Study on Active Components from the Stems of *Polyalthia plagioneura* (II)

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Abstract [**Objectives**] This study was conducted to isolate and identify the components from stems of *Polyalthia plagioneura*. [**Methods**] The compounds were isolated and purified by silica gel column, Sephadex LH-20, and C_{18} chromatography. Their chemical structures were elucidated on the basis of physicochemical properties and spectral data. [**Results**] Five compounds were isolated and identified as: di (2-ethylhexyl) phthalate (1), cinnamic anhydride (2), phthalic acid (3), citric acid (4), and syringaldehyde (5). [**Conclusions**] All compounds were isolated from this plant for the first time.

Key words *Polyalthia plagioneura*; Chemical component; Steroid

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Polyalthia plagioneura, an endemic plant to China, belongs to the genus *Polyalthia* within the Annonaceae family. It is distributed in Yunnan, Guangdong, southern Guangxi, and Hainan[1]. Previous studies have shown that the main chemical components from plants in *Polyalthia* include alkaloids[2-6] and diterpenoids[7-10], as well as small amounts of flavonoids[11] and substituted furan compounds[12]. All these compounds exhibit significant biological activity[13]. Extracts from the stems and leaves of *P. plagioneura* have been found to significantly inhibit tumor growth in S-180 tumor-bearing mice. This effect may be related to enhancing the immune function of the tumor-bearing mice themselves[14-15].

Our research group conducted antitumor activity tests on the stem extract of *P. plagioneura*. The results indicate that the extract exhibit a significant inhibitory effect on the *in-vitro* proliferation of SPCA-1 tumor cells. To further investigate the antitumor active components in the stems of *P. plagioneura*, their chemical composition was analyzed in this study. Using methods such as fraction separation and repeated column chromatography (silica gel, C_{18} reverse-phase silica gel, Sephadex LH-20), five compounds were isolated from the chloroform fraction of the stems of *P. plagioneura*. Based on spectroscopic data, these compounds were identified as di (2-ethylhexyl) phthalate (1), cinnamic anhydride (2), phthalic acid (3), citric acid (4), and syringaldehyde (5). All compounds were isolated from this genus for the first time.

Materials and Methods

Instruments and reagents

The used instruments included Bruker AV400 MHz superconducting nuclear magnetic resonance spectrometer (Bruker, Switzerland), high-performance liquid chromatography system (Elite), XT5-10 micro melting point apparatus (temperature uncorrected), 4001N electronic balance, SR5200LH ultrasonic cleaner, ultraviolet analysis dark box (YOZO-KX), silica gel H and GF_{254} (Qingdao Marine Chemical Factory), C_{18} reverse-phase silica gel (YMC, Japan), and Sephadex LH-20 gel (Amersham Biosciences). All other reagents were of analytical or chromatographic grade.

Medicinal material

P. plagioneura was collected from the Bawangling Nature Reserve in Changjiang County, Hainan Province, China. The botanical identity of the plant material was authenticated by Professor Zhong Qiongxin from the Department of Biology, Hainan Normal University, as *P. plagioneura*. A specimen is preserved at the Key Laboratory of Tropical Medicinal Resource Chemistry of the Ministry of Education, Hainan Normal University.

Experimental methods

The stems of *P. plagioneura* were air-dried in the shade and ground into coarse powder (20 kg). The powder was then extracted four times by refluxing with 75% ethanol. The combined extract was concentrated under reduced pressure to remove the solvent, yielding 520 g of crude extract. Next, the crude extract was suspended in water and successively extracted five times each with chloroform, ethyl acetate, and water-saturated n-butanol. This yielded chloroform-soluble (350 g), ethyl acetate-soluble (52 g), and n-butanol-soluble (61 g) fractions. Compounds 1 - 5 were isolated from the chloroform-soluble fraction through repeated silica gel column chromatography.

Results and Analysis

Structural identification was performed on the isolated compounds 1 - 5 mentioned above.

Compound 1

Compound 1 is a colorless to white solid (in chloroform), readily soluble in chloroform and acetone. The ^{1}H-NMR ($CDCl_3$, 400 MHz) spectrum exhibited symmetry with signals at δ: 7.71

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(1H, dd, J = 5.6, 3.6 Hz), 7.53 (1H, dd, J = 5.6, 3.6 Hz), 4.22 (2H, m), 1.67 (1H, m), 1.42 (2H, m), 1.38 – 1.26 (6H, m), 0.92 (3H, t, J = 7.6 Hz), and 0.90 (3H, t, J = 2.4 Hz). The ^{13}C-NMR ($CDCl_3$, 100 MHz) spectrum showed signals at δ: 167.8 (C-1), 132.5 (C-2), 130.9 (C-3), 128.8 (C-4), 66.2 (C-5), 38.8 (C-6), 30.4 (C-7), 29.0 (C-7a), 23.8 (C-8), 14.1 (C-8a), 23.0 (C-9), and 11.0 (C-10). Based on the spectral data and comparison with literature reports[15], compound 1 was identified as di(2-ethylhexyl) phthalate.

