Chemical Composition and Antioxidant Activity of Sugarcane Stem and Leaf: Postprint

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Abstract

To investigate the chemical composition and antioxidant activity of sugarcane (Saccharum officinarum) stems and leaves. The stems and leaves of sugarcane were extracted with methanol, and the extract was separated and purified by column chromatography (SiO2, Sephadex LH-20, Rp-18). The structures of the obtained compounds were identified by mass spectrometry and nuclear magnetic resonance techniques; the free radical scavenging capacity of the compounds was determined by the DPPH method. The results showed that: (1) A total of 22 compounds were isolated and identified from the stems and leaves of sugarcane, namely p-hydroxybenzaldehyde (1), p-methoxycinnamic acid (2), 4-methoxybenzaldehyde (3), vanillin (4), methyl 4-hydroxycinnamate (5), p-hydroxybenzoic acid (6), (2-hydroxyphenyl)(phenyl)methanone (7), p-methylbenzoic acid (8), methyl caffeate (9), aconitic acid A (10), aconitic acid E (11), 5-O-dimethoxycinnamoylquinic acid (12), quercetin (13), quercetin-3-O-α-L-arabinoside (14), quercetin-3-O-β-D-galactopyranoside (15), distearyl thiodipropionate (16), α-conidendrin (17), rel-(2α,3β)-7-O-methylcedrusin (18), methyl 3-O-feruloylquinate (19), luteolin (20), (5S,6S)-5,6-dihydro-3,8,10-trihydroxy-5-(4-hydroxy-3-methoxyphenyl)-6-hydroxymethyl-2,4-dimethoxy-7H-benzo[c]xanthen-7-one (21), and methyl 5-O-feruloylquinate (22). Among them, compounds 2-3, 7-11, 14-19, and 21-22 were isolated from this plant for the first time. (2) The free radical scavenging capacity of 15 compounds with high content (1-9, 11-16) was screened by the DPPH method, among which compound 12 (5-O-dimethoxycinnamoylquinic acid) showed good antioxidant activity (IC50 value of 49.58 g·mL-1). This study enriches the foundation of antioxidant substances in sugarcane and provides a scientific basis for its further development and utilization.

Full Text

Chemical Constituents and Antioxidant Activity from the Stems and Leaves of *Saccharum officinarum*

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Abstract

To investigate the chemical constituents and antioxidant activity from the stems and leaves of sugarcane (*Saccharum officinarum*), methanol extracts were prepared and subjected to separation and purification using column chromatography (SiO_2, Sephadex LH-20, Rp-18). The structures of isolated compounds were identified by mass spectrometry and nuclear magnetic resonance spectroscopy, and their free radical scavenging capacities were evaluated by the DPPH method. The results were as follows: (1) Twenty-two compounds were isolated and identified from the stems and leaves of *S. officinarum*, namely p-hydroxybenzaldehyde (1), p-methoxycinnamic acid (2), 4-methoxybenzaldehyde (3), vanillin (4), 4-hydroxy-cinnamic acid methylester (5), p-hydroxybenzoic acid (6), (2-Hydroxyphenyl)(phenyl)methanone (7), p-methylbenzoic acid (8), caffeic acid methyl ester (9), aconitate A (10), aconitate E (11), 5-O-dimethoxycinnamoylquinic acid (12), quercetin (13), quercetin-3-O-α-L-arabinoside (14), quercetin-3-O-β-D-galactopyranoside (15), didodecyl thiodipropionate (propionic acid, 3,3-sulfinyl di-1,1 -didodecyl ester) (16), α-conidendrin (17), rel-(2α,3β)-7-O-methylcedrusin (18), 3-O-Ferulylquinic acid methyl ester (19), luteolin (20), (5S,6S)-5,6-dihydro-3,8,10-trihydroxy-5-(4-hydroxy-3-methoxyphenyl)-6-hydroxymethyl-2,4-dimethoxy-7H-benzo[c]xanthen-7-one (21), and 5-O-Ferulylquinic acid methyl ester (22). Among these, compounds 2–3, 7–11, 14–19, and 21–22 were isolated from this plant for the first time. (2) Fifteen compounds with relatively high content (1-9, 11-16) were screened for free radical scavenging ability using the DPPH method. Compound 12 (5-O-dimethoxycinnamoylquinic acid) exhibited good antioxidant activity with an IC_{50} value of 49.58 $g \cdot mL^{-1}$. This study enriches the understanding of the antioxidant substance basis of sugarcane and provides a scientific foundation for its further development and utilization.

