Chemical Constituents of the n-Butanol Fraction from Cudrania Bark (Post-print)

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Abstract

To investigate the chemical constituents of the n-butanol fraction from the bark of Streblus ilicifolius, this study employed chromatographic methods including silica gel, ODS, Sephadex LH-20, and reversed-phase semi-preparative high-performance liquid chromatography to isolate and purify the n-butanol extract, and the structures of the compounds were identified based on comprehensive physicochemical properties and spectroscopic data. The results showed that sixteen compounds were isolated from the n-butanol extract of S. ilicifolius bark and identified as icariside E5 (1), secoisolariciresinol-9-O-β-glucopyranoside (2), 2,4,6-trimethoxyphenol-1-O-β-D-glucopyranoside (3), 9-O-β-glucopyranosyl trans-cinnamyl alcohol (4), 3,4,5-trimethoxyphenol-1-O-β-apiofuranosyl-(1" $\rightarrow$6′)− −*glucopyranoside*(5), 3 − *hydroxy* − 4, 5 − *dimethoxyphenol*− −*D* − *glucopyranoside*(6), 2, 6 − *dimethoxy* − 4 − *hydroxyphenol* − 1 − *O*− −*D* − *glucopyranoside*(7), *isotachioside*(8), *ficuscarpanoside A*(9), *uridine*(10), *methylsyringate*4− *O*− −*D*−*glucopyranoside*(11), 3, 4, 5−*trimethoxyphenol*− −*D*−*glucopyranoside*(12), *luteolin*(13), *ginsenosi* *lyonirenisol*−3 −*O*− $-D-glucopyranoside (15), and myricetin 3-neohesperidoside (16). All compounds were isolated from this genus for the first time.

Full Text

Preamble

Chemical Constituents from the n-Butanol Fraction of Streblus ilicifolius Bark

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Abstract

To investigate the chemical constituents of the n-butanol extract from the bark of *Streblus ilicifolius*, this study employed various chromatographic methods including silica gel, ODS, Sephadex LH-20, and reversed-phase semi-preparative HPLC for separation and purification. The structures of the isolated compounds were identified based on physicochemical properties and spectroscopic data. The results showed that sixteen compounds were isolated from the n-butanol extract of *S. ilicifolius* bark and identified as icariside E5 (1), secoisolariciresinol 9-O-β-glucopyranoside (2), 2,4,6-trimethoxyphenol-1-O-β-D-glucoside (3), 9-O-β-glucopyranosyl trans-cinnamyl alcohol (4), 3,4,5-trimethoxyphenyl-1-O-β-apiofuranosyl-(1" $\rightarrow$6′)− −*glucopyranoside*(5), 3 − *hydroxy* − 4, 5 − *dimethoxyphenyl*− −*D* − *glucopyranoside*(6), 2, 6 − *dimethoxy* − 4 − *hydroxyphenol* − 1 − *O*− −*D* − *glucopyranoside*(7), *isotachioside*(8), *ficuscarpanoside A*(9), *uridine*(10), *methylsyringate*4− *O*− −*D*−*glucopyranoside*(11), 3, 4, 5−*trimethoxyphenyl*− −*D*−*glucopyranoside*(12), *luteolin*(13), *ginsenosi lyonirenisol*−3 −*O*− $-D-glucopyranoside (15), and myricetin 3-neohesperidoside (16). All compounds were isolated from the genus *Streblus* for the first time.

Keywords: *Streblus ilicifolius*, chemical constituents, icariside E5, secoisolariciresinol 9-O-β-glucopyranoside, ginsenoside Rg1

Introduction

Medicinal plants and other natural products represent a treasure trove of diverse chemical constituents and pharmacological activities, serving as valuable sources of lead compounds for drug discovery. Consequently, the identification of lead compounds from natural plants, particularly folk medicinal herbs, has become a hotspot in pharmaceutical research. Plants from the genus *Streblus* Lour., belonging to the Moraceae family, have long been used as folk medicines in China. The genus comprises approximately 22 species worldwide, with seven species distributed in China, including *S. asper*, *S. indicus*, *S. ilicifolius*, *S. macrophyllus*, *S. zeylanicus*, *S. taxoides*, and *S. tonkinensis*. These species are primarily found in Hainan, Guangxi, and southeastern to southwestern Yunnan provinces.

Streblus species exhibit diverse chemical constituents and various pharmacological activities, making them important traditional medicines. For instance, leaf extracts of *S. asper* have demonstrated anticancer properties, while chemical constituents from its heartwood and bark exhibit antibacterial, anti-hepatitis B virus, antioxidant, and anti-inflammatory effects. The bark of *S. indicus* is commonly used to treat traumatic bleeding, bruises, and gastrointestinal hemorrhage, earning it the folk names "hemostatic bark" and "slippery leaf bruise

remedy." Previous phytochemical and pharmacological studies have revealed that *Streblus* plants mainly contain phenylpropanoids, steroids, flavonoids, terpenoids, and other compounds.

Streblus ilicifolius is a species within this genus. Based on the genetic relationships among congeneric plants, its chemical constituents and pharmacological activities may share similarities with other *Streblus* species. However, prior to this study, no reports on the chemical constituents or biological activities of *S. ilicifolius* were available in the literature. Building upon the known chemical diversity and pharmacological potential of *S. asper* and *S. indicus*, this research aimed to systematically investigate the chemical constituents of the n-butanol fraction from *S. ilicifolius* bark using multiple chromatographic techniques. Sixteen compounds were successfully identified, predominantly phenolic glycosides, with their structures illustrated in Figure 1 [Figure 1: see original paper]. All compounds represent first-time isolations from the genus *Streblus*.

