23**) (Chen et al., 2022), caffeic acid methyl ester (**24**) (Hu et al., 2023), zingerone β-*D*-glucopyranoside (**25**) (Nagatani et al., 2001), myzodendrone (**26**) (Nagatani et al., 2001), benzyl-β-*D*-glucopyranoside (**27**) (Zhang et al., 2022), icariside F₂ (**28**) (Zhang et al., 2022), salicylic acid (**29**) (Yang et al., 2024), (-)-nectandrin B-β-*D*-glucopyranoside (**30**) (Bu et al., 2013), daucosterol (**31**) (Zheng et al., 2024), and (9*S*,10*E*,12*R*,13*S*,15*Z*)-9,12,13-trihydroxy-10,15-octadecadienoic acid (**32**) (Lopes et al., 2022).

4. Chemotaxonomic significance

In this study, a total of 32 compounds, including six flavonols (**1**-**6**), five dihydroflavones (**7**-**11**), three dihydroflavonols (**12**-**14**), three megastigmane glycosides (**15**-**17**), two triterpenoids (**18**-**19**), four sugars (**20**-**23**), one phenylpropanoid (**24**), two phenolic glycosides (**25**-**26**), two aromatic glycosides (**27**-**28**), one phenolic (**29**), one lignan glycoside (**30**), one steroid glycoside (**31**), and one fatty acid (**32**) were isolated from *U. pumila*. The isolation of four flavonoids (**4** and **8**-**10**) was reported for the first time from this plant; the flavonoid (2*R*,3*R*)-dihydrokaempferol (**12**) was first identified from the genus *Ulmus*; and twenty compounds (**2**, **6**, **7**, **11**, **13**, **15**-**17**, **19**-**28**, **30**, and **32**) were originally isolated from the family Ulmaceae. These findings improved our understanding of the family Ulmaceae and genus *Ulmus*.

Fourteen flavonoids were purified from *U. pumila*, being flavonols (**1**-**6**) predominant, followed by dihydroflavones (**7**-**11**), and dihydroflavonols (**12**-**14**) ones. Among the flavonoids of *U. pumila*, glycosylation at C-7 of the flavonoids nucleus has been found to be the most common feature (Cheng et al., 2020), and compounds **2**, **6**-**8**, **10**-**11**, and **13** isolated from the leaves of *U. pumila* were in good agreement with the above previous statement. Flavonoids **2**, **6**, **11**, and **13** have been obtained in a variety of plants; however, they were first identified as novel constituents of the family Ulmaceae that could serve as potential chemotaxonomic biomarkers to distinguish *U. pumila* from other species of the Ulmaceae family. (2*R*)-Naringenin 7-*O*-β-*D*-glucopyranoside (**7**) was previously obtained from *Helichrysum arenarium* (Asteraceae) (Yang et al., 2021), *Viburnum macrocephalum* (Viburnaceae) (Xu et al., 2017), and *Paeonia lactiflora* (Paeniaceae) (Shi et al., 2016). It was the first to be recorded from the family Ulmaceae. Thus, the biosynthetic processes may be the same in *U. pumila* and the plants mentioned above. To the best of our knowledge, (2*S*)-naringenin 7-*O*-β-*D*-glucopyranoside (**8**) and eriodictyol 7-*O*-β-*D*-glucopyranoside (**10**) have only been reported from *U. macrocarpa* in the Ulmaceae family. The first discovery of these two compounds from this plant may serve as a potential chemotaxonomic marker for *Ulmus*, in view of the fact that they have not been isolated from other genera of the Ulmaceae family. It is also possible to distinguish the genus *Ulmus* and most genera in the Ulmaceae family. (2*R*,3*R*)-Dihydrokaempferol (**12**) was previously isolated only from the genera *Hemiptelea* and *Zelkova* in the Ulmaceae family (Table 1); however, it has never been reported in *Ulmus*. Compound **12** may be useful in distinguishing *U. pumila* from other species of the genus *Ulmus*, as well as providing additional evidence of the close relationship between *Hemiptelea*, *Zelkova*, and *Ulmus*. Quercitrin (**4**) was previously obtained in the genus *Ulmus* and *Zelkova* among in the family Ulmaceae, such as *U. minor*, *U. parvifolia*, and *Zelkova serrata*, and eriodictyol (**9**) was also formerly derived in the genus *Ulmus* solely in *U. macrocarpa* among the Ulmaceae family. Both compounds were the first time isolated from *U. pumila*, providing fresh information on the chemistry of *U. pumila*. This evidence suggests a chemical correlation within the same genus. Kaempferol (**1**) and quercetin (**3**) have been found in numerous plant species. By contrast, quercetin 3-*O*-glucoside (**5**) and taxifolin (**14**) have only been found in the genus *Ulmus* within the Ulmaceae family. Notably, the narrow distribution of compounds **5** and **14** in the genus might have chemotaxonomic significance in the Ulmaceae family.

Five terpenoids (**15**-**19**) were purified from this plant, comprising three megastigmane glycosides (**15**-**17**) and two triterpenoids (**18**-**19**). All of the isolated terpenoids, except maslinic acid (**18**), are novel to the family Ulmaceae. The discovery of these compounds has enriched the chemical composition of terpenoids in the family Ulmaceae and may be useful in distinguishing *U. pumila* from other species in the family. As for triterpenoid **18** has previously been reported in the bark of *U. pumila*, and this is the first time it has been found in the leaves of this plant.