Keywords: chemical constituents, *Saccharum officinarum*, stems and leaves, antioxidant, activity

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Introduction

Saccharum officinarum (sugarcane) belongs to the family Gramineae and genus *Saccharum*, distributed across temperate and subtropical regions and cultivated in over 100 countries worldwide. China possesses abundant sugarcane resources, with concentrated cultivation in Guangxi, Yunnan, and Fujian provinces (Editorial Committee of Flora of China, Chinese Academy of Sciences, 2014), serving as the most important raw material for the sugar industry. After sugar extraction, the remaining leaves and bagasse constitute agricultural byproducts that account for approximately 40% of the total biomass. Currently, only small quantities are processed into animal feed or yeast (Wei et al., 2016), while the majority is burned in the field, causing resource waste and severe environmental pollution. As China' s sugar industry continues to develop healthily and steadily, sugarcane byproducts will increase accordingly, making efficient utilization of these resources a major societal concern.

According to the *Dictionary of Traditional Chinese Medicine*, sugarcane is sweet in taste and cool in nature, with effects of clearing heat, promoting fluid production, and relieving alcohol toxicity (Jiangsu New Medical College, 2006). Folk medicine has used sugarcane to treat bladder stones, kidney stones, and gonorrhea with good efficacy. Sugarcane juice functions to clear heat, promote fluid production, eliminate phlegm, relieve cough, and stop hiccups and vomiting. Current research indicates that the chemical constituents of sugarcane leaves are primarily carbohydrates, flavonoids, phenols, and polysaccharides (Feng, 2017; Hou, 2014), exhibiting various biological activities including antioxidant, antitumor, hypoglycemic, and anti-inflammatory effects (Jiang et al., 2012; Gui et al., 2012; He et al., 2015). However, reports on individual active compounds remain limited. To further elucidate the active constituents of sugarcane and better develop the medicinal value of its byproducts, this study conducted systematic chemical investigations on the stems and leaves using methanol extraction. Twenty-two compounds were isolated from the ethyl acetate fraction (Figure 1 [Figure 1: see original paper]), among which compounds 2–3, 7–11, 14–19, and 21–22 were obtained from this plant for the first time. Compound 12 was a known compound previously isolated by our group (Lou et al., 2021). The DPPH method was employed to screen fifteen compounds with relatively high content (1–9, 11–16) for free radical scavenging capacity. Compound 12 (5-O-dimethoxycinnamoylquinic acid) demonstrated good antioxidant activity with an IC_{50} value of 49.58 $\text{g} \cdot \text{mL}^{-1}$. These results provide a material basis for subsequent bioactivity studies and resource development of sugarcane byproducts.

1. Instruments and Materials

Instruments: JP-03OS ultrasonic instrument (Shenzhen Yujie Cleaning Equipment Co., China), HC-2500Y304 grinder (Wuyi Haina Electric Appliance Co., China), VG-autospec 3000 mass spectrometer (Micromass, UK), Bruker Avance-600 MHz NMR spectrometer (Bruker, Switzerland), OSB-2100 rotary evaporator (Shanghai Ailang Instrument Co., China), Rp-18 reversed-phase silica gel and Sephadex LH-20 gel (Shanghai Yika Biotechnology Co., China).

Materials: SiO_2 (200–300 mesh) and silica gel plates GF_{254} (Qingdao Marine Chemical Co., China); conventional reagents (methanol, acetone, ethyl acetate, petroleum ether, etc.) were analytically pure and made in China; purified water.

Sugarcane stems and leaves were collected in August 2018 from the Yunnan Provincial Sugarcane Germplasm Resource Nursery (located on the campus of Yunnan Agricultural University, altitude 1950 m) and identified as *Saccharum officinarum* by Professor Li Fusheng of Yunnan Agricultural University.

2.1 Extraction and Separation

Dried sugarcane stems and leaves (80 kg) were pulverized, soaked in methanol for 1 day, and extracted by reflux. The methanol was recovered to obtain a concentrated extract, which was dissolved in water and adjusted to pH 3-4 with 5% HCl. The solution was then continuously extracted three times with ethyl acetate. After solvent recovery, 500 g of crude extract was obtained. The crude extract was subjected to silica gel column chromatography using a gradient elution of $CHCl_3$-CH_3OH-glacial acetic acid (10:0.1:0.02 to 10:3.5:0.02). Fractions with identical spots were combined to yield eight fractions (Fr.1–8).