Experimental

Materials

The bark of *Streblus ilicifolius* was collected on August 12, 2019, from Jiaxi, Lingshui County, Hainan Province, China. The plant material was authenticated by Professor Guodong Li from the School of Traditional Chinese Medicine, Yunnan University of Chinese Medicine. A voucher specimen (ZFC201903014e) has been deposited in the Natural Products Research Laboratory, State Key Laboratory of Medicinal Resources Chemistry and Molecular Engineering, School of Chemistry and Pharmaceutical Sciences, Guangxi Normal University.

Instruments and Reagents

The following instruments were used: Agilent 6545 Q-TOF LC-MS high-resolution mass spectrometer (Agilent, USA); Bruker AVANCE 400/600 MHz NMR spectrometer (Bruker BioSpin AGFacilities); LC3000 semi-preparative HPLC system (Beijing Chuangxintongheng Technology); LC1260 semi-preparative HPLC system (Agilent, USA). Column chromatography was performed using silica gel (200-400 mesh) and TLC plates (G254) from Qingdao Marine Chemical Factory; ODS, Sephadex LH-20, and MCI gel (Merck, Germany) from Beijing Green Herbs Science and Technology. All analytical-grade reagents including methanol, ethanol, acetone, ethyl acetate, chloroform, and dichloromethane were purchased from Xilong Chemical Co., Ltd. The 5% sulfuric acid-ethanol developing reagent was prepared in-house.

Methods

Dried *S. ilicifolius* bark (20 kg) was pulverized into coarse powder and extracted four times with 75% ethanol (80 L each time) by heating reflux at 70°C after overnight soaking. The combined extracts were concentrated under reduced

pressure to remove ethanol, yielding 1.8 kg of crude ethanol extract. The extract was suspended in water and successively partitioned with ethyl acetate and n-butanol to obtain the ethyl acetate fraction (523.8 g) and n-butanol fraction (374.6 g).

The n-butanol fraction (374.6 g) was mixed with an equal amount of silica gel (200-300 mesh), thoroughly blended, and dried. Gradient elution with dichloromethane-methanol (v/v 100:0 to 1:1) on a silica gel column yielded seven fractions (Fr.1-Fr.7). Fraction Fr.3 (28.3 g) was mixed with RP-C18 support and subjected to RP-C18 column chromatography with a methanol-water gradient (v/v 5:95 to 100:0) to afford eight subfractions (Fr.3-1 to Fr.3-8).

Fr.3-3 was further purified by RP-C18 column chromatography using methanol-water (v/v 5:95 to 50:50) gradient elution to obtain eight components (Fr.3-3-1 to Fr.3-3-8). Fr.3-3-1 was passed through Sephadex LH-20 with methanol-water (v/v 20:80) as mobile phase, followed by semi-preparative HPLC with methanol-water (v/v 20:80) to yield compound 1 (3.8 mg). Compound 2 (4.6 mg) was obtained from Fr.3-3-1 after Sephadex LH-20 separation (methanol) and semi-preparative HPLC purification with methanol-water (v/v 30:70). Compound 4 (3.7 mg) was isolated from Fr.3-3-8 by semi-preparative HPLC using methanol-water (v/v 28:72) as eluent.

Fr.3-4 was separated into six fractions (Fr.3-4-1 to Fr.3-4-6) by RP-C18 column chromatography. Fr.3-4-2 yielded compound 3 (5.1 mg) through semi-preparative HPLC with methanol-water (v/v 20:80). Fr.3-5 was initially separated on Sephadex LH-20 with methanol to give six fractions (Fr.3-5-1 to Fr.3-5-6). Fr.3-5-3 was further purified by Sephadex LH-20 gradient elution with methanol-water (v/v 10:90 to 80:20), followed by semi-preparative HPLC with methanol-water (v/v 18:82) to afford compound 5 (3.4 mg). Fr.3-5-5 was subjected to Sephadex LH-20 gradient elution with methanol-water (v/v 10:90 to 80:20) to obtain five subfractions, from which Fr.3-5-5-2 and Fr.3-5-5-3 yielded compounds 13 (3.5 mg) and 6 (3.0 mg), respectively, via semi-preparative HPLC with methanol-water (v/v 16:84).

Fr.4 was separated by silica gel column chromatography using a dichloromethane-methanol gradient (v/v 20:1 to 1:1) to give five fractions (Fr.4-1 to Fr.4-5). Fr.4-2 was chromatographed on Sephadex LH-20 with methanol to obtain seven subfractions (Fr.4-2-1 to Fr.4-2-7). Fr.4-2-2 was further separated on Sephadex LH-20 with dichloromethane-methanol (v/v 1:1) to yield seven portions (Fr.4-2-2-1 to Fr.4-2-2-7). Compounds 8 (3.4 mg), 9 (3.5 mg), and 10 (4.1 mg) were obtained from Fr.4-2-2-2, Fr.4-2-2-3, and Fr.4-2-2-5, respectively, by semi-preparative HPLC with acetonitrile-water (v/v 8:92). Fr.4-2-5 was purified on Sephadex LH-20 with methanol-water (v/v 30:70), followed by semi-preparative HPLC with methanol-water (v/v 16:84) to give compound 12 (2.8 mg). Fr.4-4 was separated on Sephadex LH-20 with methanol to afford five fractions (Fr.4-4-1 to Fr.4-4-5). Fr.4-4-2 was subjected to Sephadex LH-20 gradient elution with methanol-water (v/v 10:90 to 80:20) to yield four subfractions (Fr.4-4-2-1 to Fr.4-4-2-4), from which Fr.4-4-2-2 gave compound 7