Sugars (**20**-**23**), phenylpropanoid (**24**), phenolic glycosides (**25**-**26**), aromatic glycosides (**27**-**28**), lignan glycoside (**30**), and fatty acid (**32**) were identified as novel constituents of the family Ulmaceae. The

Table 1
Repartition of the compounds isolated from *Ulmus pumila* in the genus and family.

Phytochemical class	Compounds	<i>Ulmus</i> species	References	Ulmaceae genus	References			
Flavonoids	kaempferol (1)	<i>U. americana</i>	Sherman and Giannasi (1987)	<i>Hemiptelea</i> <i>Zelkova</i>	Chang et al. (2004) Won et al. (2018)			
		<i>U. davidiana</i>	Park et al. (2022)					
		<i>U. davidiana</i> var. <i>japonica</i>	Lee et al. (2023)					
		<i>U. glabra</i>	Varfalvyova et al. (2023)					
		<i>U. laevis</i>	Drzewiecka et al. (2018)					
		<i>U. macrocarpa</i>	Li and Bao (2022)					
		<i>U. minor</i>	Schott et al. (2023)					
		<i>U. parvifolia</i>	Cho et al. (2003)					
		<i>U. pumila</i>	Li and Bao (2022)					
		<i>U. rubra</i>	Sherman and Giannasi (1987)					
		quercetin (3)	<i>U. davidiana</i> var. <i>japonica</i> <i>U. parvifolia</i> <i>U. pumila</i>			Lee et al. (2023)	<i>Holoptelea</i> <i>Zelkova</i>	Anju et al. (2020) Won et al. (2018)
						Cho et al. (2003)		
Zhou et al. (2017)								
quercitrin (4)	<i>U. minor</i> <i>U. parvifolia</i>	D'Angiolo et al. (2022)	<i>Zelkova</i>	Sun et al. (2015)				
		Cho et al. (2003)						
quercetin 3-O-glucoside (5)	<i>U. davidiana</i> <i>U. laciniata</i> <i>U. minor</i> <i>U. parvifolia</i> <i>U. pinnato-ramosa</i> <i>U. pumila</i> <i>U. rubra</i>	Park et al. (2022)	<i>Zelkova</i>					
		Gu et al. (2019)						
		D'Angiolo et al. (2022)						
		Cho et al. (2003)						
		Chumbalov et al. (1972)						
		Gu et al. (2019)						
		Saleem et al. (2009)						
(2S)-naringenin 7-O- β -d-glucopyranoside (8)	<i>U. macrocarpa</i>	Kwon et al. (2022)	<i>Hemiptelea</i> <i>Zelkova</i>	Chang et al. (2004) Niklas and Giannasi (1977)				
eriodictyol (9)	<i>U. macrocarpa</i>	Li and Bao (2022)						
eriodictyol 7-O- β -d-glucopyranoside (10)	<i>U. macrocarpa</i>	Kwon et al. (2022)						
(2R,3R)-dihydrokaempferol (12)								
taxifolin (14)	<i>U. davidiana</i> <i>U. glabra</i> <i>U. macrocarpa</i> <i>U. pumila</i>	Kim et al. (2000)	<i>Zelkova</i>	Choi et al. (2019)				
		Varfalvyova et al. (2023)						
		Kim et al. (2000)						
		Kim et al. (2000)						
		Yang et al. (2011)						
maslinic acid (18)	<i>U. davidiana</i> <i>U. davidiana</i> var. <i>japonica</i>	Zheng et al. (2010)	<i>Zelkova</i>					
		Wang et al. (2006)						
salicylic acid (29)	<i>U. davidiana</i> <i>U. minor</i> <i>U. pumila</i>	Ham and Kim (2020)	<i>Hemiptelea</i>	Yang et al. (2024)				
		Lortzing et al. (2024)						
		Huang et al. (2016)						
Steroid glycoside	daucosterol (31)	<i>U. davidiana</i>	Yang et al. (2011)	<i>Holoptelea</i>	Nadella et al. (2012)			
		<i>U. davidiana</i> var. <i>japonica</i>	Zheng et al. (2010)					
		<i>U. parvifolia</i>	Moon and Rim (1995)					
		<i>U. pumila</i>	Zheng et al. (2024)					
		<i>U. pumila</i>	Wang et al. (2012)					

discovery of these compounds has enriched the knowledge of the chemical diversity of the Ulmaceae, and may serve as potential chemotaxonomic biomarkers to differentiate *U. pumila* from other species of the Ulmaceae family. Although salicylic acid (29) and daucosterol (31) have been found in numerous plant species, they were found only in *Ulmus*, *Hemiptelea* and *Holoptelea* among the family Ulmaceae (Table 1), which may indicate chemical correlation between the genera mentioned above.

Our paper has investigated the diversity of the phytochemical composition of *U. pumila* leaves. Chemotaxonomic studies have shown that the genus *Ulmus* may be closely related to the genera *Hemiptelea*, *Zelkova*, and *Holoptelea*. Overall, this phytochemical investigation enhanced our knowledge of the chemical diversity of *U. pumila*.

CRedit authorship contribution statement

Deri Hu: Writing – original draft, Investigation. **Zhenyu Liu:** Writing – original draft, Investigation. **Yue Yu:** Writing – original draft, Investigation. **Chenghao Wu:** Investigation. **Jinze Liu:** Investigation. **Dongzhou Kang:** Investigation. **Junzhe Min:** Writing – review &

editing, Conceptualization. **Mingshan Zheng:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bse.2024.104907>.

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