Fr.1 was further separated by silica gel column chromatography with petroleum ether–ethyl acetate-glacial acetic acid (5:0.5–1.2:0.02) gradient elution to obtain three subfractions (Fr.1-A, 1-B, 1-C). Fr.(1-A) was repeatedly purified by silica gel column chromatography with petroleum ether-acetone-glacial acetic acid (5:0.5:0.02) and Sephadex LH-20 ($CHCl_3$-MeOH 1:1) to yield compounds **1** (0.43 g), **2** (0.58 g), **3** (0.33 g), **4** (0.19 g), and **5** (0.38 g). Fr.(1-B) was separated by repeated silica gel column chromatography with $CHCl_3$-MeOH-glacial acetic acid (10:0.1:0.01) and purified by Sephadex LH-20 ($CHCl_3$-CH_3OH 1:1) to afford compounds **6** (0.32 g) and **7** (0.87 g). Fr.(1-C) was subjected to silica gel column chromatography with $CHCl_3$-MeOH-glacial acetic acid (10:0.1:0.02) gradient elution and further purified by Rp-18 silica gel with a methanol–water system (5:95–90:10) to obtain compounds **8** (0.29 g), **9** (0.20 g), **10** (0.05 g), and **11** (0.33 g).

Fr.4 was repeatedly separated and purified by silica gel column chromatography with $CHCl_3$-MeOH-glacial acetic acid (10:0.3:0.02) to yield compound **12** (0.38 g).

Fr.6 was eluted through an Rp-18 silica gel column with a methanol–water system (5:95–80:20) and repeatedly purified by Sephadex LH-20 ($CHCl_3$-CH_3OH 1:1) to obtain compounds **13** (0.19 g), **14** (0.24 g), and **15** (0.22 g).

Fr.7 was separated by silica gel column chromatography using petroleum ether-ethyl acetate-glacial acetic acid (5:5.0:0.02) gradient elution to yield three subfractions (Fr.7-A, 7-B, 7-C). Fr.(7-A) was separated by Sephadex LH-20 ($CHCl_3$-MeOH 1:1) and eluted with an Rp-18 silica gel methanol–water system (30:70–70:20) to afford compounds **16** (0.17 g) and **17** (0.025 g). Fr.(7-B) was subjected to silica gel column chromatography with $CHCl_3$-MeOH-glacial acetic acid (10:0.8:0.02) and repeatedly purified by Sephadex LH-20 ($CHCl_3$-MeOH 1:1) to yield compounds **18** (0.057 g), **19** (0.076 g), and **20** (0.055 g). Fr.(7-C) was separated by repeated silica gel column chromatography using an Rp-18 silica gel methanol–water system (30:70–80:20) gradient elution to obtain compounds **21** (0.04 g) and **22** (0.068 g).

2.2 DPPH Assay for Free Radical Scavenging Capacity

Fifteen compounds with relatively high content (1-9, 11–16) were diluted with anhydrous ethanol to the required concentrations. DPPH was prepared as a 0.2 $\text{mmol} \cdot \text{L}^{-1}$ solution in anhydrous ethanol. In a 96-well plate, 100 µL of each test compound solution at various concentrations (1000, 500, 250, 125, 62.5, 31.25, and 15.625 $\mu\text{g} \cdot \text{mL}^{-1}$) was mixed with 100 µL of DPPH solution. The mixture was gently agitated and allowed to react in the dark at room temperature for 30 min. The absorbance (As) was measured at 517 nm. Simultaneously, the absorbance of 100 µL DPPH solution mixed with 100 µL anhydrous ethanol (Ab) and 200 µL anhydrous ethanol (Aref) was determined. The DPPH radical scavenging rate (I) was calculated using the following formula, and IC_{50} values were calculated using Origin software.

$$I(\%) = \frac{(A_{\text{ref}} - A_{\text{b}}) - (A_{\text{s}} - A_{\text{ref}})}{A_{\text{ref}} - A_{\text{b}}} \times 100\%$$

Where: I represents the DPPH radical scavenging rate; As represents the OD value of the test sample mixed with DPPH solution; Ab represents the OD value of DPPH solution mixed with anhydrous ethanol; and Aref represents the OD value of anhydrous ethanol.

3.1 Structure Identification

Compound 1 was obtained as a white powder. ESI-MS m/z: 123.1 $[M+H]^+$, molecular formula $C_7H_6O_2$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 9.75 (1H, s, H-4), 7.75 (2H, s, H-2,6), 6.91 (2H, m, H-3,5); ^{13}C-NMR (150 MHz, CD_3OD) δ: 192.8 (C-6), 165.2 (C-4), 133.4 (C-1), 130.3 (C-2,6), 116.9 (C-3,5). These data were consistent with literature values (Li et al., 2022), and the compound was identified as p-hydroxybenzaldehyde.

Compound 2 was isolated as white needle-like crystals. ESI-MS m/z: 179.2 $[M+H]^+$, molecular formula $C_{10}H_{10}O_3$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.57 (1H, d, J = 15.1 Hz, H-7), 7.41 (2H, dd, J = 6.1, 1.7 Hz, H-2,6), 6.78 (2H, dd, J = 6.5, 1.7 Hz, H-3,5), 6.28 (1H, d, J = 15.3 Hz, H-8), 3.73 (3H, s, $-OCH_3$); ^{13}C-NMR (150 MHz, CD_3OD) δ: 169.7 (-COOH), 161.2 (C-4), 146.5 (C-7), 131.1 (C-2,6), 127.1 (C-1), 116.8 (C-3,5), 114.9 (C-8), 51.9 ($-OCH_3$). These data matched literature values (Zhou et al., 2020), and the compound was identified as p-methoxycinnamic acid.