(4.3 mg) by semi-preparative HPLC with methanol-water (v/v 18:82). Fr.4-4-3 was purified on Sephadex LH-20 with methanol-water (v/v 10:90 to 80:20) to obtain five fractions (Fr.4-4-3-1 to Fr.4-4-3-5). Fr.4-4-3-1 yielded compounds 11 (2.9 mg) and 14 (4.0 mg) via semi-preparative HPLC with methanol-water (v/v 16:84). Fr.4-3 was combined and separated on Sephadex LH-20 with methanol-water gradient (v/v 10:90 to 80:20) to give six portions (Fr.4-3-1 to Fr.4-3-6). Fr.4-3-2 afforded compound 15 (6.6 mg) by semi-preparative HPLC with methanol-water (v/v 18:82), while Fr.4-3-3 yielded compound 16 (3.1 mg) using methanol-water (v/v 10:90).

Structural Identification of Compounds

Compound 1 was obtained as an amorphous powder. HR-ESI-MS m/z: 545.1993 $[M + Na]^+$. ^{1}H-NMR (400 MHz, CD_3OD) δH 6.58 (1H, d, J = 8.0 Hz, H-2), 6.56 (1H, d, J = 1.9 Hz, H-5), 6.47 (1H, dd, J = 8.0, 1.9 Hz, H-6), 2.96 (1H, dd, J = 13.8, 5.6 Hz, H-7a), 2.72 (1H, dd, J = 13.8, 9.4 Hz, H-7b), 3.95 (1H, d, J = 6.7, 2.7 Hz, H-8a), 3.76 (1H, m, H-9a), 3.65 (1H, m, H-9b), 6.93 (1H, d, J = 1.9 Hz, H-2), 6.91 (1H, d, J = 2.0 Hz, H-6), 6.54 (1H, dd, J = 16.0 Hz, H-7), 6.31 (1H, dd, J = 16.0 Hz, H-8), 4.22 (1H, d, J = 5.6, 1.6 Hz, H-9 a), 4.67 (1H, dd, J = 7.3, H-1"), 3.43 (1H, m, H-2"), 3.39 (1H, m, H-3"), 3.36 (1H, m, H-4"), 3.11 (1H, m, H-5"), 3.79 (1H, d, J = 1.9, H-6" a), 3.65 (1H, d, J = 1.9, H-6" b), 3.68 (3H, s, 3-OCH_3), 3.82 (3H, s, 3 -OCH_3); ^{13}C-NMR (100 MHz, CD_3OD) δC 133.16 (C-1), 115.61 (C-2), 148.37 (C-3), 145.32 (C-4), 113.68 (C-5), 122.55 (C-6), 39.13 (C-7), 42.77 (C-8), 66.80 (C-9), 135.35 (C-1), 109.03 (C-2), 153.42 (C-3), 144.95 (C-4), 138.91 (C-5), 119.09 (C-6), 131.46 (C-7), 129.62 (C-8), 63.65 (C-9), 105.31 (C-1"), 75.91 (C-2"), 78.04 (C-3"), 71.20 (C-4"), 77.82 (C-5"), 62.40 (C-6"), 56.32 (3-OCH_3), 56.20 (3 -OCH_3). These data were consistent with literature values (Lee et al., 2009), leading to the identification of compound 1 as icariside E5.

Compound 2 was isolated as a light yellow oil. HR-ESI-MS m/z: 443.1676 $[M + Na]^+$. ^{1}H-NMR (400 MHz, CD_3OD) δH 6.68 (1H, d, J = 2.5 Hz, H-2), 6.64 (1H, d, J = 1.9 Hz, H-5), 6.58 (1H, dd, J = 3.6, 1.9 Hz, H-6), 2.70 (1H, dd, J = 13.7, 7.8 Hz, H-7a), 2.59 (1H, m, H-7b), 2.08 (1H, dd, J = 7.8, 4.3 Hz, H-8), 3.89 (1H, m, H-9a), 3.54 (1H, m, H-9b), 6.66 (1H, d, J = 2.5 Hz, H-2), 6.62 (1H, d, J = 1.9 Hz, H-5), 6.56 (1H, dd, J = 3.6, 1.9 Hz, H-6), 2.61 (2H, m, H-7), 2.00 (1H, dd, J = 8.2, 5.1 Hz, H-8), 3.65 (1H, m, H-9 a), 3.57 (1H, dd, J = 5.8, 2.7, H-9 b), 4.19 (1H, d, J = 7.8 Hz, H-1"), 3.21 (1H, m, H-2"), 3.33 (1H, m, H-3"), 3.33 (1H, m, H-4"), 3.28 (1H, m, H-5"), 3.86 (1H, m, H-6" a), 3.68 (1H, d, J = 5.2 Hz, H-6" b), 3.75 (6H, s, 3, 3 -OCH_3); ^{13}C-NMR (100 MHz, CD_3OD) δC 133.98 (C-1), 113.52 (C-2), 148.80 (C-3), 145.41 (C-4), 115.74 (C-5), 122.77 (C-6), 35.35 (C-7), 41.56 (C-8), 70.38 (C-9), 133.94 (C-1), 113.35 (C-2), 148.75 (C-3), 145.39 (C-4), 115.74 (C-5), 122.71 (C-6), 104.62 (C-1"), 78.15 (C-2"), 77.95 (C-3"), 71.67 (C-4"), 75.20 (C-5"), 62.72 (C-6"), 56.30 (3, 3 -OCH_3). These data matched literature values (Jiang et al., 2018), identifying compound 2 as secoisolariciresinol 9-O-β-glucopyranoside.

