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Chemical constituents of *Zanthoxylum schinifolium* (Rutaceae)

Wei Li [a], Wei Zhou [b], Sang Hee Shim [a,**], Young Ho Kim [b,*]

[a] School of Biotechnology, Yeungnam University, 214-1 Dae-dong, Gyeongsan, Gyeongbuk 712-749, Republic of Korea
[b] College of Pharmacy, Chungnam National University, Daejeon 305-764, Republic of Korea

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1. Subject and source

Zanthoxylum schinifolium is a dioecious shrub with hooked prickly branchlets belonging to the Rutaceae family, which is found in China, Korea and Japan (Paik et al., 2005). Pharmacological studies on the fruits and leaves of *Z. schinifolium* have revealed medicinal activities including anti-platelet aggregation, anti-oxidant, anti-inflammatory, and anti-tumour effects (Tsai et al., 2000; Cao et al., 2009; Min et al., 2011). Moreover, *Z. schinifolium* is used widely as a pungent condiment and seasoning in Korea, China, Japan and other East Asian countries because of its unique aroma and taste (Yang, 2008). Dried stems of *Z. schinifolium* were collected from Daejeon, Korea in September 2012 (identified by Prof. Young Ho Kim). A voucher specimen (CNU 12102) was deposited at the Herbarium of the College of Pharmacy, Chungnam National University.

2. Previous work

The genus *Zanthoxylum* includes more than 200 species, and the fruits of these species have a distinctive aroma due to their essential oils. Previous phytochemical studies on *Z. schinifolium* have explored the essential oils, coumarins, flavonoids, and alkaloids from the fruits and leaves (Tsai et al., 2000; Cheng et al., 2002; Fang et al., 2010). However, components of the stems have not been characterised.

* Corresponding author. Tel.: +82 42 821 5933; fax: +82 42 823 6566.
** Corresponding author. Tel.: +82 53 810 3028.
E-mail addresses: shshim29@ynu.ac.kr (S.H. Shim), yhk@cnu.ac.kr (Y.H. Kim).

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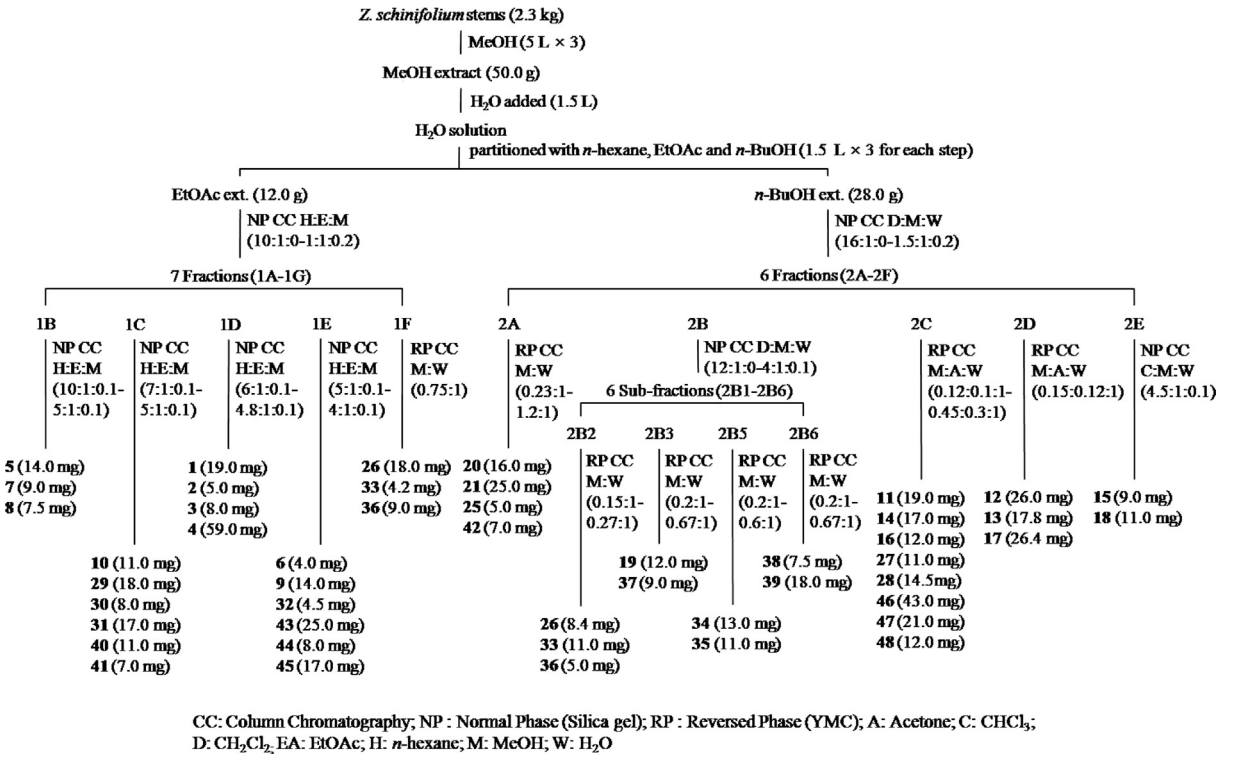

Fig. 1. Isolation scheme of compounds 1–48.