Compound 2

Compound 2 is a white waxy solid, readily soluble in chloroform and acetone. MS: m/z 301 [(M + Na)$^+$], 579 [(2M + Na)$^+$], molecular weight: 278, molecular formula: $C_{18}H_{14}O_3$. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.46 (d, 1H, J = 15.6 Hz), 7.81 (d, 2H, J = 16.0 Hz), 7.56 (dd, 2H, J = 6.0, 1.6 Hz), 7.41 – 7.42 (m, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 117.3 (C-1), 147.0 (C-2), 134.1 (C-3), 128.4 (C-4,8), 129.0 (C-5,7), 132.4 (C-6), 164.9 (C-9). HMBC: HMBC analysis showed correlations: H-1 with C-2, C-3, and C-9; H-2 with C-3 and C-9; H-4 with C-2 and C-3; H-5 with C-3 and C-4; H-6 with C-7 and C-8. These spectral data are consistent with those reported in the literature[16], confirming the compound as 3-phenyl-2-propenoic anhydride.

Compound 3

Compound 3 is a colorless crystal (obtained from methanol), readily soluble in methanol and acetone, and also soluble in chloroform. ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum, low-field signals at δ 8.03 (2H, dd, J = 6.4, 2.8 Hz) and 7.92 (2H, dd, J = 6.4, 2.8 Hz) constitute an AA′BB′ coupling system on a benzene ring, indicating ortho-disubstitution. The fact that each signal corresponds to 2H suggests that the two substituents are identical. The chemical shifts at δ 8.03 and 7.92 are characteristic of a benzene ring directly attached to carboxyl groups. These spectral data are consistent with those reported in the literature[17], confirming the compound as phthalic acid.

Compound 4

Compound 4 was obtained as colorless cluster-like crystals (from methanol). ^{1}H-NMR (400 MHz, CD_3OD) δ: 2.82 (2H, d, J = 15.6 Hz) and 2.95 (2H, d, J = 15.6 Hz). ^{13}C NMR (100 MHz, CD_3OD) δ: 43.9 (C-1), 74.2 (C-2), 43.9 (C-3), 173.6 (1, 3-COOH), and 176.9 (2-COOH). These data are in good agreement with those reported in the literature[18], confirming the structure of the compound as 2-hydroxy-1, 2, 3-propanetricarboxylic acid, commonly known as citric acid.

Compound 5

Compound 5 is a white needle-like crystal (obtained from chloroform), readily soluble in chloroform and acetone. ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz), the signal at δ 9.82 (1H, s) suggests the possible presence of an aldehyde group in the molecule. The signal at δ 7.15 (2H, s) likely corresponds to hydrogen atoms in equivalent chemical environments on a benzene ring, indicating a potentially symmetrical substitution pattern. The signal at δ 3.97 (6H, s) indicates the presence of two methoxy groups in identical chemical environments. Based on the above data and comparison with literature reports, compound 5 was identified as syringaldehyde[19].

Conclusions and Discussion

Five known compounds were isolated from *P. plagioneura*, namely di (2-ethylhexyl) phthalate (1), cinnamic anhydride (2), phthalic acid (3), citric acid (4), and syringaldehyde (5). All compounds were obtained from this genus for the first time.

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(Continued on page 69)

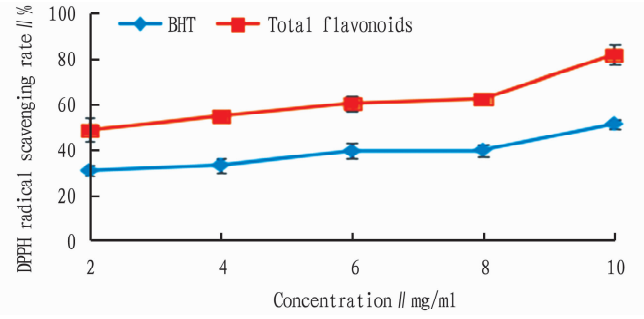

Fig. 6 DPPH radical scavenging activity of total flavonoids from *H. undatus*

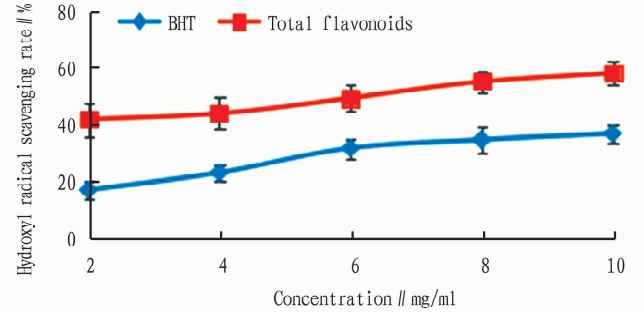

Fig. 7 Hydroxyl radical scavenging activity of total flavonoids from *H. undatus*

Conclusions and Discussion

In this study, *H. undatus* was selected as the raw material to optimize the ultrasonic-assisted extraction process for total flavonoids and investigate their antioxidant activity. The findings provide scientific support for the high-value development and diversified application of flavonoids from this plant. Through single-factor and orthogonal experiments, with the total flavonoid yield as the evaluation criterion, the optimal extraction process was determined as follows: ethanol concentration 75%, extraction temperature 60 ℃, extraction time 30 min, and solid-to-liquid ratio 1∶50 (ml/g). Under these conditions, the total flavonoid yield reached 3.08%. The optimized process significantly reduced the extraction duration while enhancing the flavonoid yield, thereby validating the superiority of ultrasonic-assisted technology in extracting flavonoids from plant materials and providing a feasible reference for potential large-scale production. Antioxidant activity assays revealed that the flavonoid extract obtained under the optimal conditions exhibited superior DPPH and hydroxyl radical scavenging capacities compared with the commonly used antioxidant BHT. This confirms its excellent potential as a natural antioxidant and lays a foundation for subsequent new product development.

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(Continued from page 64)

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