Compound 3 was obtained as a white powder. HR-ESI-MS m/z: 369.1156 [M + Na]$^+$. ^{1}H-NMR (400 MHz, CD_3OD) δH 6.49 (2H, s, H-3,5), 4.81 (1H, d, J = 7.2, H-1), 3.33 (2H, m, H-2 ,3), 3.44 (2H, m, H-4 ,5), 3.92 (1H, dd, J = 12.0, 2.3, H-6 a), 3.66 (1H, dd, J = 12.0, 6.7, H-6 b); ^{13}C-NMR (100 MHz, CD_3OD) δC 134.36 (C-1), 154.80 (C-2,6), 96.02 (C-3,5), 156.10 (C-4), 103.20 (C-1), 74.95 (C-2), 78.08 (C-3), 71.71 (C-4), 78.45 (C-5), 62.73 (C-6), 56.52 (2,6-OCH_3), 61.23 (4-OCH_3). These data were consistent with literature values (Chang et al., 2013), identifying compound 3 as 2,4,6-trimethoxyphenol-1-O-β-D-glucoside.

Compound 4 was isolated as a light yellow powder. HR-ESI-MS m/z: 297.1333 [M + H]$^+$. ^{1}H-NMR (400 MHz, CD_3OD) δH 7.40 (2H, d, J = 7.6 Hz, H-2,6), 7.29 (2H, d, J = 8.5 Hz, H-3,5), 7.21 (1H, m, H-4), 6.72 (1H, d, J = 16.3 Hz, H-7), 6.15 (1H, dd, J = 16.3, 8.1 Hz, H-8), 4.35 (1H, d, J = 7.9, H-1); ^{13}C-NMR (100 MHz, CD_3OD) δC 137.76 (C-1), 126.58 (C-2,6), 127.72 (C-3,5), 128.99 (C-4), 135.15 (C-7), 128.63 (C-8), 71.69 (C-9), 100.89 (C-1), 78.06 (C-2), 77.95 (C-3), 775.01 (C-4), 75.98 (C-5), 62.83 (C-6). These data matched literature values (Abd-ellah et al., 2014), identifying compound 4 as 9-O-β-glucopyranosyl trans-cinnamyl alcohol.

Compound 5 was obtained as a colorless oil. HR-ESI-MS m/z: 479.1759 [M + H]$^+$. ^{1}H-NMR (400 MHz, CD_3OD) δH 6.46 (2H, s, H-2,6), 4.80 (1H, d, J = 7.1 Hz, H-1), 3.44 (2H, m, H-2 ,3), 3.35 (1H, m, H-4), 3.59 (1H, m, H-5), 4.04 (1H, d, J = 9.3, 4.8 Hz, H-6 a), 3.59 (1H, m, H-6 b), 4.97 (1H, d, J = 2.7 Hz, H-1"), 3.88 (1H, d, J = 2.6 Hz, H-2"), 3.59 (1H, m, H-3"), 3.95 (1H, d, J = 9.7 Hz, H-4" a), 3.74 (1H, d, J = 9.7 Hz, H-4" b), 3.55 (2H, m, H-5"), 3.82 (6H, s, 3,5-OCH_3), 3.71 (3H, s, 4-OCH_3); ^{13}C-NMR (100 MHz, CD_3OD) δC 134.59 (C-1), 96.31 (C-2,6), 154.80 (C-3,5), 155.95 (C-4), 103.17 (C-1), 74.87 (C-2), 77.95 (C-3), 71.58 (C-4), 77.00 (C-5), 68.74 (C-6), 110.84 (C-1"), 77.93 (C-2"), 80.48 (C-3"), 74.91 (C-4"), 65.34 (C-5"), 56.30 (3,5-OCH_3), 56.20 (4-OCH_3). These data were consistent with literature values (Kanchanapoom et al., 2002), identifying compound 5 as 3,4,5-trimethoxyphenyl-1-O-β-apiofuranosyl-(1" $→6′)− $-glucopyranoside.

Compound 6 was isolated as a white amorphous powder. HR-ESI-MS m/z: 355.0999 [M + Na]$^+$. ^{1}H-NMR (400 MHz, CD_3OD) δH 6.28 (1H, d, J = 2.8 Hz, H-2), 6.34 (1H, d, J = 2.7 Hz, H-6), 4.78 (1H, d, J = 7.2 Hz, H-1), 3.48-3.33 (4H, m, H-2 ,3 ,4 ,5), 3.91 (1H, dd, J = 12.0, 2.2 Hz, H-6 a), 3.69 (1H, dd, J = 12.0, 5.7 Hz, H-6 b), 3.72 (3H, s, 4-OCH_3), 3.80 (3H, s, 5-OCH_3); ^{13}C-NMR (100 MHz, CD_3OD) δC 155.85 (C-1), 98.70 (C-2), 151.90 (C-3), 133.21 (C-4), 154.90 (C-5), 94.81 (C-6), 102.90 (C-1), 74.90 (C-2), 78.03 (C-3), 71.48 (C-4), 78.23 (C-5), 62.59 (C-6), 61.11 (4-OCH_3), 56.35 (5-OCH_3). These data matched literature values (Takara et al., 2002), identifying compound 6 as 3-hydroxy-4,5-dimethoxyphenyl-β-D-glucopyranoside.