3. Present study

Dried stems of *Z. schinifolium* (2.3 kg) were extracted with MeOH (5 L × 3) under reflux. The MeOH extract (102.0 g) was suspended in water and partitioned with *n*-hexane, EtOAc and *n*-BuOH (Fig. 1). The EtOAc fraction (12.0 g) was subjected to silica gel (5 × 30 cm) column chromatography with a gradient of hexane-EtOAc-MeOH (10:1:0, 6:1:0, 3:1:0, 1.5:1:0.1, 1:1:0.2; 1.5 L for each step) to yield six fractions (Fr. 1A–1F). Fraction 1B was separated using silica gel (1.5 × 80 cm) column chromatography with a gradient of hexane-EtOAc-MeOH (10:1:0.1, 8:1:0.1, 6:1:0.1, 5:1:0.1; 800 mL for each step) elution solvent to yield lacinartin (**5**) (14.0 mg, Deng et al., 2007), collinin (**7**) (9.0 mg, Tsai et al., 2000), and 8-methoxyanisocoumarin H (**8**) (7.5 mg, Tsai et al., 2000). Fraction 1C was separated using silica gel (1.5 × 80 cm) column chromatography with a gradient of hexane-acetone-MeOH (7:1:0.1, 6:1:0.1, 5:1:0.1; 1.0 L for each step) elution solvent to yield schinifolisatin (**10**) (11.0 mg, Li et al., 2014a), (+)-lariciresinol (**29**) (18.0 mg, Della Greca et al., 2010), 5′-methoxylariciresinol (**30**) (8.0 mg, Fiorentino et al., 2007), (+)-5,5′-dimethoxylariciresinol (**31**) (17.0 mg, Yang et al., 2007), skimmianine (**40**) (11.0 mg, Chakravarty et al., 1999), and haplopine (**41**) (7.0 mg, Yang et al., 2002). Fraction 1D was separated using silica gel (1.0 × 80 cm) column chromatography with a gradient of hexane-EtOAc-MeOH (6:1:0.1, 5.5:1:0.1, 4.8:1:0.1; 1.0 L for each step) elution solvent to yield scopoletin (**1**) (19.0 mg, Zakaria et al., 2012), phytodolor (**2**) (5.0 mg, Deng et al., 2007), daphnetin 7-methyl ether (**3**) (8.0 mg, Fang et al., 2010), and scoparone (**4**) (59.0 mg, Kuo et al., 2011). Fraction 1E was separated using silica gel (1.0 × 80 cm) column chromatography with a gradient of hexane-EtOAc-MeOH (5:1:0.1, 4.5:1:0.1, 4:1:0.1; 2.0 L) elution solvent to yield puberulin (**6**) (4.0 mg, Kanlayavattanakul et al., 2003), acetoxyschinifolin (**9**) (14.0 mg, Tsai et al., 2000), lariciresinol acetate (**32**) (4.5 mg, Pullela et al., 2005), norchelerythrine (**43**) (25.0 mg, Ishihara et al., 2011), nornitidine (**44**) (8.0 mg, Ishihara et al., 2011), and decarine (**45**) (17.0 mg, Ishihara et al., 2011). Fraction 1F was separated using YMC (1.0 × 80 cm) column chromatography with a MeOH-H_2O (0.75:1; 850 mL) elution solvent to yield epipinoresinol (**26**) (18.0 mg, Yang et al., 2009), (+)-9′-*O*-*trans*-feruloyl-5,5′-dimethoxylariciresinol (**33**) (4.2 mg, Kwon et al., 1999), and schinifolisatin A (**36**) (9.0 mg, Li et al., 2013).

The *n*-BuOH fraction (28.0 g) was subjected to silica gel (3.0 × 30 cm) column chromatography with a gradient of CH_2Cl_2-MeOH-H_2O (16:1:0, 10:1:0, 7.5:1:0.1, 3:1:0.15, 1.5:1:0.2; 2.5 L for each step) to yield six fractions (Fr. 2A–2F). Fraction 2A was separated using YMC (1 × 80 cm) column chromatography with a MeOH-H_2O (0.23:1, 1.2:1; 750 mL for each step) elution solvent to yield betulalbuside A (**20**) (16.0 mg, Morikawa et al., 2004), 2-hydroxy-4-(2-hydroxyethyl)phenyl, 6-(4-hydroxy-3,5-dimethoxybenzoate) *O*-*β*-D-glucopyranoside (**21**) (25.0 mg, Furukawa et al., 2011), roseoside A (**25**) (5.0 mg, Faiella et al., 2012), and glycohaplopine (**42**) (7.0 mg, Li et al., 2014b). Fraction 2B was subjected to silica gel (1.0 × 70 cm) column chromatography with a gradient of CH_2Cl_2-MeOH-H_2O (12:1:0, 10:1:0, 8.5:1:0.1, 6.5:1:0.1, 4:1:0.1; 1.5 L for each step) to yield six