Compound 7 was obtained as a white amorphous powder. HR-ESI-MS m/z: 355.0999 [M + Na]$^+$. ^{1}H-NMR (400 MHz, CD_3OD) δH 6.13 (2H, s, H-3,5), 4.67 (1H, d, J = 7.1 Hz, H-1), 3.21 (1H, m, H-2), 3.48-3.33 (3H, m, H-3 ,4 ,5), 3.81 (1H, d, J = 2.1 Hz, H-6 a), 3.69 (1H, d, J = 2.1, 5.0 Hz, H-6 b), 3.79 (6H,

s, 2,6-OCH_3); ^{13}C-NMR (100 MHz, CD_3OD) δC 129.57 (C-1), 154.74 (C-2,6), 94.47 (C-3,5), 156.01 (C-4), 106.19 (C-1), 75.70 (C-2), 77.79 (C-3), 71.29 (C-4), 78.26 (C-5), 62.58 (C-6), 56.76 (2,6-OCH_3). These data were consistent with literature values (Ishimaru et al., 1990), identifying compound 7 as 2,6-dimethoxy-4-hydroxyphenol-1-O-β-D-glucopyranoside.

Compound 8 was isolated as a white amorphous powder. HR-ESI-MS m/z: 303.1074 $[M + H]^+$. ^{1}H-NMR (400 MHz, CD_3OD) δH 6.47 (1H, d, J = 2.7 Hz, H-2), 7.01 (1H, d, J = 8.7 Hz, H-5), 6.30 (1H, dd, J = 8.7, 2.7 Hz, H-6), 4.70 (1H, d, J = 7.8 Hz, H-1), 3.46-3.32 (3H, m, H-2 ,3 ,4 ,5), 3.86 (1H, dd, J = 12.0, 2.4 Hz, H-6 a), 3.69 (1H, dd, J = 12.0, 5.5 Hz, H-6 b), 3.81 (3H, s, 3-OCH_3); ^{13}C-NMR (100 MHz, CD_3OD) δC 141.02 (C-1), 151.98 (C-2), 101.23 (C-3), 154.91 (C-4), 107.55 (C-5), 120.46 (C-6), 104.28 (C-1), 75.05 (C-2), 78.12 (C-3), 71.35 (C-4), 77.81 (C-5), 62.35 (C-6), 56.76 (3-OCH_3). These data matched literature values (Liu et al., 2014), identifying compound 8 as isotachioside.

Compound 9 was obtained as a white powder. HR-ESI-MS m/z: 381.1156 $[M + Na]^+$. ^{1}H-NMR (400 MHz, CD_3OD) δH 6.99 (1H, d, J = 1.9 Hz, H-2), 6.78 (1H, d, J = 8.1 Hz, H-5), 6.86 (1H, dd, J = 8.1, 1.9 Hz, H-6), 4.45 (1H, d, J = 9.5 Hz, H-7), 3.80 (1H, ddd, J = 9.6, 5.2, 2.3 Hz, H-8), 3.45-3.34 (2H, m, H-9), 4.60 (1H, d, J = 7.7 Hz, H-1), 3.15 (1H, dd, J = 9.7, 7.7 Hz, H-2), 3.58 (1H, t, J = 9.1 Hz, H-3), 3.48 (1H, ddd, J = 11.5, 5.7, 2.5 Hz, H-4), 3.45-3.34 (1H, m, H-5), 3.91 (1H, dd, J = 11.9, 2.2 Hz, H-6 a), 3.73 (1H, dd, J = 11.9, 5.6 Hz, H-6 b); ^{13}C-NMR (100 MHz, CD_3OD) δC 130.11 (C-1), 112.28 (C-2), 148.96 (C-3), 148.02 (C-4), 116.03 (C-5), 121.85 (C-6), 80.22 (C-7), 82.67 (C-8), 62.08 (C-9), 99.79 (C-1), 80.75 (C-2), 75.07 (C-3), 71.85 (C-4), 79.79 (C-5), 62.55 (C-6), 56.40 (4-OCH_3). These data were consistent with literature values (Wang et al., 2017), identifying compound 9 as ficuscarpanoside A.

Compound 10 was isolated as a white crystalline substance. HR-ESI-MS m/z: 245.0786 $[M + H]^+$. ^{1}H-NMR (400 MHz, CD_3OD) δH 5.70 (1H, d, J = 8.1 Hz, H-2,6), 8.02 (1H, d, J = 7.1 Hz, H-6), 5.90 (1H, d, J = 4.7 Hz, H-1), 4.18 (1H, d, J = 5.0 Hz, H-2), 4.15 (1H, d, J = 4.9 Hz, H-3), 4.01 (1H, d, J = 4.0 Hz, H-4), 3.84 (1H, dd, J = 12.3, 2.8 Hz, H-5 a), 3.73 (1H, dd, J = 12.3, 3.2 Hz, H-5 b); ^{13}C-NMR (100 MHz, CD_3OD) δC 152.47 (C-2), 166.21 (C-4), 102.64 (C-5), 142.73 (C-6), 90.64 (C-1), 71.31 (C-2), 75.73 (C-3), 86.37 (C-4), 62.26 (C-5). These data matched literature values (Ma et al., 2010), identifying compound 10 as uridine.

Compound 11 was obtained as colorless crystals. HR-ESI-MS m/z: 375.1286 $[M + H]^+$. ^{1}H-NMR (400 MHz, CD_3OD) δH 7.35 (2H, s, H-2,6), 5.08 (1H, d, J = 7.5, H-1), 3.40 (1H, m, H-2), 3.22 (1H, ddd, J = 9.5, 5.5, 2.4 Hz, H-3), 3.40 (1H, m, H-4), 3.49 (1H, m, H-5), 3.77 (1H, dd, J = 12.0, 2.4 Hz, H-6 a), 3.65 (1H, dd, J = 12.0, 5.3 Hz, H-6 a), 3.90 (6H, s, 3,5-OCH_3), 3.89 (3H, s, 7-OCH_3); ^{13}C-NMR (100 MHz, CD_3OD) δC 127.07 (C-1), 108.40 (C-2,6), 154.19 (C-3,5), 140.23 (C-4), 168.03 (C=O), 104.44 (C-1), 78.45 (C-2), 71.34 (C-3), 75.70 (C-4), 77.86 (C-5), 62.52 (C-6), 57.05 (3,5-OCH_3), 52.78 (4-OCH_3). These data were consistent with literature values (Fujimatu et al., 2003), identifying

compound 11 as methyl syringate 4-O-β-D-glucopyranoside.

Compound 12 was isolated as a white powder. HR-ESI-MS m/z: 369.1105 $[M + Na]^+$. ^{1}H-NMR (600 MHz, CD_3OD) δH 6.46 (2H, s, H-2,6), 4.81 (1H, m, J = 7.8 Hz, H-1), 3.92 (1H, dd, J = 12.0, 2.2 Hz, H-6 a), 3.81 (6H, s, 3,5-OCH_3), 3.70 (3H, s, 4-OCH_3); ^{13}C-NMR (150 MHz, CD_3OD) δC 156.06 (C-1), 96.05 (C-2,6), 154.79 (C-3,5), 134.38 (C-4), 103.18 (C-1), 74.93 (C-2), 78.42 (C-3), 71.69 (C-4), 78.06 (C-5), 62.72 (C-6), 56.52 (3,5-OCH_3), 61.21 (4-OCH_3). These data matched literature values (Xuan et al., 2006), identifying compound 12 as 3,4,5-trimethoxyphenyl-β-D-glucopyranoside.

Compound 13 was obtained as a yellow powder. ^{1}H-NMR (400 MHz, CD_3OD) δH 6.53 (1H, s, H-3), 6.20 (1H, d, J = 2.1 Hz, H-6), 6.43 (1H, d, J = 2.1 Hz, H-8), 7.37 (1H, s, H-2), 6.90 (1H, d, J = 8.5 Hz, H-5), 7.39 (1H, d, J = 2.2 Hz, H-6). These data were consistent with literature values (Liu et al., 2018), identifying compound 13 as luteolin.

Compound 14 was isolated as a white powder. HR-ESI-MS m/z: 615.3867 $[M + Na]^+$. ^{1}H-NMR (400 MHz, CD_3OD) δH 1.00 (3H, s, H-18), 1.00 (3H, s, H-19), 1.62 (3H, s, H-21), 1.10 (3H, s, H-26), 1.34 (3H, s, H-27), 1.68 (3H, s, H-28), 1.33 (3H, s, H-29), 0.95 (3H, s, H-30), 4.35 (1H, d, J = 7.8 Hz, H-1”), 4.81 (1H, d, J = 7.8 Hz, H-1); ^{13}C-NMR (100 MHz, CD_3OD) δC 40.17 (C-1), 25.57 (C-2), 77.63 (C-3), 40.47 (C-4), 61.74 (C-5), 80.92 (C-6), 42.25 (C-7), 41.84 (C-8), 50.57 (C-9), 40.34 (C-10), 31.37 (C-11), 71.15 (C-12), 50.57 (C-13), 52.42 (C-14), 30.93 (C-15), 27.23 (C-16), 53.10 (C-17), 17.63 (C-18), 17.82 (C-19), 84.90 (C-20), 22.83 (C-21), 36.61 (C-22), 24.22 (C-23), 125.83 (C-24), 132.28 (C-25), 25.89 (C-26), 17.96 (C-27), 31.51 (C-28), 16.10 (C-29), 17.11 (C-30), 105.54 (C-1), 75.45 (C-2), 79.82 (C-3), 71.84 (C-4), 78.18 (C-5), 62.50 (C-6), 98.26 (C-1”), 75.35 (C-2”), 79.04 (C-3”), 71.66 (C-4”), 77.90 (C-5”), 62.88 (C-6”). These data matched literature values (Yang et al., 2018), identifying compound 14 as ginsenoside Rg1.

Compound 15 was obtained as a light yellow oil. HR-ESI-MS m/z: 465.1755 $[M + H]^+$. ^{1}H-NMR (400 MHz, CD_3OD) δH 2.63 (1H, dd, J = 15.1 Hz, H-1a), 2.74 (1H, dd, J = 15.1, 4.8 Hz, H-1b), 1.72 (1H, m, H-2), 2.08 (1H, m, H-3), 4.42 (1H, d, J = 6.2 Hz, H-4), 6.58 (1H, s, H-8), 3.65 (1H, dd, J = 5.2, 11.8 Hz, H-11a), 3.55 (1H, dd, J = 10.9, 6.6 Hz, H-11b), 3.90 (1H, dd, J = 9.8, 4.4 Hz, H-12a), 3.45 (1H, dd, J = 4.1, 9.8 Hz, H-12b), 6.43 (2H, s, H-2 ,6), 4.28 (1H, d, J = 7.7, H-1”), 3.24 (1H, m, H-2”), 3.45 (1H, m, H-3”), 3.38 (1H, m, H-4”), 3.24 (1H, m, H-5”), 3.65 (1H, dd, H-6” a), 3.83 (1H, dd, H-6” b), 3.34 (3H, s, 5-OCH_3), 3.86 (1H, s, 7-OCH_3), 3.75 (6H, s, 3 ,5 -OCH_3); ^{13}C-NMR (100 MHz, CD_3OD) δC 34.9 (C-1), 41.02 (C-2), 47.15 (C-3), 43.24 (C-4), 148.01 (C-5), 139.78 (C-6), 149.42 (C-7), 108.26 (C-8), 130.62 (C-9), 126.87 (C-10), 66.64 (C-11), 71.86 (C-12), 139.35 (C-1), 107.32 (C-2 ,6), 149.07 (C-3 ,5), 134.90 (C-4), 105.28 (C-1”), 75.62 (C-2”), 78.68 (C-3”), 72.10 (C-4”), 78.39 (C-5”), 63.27 (C-6”), 60.60 (5-OCH_3), 57.02 (7-OCH_3), 57.28 (3 ,5 -OCH_3). These data were consistent with literature values (Balázs et al., 2002), identifying compound 15 as (+)-lyonirenisol-3α-O-β-D-glucopyranoside.

Compound 16 was isolated as a yellow amorphous powder. HR-ESI-MS m/z: 611.1606 $[M + H]^+$. ^{1}H-NMR (400 MHz, CD_3OD) δH 6.21 (d, J = 2.0 Hz, 1H, H-6), 6.40 (d, J = 2.0 Hz, 1H, H-8), 7.66 (d, J = 2.1 Hz, 1H, H-2), 6.87 (d, J = 8.5 Hz, 1H, H-5), 7.62 (dd, J = 2.1, 8.5 Hz, 1H, H-6), 5.10 (d, J = 7.7 Hz, 1H, H-1), 3.46 (dd, J = 7.7, 8.9 Hz, 1H, H-2), 3.40 (t, J = 8.9 Hz, 1H, H-3), 3.26 (t, J = 8.9 Hz, 1H, H-4), 3.32 (ddd, J = 1.2, 6.1, 8.9 Hz, 1H, H-5), 3.80 (dd, J = 1.2, 11.0 Hz, 1H, H-6a), 3.38 (dd, J = 6.1, 11.0 Hz, 1H, H-6b), 4.51 (d, J = 1.5 Hz, 1H, H-1), 3.62 (dd, J = 1.5, 3.4 Hz, 1H, H-2), 3.53 (dd, J = 3.4, 9.6 Hz, 1H, H-3), 3.27 (t, J = 9.6 Hz, 1H, H-4), 3.44 (dq, J = 6.2, 9.6 Hz, 1H, H-5), 1.11 (d, J = 6.2 Hz, 3H, H-6). ^{13}C-NMR (100 MHz, CD_3OD) δC 179.4 (C-4), 166.1 (C-7), 162.9 (C-5), 159.4 (C-9), 158.5 (C-2), 149.8 (C-4), 145.9 (C-3), 135.6 (C-3), 123.5 (C-6), 123.1 (C-1), 117.7 (C-2), 116.1 (C-5), 105.6 (C-10), 104.7 (C-1), 102.4 (C-1), 99.9 (C-6), 94.9 (C-8), 78.2 (C-3), 77.2 (C-5), 75.7 (C-2), 73.9 (C-4), 72.2 (C-3), 72.1 (C-2), 71.4 (C-4), 69.7 (C-5), 68.5 (C-6), 17.9 (C-6). These data were consistent with literature values (Kohei et al., 2003), identifying compound 16 as myricetin 3-neohesperidoside.

Discussion and Conclusion

This study investigated the chemical constituents of the n-butanol fraction from *Streblus ilicifolius* bark, leading to the isolation and identification of sixteen compounds for the first time from this plant species. The identified compounds encompass phenylpropanoids, flavonoids, saponins, and phenolic glycosides, with phenolic glycosides being the predominant class. Literature reports indicate that icariside E5 (1) exhibits DPPH radical scavenging activity with an IC_{50} value of 42.1 $mol \cdot L^{-1}$, demonstrating significant antioxidant potential. Luteolin (13) possesses antitumor, antioxidant, and anti-inflammatory properties, while ginsenoside Rg1 (14) shows anti-fatigue, anti-aging, angiogenic-promoting, and vascular protective effects. This research represents the first comprehensive chemical investigation of *S. ilicifolius*, enriching our understanding of its material basis and filling a gap in the phytochemical knowledge of this species while expanding the chemical diversity known for the genus *Streblus*. Pharmacological studies on these compounds are currently underway to identify promising lead compounds with potent biological activities, which may provide new sources for drug discovery and offer a theoretical foundation for the development and utilization of *Streblus* plants.

References

ABD-ELLAH AE, MOHAMED KM, BACKHEET EY, et al., 2014. Cinnamyl alcohol, benzyl alcohol, and flavonoid glycosides from *Sanchezia nobilis* [J]. Chem Nat Comp, 50(5): 823-826.

BALÁZS B, TÓTH G, DUDDECK H, et al., 2006. Iridoid and lignan glycosides from *Citharexylum spinosum* L. [J]. Nat Prod Res, 20(2): 201-205.

CHANG RJ, WANG CH, ZENG Q, et al., 2013. Chemical constituents of the

stems of *Celastrus rugosus* [J]. Arch Pharm Res, 36: 1291-1301.

CHEN JM, QIN YN, CANG HD, 1983. Analgesic and sedative effects of *Pseudostreblus indica* Bur. [J]. J Xuzhou Med Univ, 4: 20-22.

Editorial Committee of Flora of China Chinese Academy of Sciences, 1998. *Flora Reipublicae Popularis Sinicae* [M]. Beijing: Science Press, 23: 38-41.

FUJIMATU E, ISHIKAWA T, KITAJIMA J, 2003. Aromatic compound glucosides, alkyl glucoside and glucide from the fruit of anise [J]. Phytochemistry, 63: 609-616.

HE RJ, ZHANG YJ, WU LD, et al., 2017. Benzofuran glycosides and coumarins from the bark of *Streblus indicus* (Bur.) Corner [J]. Phytochemistry, 138: 170-177.

HUANG JG, LI J, WU Q, et al., 2012. Constituents from heartwood of *Streblus asper* and their antibacterial activity [J]. Nat Prod Res Dev, 24: 780-783.

ISHIMARU K, SUDO H, SATAKE M, et al., 1990. Phenyl glucosides from a hairy root culture of *Swertia japonica* [J]. Phytochemistry, 29(12): 3823-3825.

JIANG H, HUANG CW, LIAO HB, et al., 2018. Two new lignan glucosides from caulis of *Tinospora sinensis* [J]. Chin Trad Herb Drugs, 49(10): 2334-2344.

KANCHANAPOOM T, KASAIA R, YAMASAKIA K, et al., 2002. Iridoid and phenolic glycosides from *Morinda coreia* [J]. Phytochemistry, 59: 551-556.

KOHEI K, NAONOBU N, MASAHIKO S, 2003. Malonylated flavonol glycosides from the petals of *Clitoria ternatea* [J]. Phytochemistry, 62: 229-237.

LEE DY, LEE DG, CHO JG, et al., 2009. Lignans from the fruits of the red pepper (*Capsicum annuum* L.) and their antioxidant effects [J]. Arch Pharm Res, 32(10): 1345-1349.

LIANG CQ, DAI ZK, WANG Z, et al., 2010. Anticancer effect of petroleum ether extract from leaves of *Streblus asper* Lour and its components [J]. J Anhui Agric Sci, 38(8): 2340-2341.

LI C, HUANG CP, LU TL, et al., 2014. Tandem mass spectrometric fragmentation behavior of lignans, flavonoids and triterpenoids in *Streblus asper* [J]. Rapid Comm Mass Sp, 28: 2363-2370.

LI J, MENG AP, GUAN XL, et al., 2013. Anti-hepatitis B virus lignans from the root of *Streblus asper* [J]. Bioorg Med Chem Lett, 23: 2238-2244.

LI J, TANG MT, WU Q, et al., 2012. Water-soluble constituents of the heartwood of *Streblus asper* [J]. Nat Prod Comm, 7(5): 599-602.

LI LQ, LI J, HUANG Y, et al., 2012. Lignans from the heartwood of *Streblus asper* and their inhibiting activities against hepatitis B virus [J]. Fitoterapia, 83: 303-309.

LIU WB, DONG LM, LUO B, et al., 2018. Chemical constituents from the leaves of *Cyclocarya paliurus* [J]. J Trop Subtrop Bot, 26(3): 317-322.

LIU YF, LIANG D, LUO H, et al., 2014. Chemical constituents from root tubers of *Rehmannia glutinosa* [J]. Chin Trad Herb Drugs, 45(1): 16-22.

MA YT, QIAO LR, SHI WQ, et al., 2010. Metabolites produced by an endophyte *Alternaria alternata* isolated from *Maytenus hookeri* [J]. Chem Nat Comp, 46(3): 504-506.

PRAKASH K, DEEPAK D, KHARE A, et al., 1992. A pregnane glycoside from *Streblus asper* [J]. Phytochemistry, 31(3): 1056-1057.

REN YL, CHEN WL, LANTVIT DD, et al., 2017. Cardiac glycoside constituents of *Streblus asper* with potential antineoplastic activity [J]. J Nat Prod, 80: 648-658.

SINGH SP, SINGH AP, SINGH R, et al., 2015. A brief study on *Strebulus asper* L. [J]. Res J Phytomed, 1(2): 1-5.

TAKARA K, MATSUI D, WADA K, et al., 2002. New antioxidative phenolic glycosides isolated from Kokuto non-centrifuged cane sugar [J]. Biol Biotechnol Biochem, 66(1): 29-35.

WANG JS, HE Y, ZHANG WJ, et al., 2013. Advances in studies on pharmacological effects of luteolin [J]. Chin Bull Life Sci, 25(6): 560–565.

WANG LY, CHEN MH, WU J, et al., 2017. Bioactive glycosides from the twigs of *Litsea cubeba* [J]. J Nat Prod, 80: 1808-1818.

XUAN WD, CHEN HS, BIAN J, 2006. A new indole alkaloid glycoside from stem of *Nauclea officinalis* [J]. Acta Pharm Sin, 41(11): 1064-1067.

YANG BY, YANG CL, LIU Y, et al., 2018. Chemical constituents from roots of *Datura metel* [J]. Chin J Chin Mat Med, 43(8): 1654-1661.

YANG YT, YANG Y, XIAO JM, et al., 2021. Research advances on the effect of ginsenoside Rg1 on angiogenesis and vascular protection [J]. Acta Pharm Sin, 56(8): 2146-2153.

ZHANG GR, HUANG XS, HUANG Y, et al., 2021. Research progress on chemical constituents and pharmacological activities of plants from *Streblus* [J]. Chin Trad Herb Drugs, 52(19): 6066-6075.

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