Compound 3 was obtained as a white solid. ESI-MS m/z: 135.0 $[M-H]^-$, molecular formula $C_8H_8O_2$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 9.75 (1H, s, H-4), 7.75 (2H, s, H-2,6), 6.91 (2H, m, H-3,5); ^{13}C-NMR (150 MHz, CD_3OD) δ: 192.8

(C-1), 165.2 (C-4), 133.2 (C-2,6), 115.9 (C-3,5). These data were consistent with literature values (Huang et al., 2017), and the compound was identified as 4-methoxybenzaldehyde.

Compound 4 was isolated as a white powder. ESI-MS m/z: 151.2 $[M-H]^-$, molecular formula $C_8H_8O_3$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 9.71 (1H, s, -CHO), 7.42 (1H, dd, J = 8.6, 1.3 Hz, H-6), 7.41 (1H, d, J = 1.5 Hz, H-2), 6.92 (1H, d, J = 8.2 Hz, H-5), 6.90 (1H, brs, -OH), 3.87 (3H, s, $-OCH_3$); ^{13}C-NMR (150 MHz, CD_3OD) δ: 192.8 (-CHO), 155.4 (C-4), 149.9 (C-3), 130.3 (C-1), 128.1 (C-6), 116.4 (C-2), 111.2 (C-5), 56.4 ($-OCH_3$). These data matched literature values (Wu et al., 2021), and the compound was identified as vanillin.

Compound 5 was obtained as a white powder. ESI-MS m/z: 177.2 $[M-H]^-$, molecular formula $C_{10}H_{10}O_3$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.60 (1H, d, J = 16.2 Hz, H-3), 7.58 (2H, d, J = 8.4 Hz, H-5,9), 6.79 (2H, d, J = 8.6 Hz, H-6,8), 6.78 (1H, d, J = 15.8 Hz, H-2), 3.29 (3H, s, $-OCH_3$); ^{13}C-NMR (150 MHz, CD_3OD) δ: 169.7 (C-9), 161.3 (C-4), 146.5 (C-7), 133.6 (C-2), 131.1 (C-6), 127.1 (C-1), 116.8 (C-3), 116.8 (C-5), 114.9 (C-8), 51.9 ($-OCH_3$). These data were consistent with literature values (El-Kader et al., 2020), and the compound was identified as 4-hydroxy-cinnamic acid methylester.

Compound 6 was isolated as a white solid. ESI-MS m/z: 137.1 $[M-H]^-$, molecular formula $C_7H_6O_3$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.85 (2H, d, J = 8.6 Hz, H-2,6), 6.79 (2H, d, J = 8.4 Hz, H-3,5); ^{13}C-NMR (150 MHz, CD_3OD) δ: 170.1 (C-7), 163.3 (C-4), 132.9 (C-2,6), 122.7 (C-1), 116.0 (C-3,5). These data matched literature values (Ren et al., 2020), and the compound was identified as p-hydroxybenzoic acid.

Compound 7 was obtained as a white powder. ESI-MS m/z: 197.2 $[M-H]^-$, molecular formula $C_{13}H_{10}O_2$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 9.76 (1H, s, H-6), 7.78 (2H, d, J = 7.8 Hz, H-3,5), 7.75 (2H, m, H-2 ,6), 6.91–7.23 (3H, t, J = 7.2 Hz, H-3 ,4 ,5), 6.89 (1H, d, J = 8.5 Hz, H-2), 6.37 (1H, d, J = 7.8 Hz, H-4); ^{13}C-NMR (150 MHz, CD_3OD) δ: 192.8 (C-7), 165.2 (C-6), 158.8 (C-1), 133.4 (C-3 ,5), 130.4 (C-2,4), 129.0 (C-1), 116.9 (C-3,5), 116.8 (C-1), 115.8 (C-4), 104.8 (C-2 ,6). These data were consistent with literature values (Ang et al., 2014), and the compound was identified as (2-Hydroxyphenyl)(phenyl)methanone.

Compound 8 was isolated as a white crystalline solid. ESI-MS m/z: 135.2 $[M-H]^-$, molecular formula $C_8H_8O_2$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.25 (2H, br.s, H-2 ,6), 6.67 (1H, s, H-3), 6.49 (1H, s, H-6), 6.32 (1H, s, H-8), 3.97 (6H, s, $2CH_3$); ^{13}C-NMR (150 MHz, CD_3OD) δ: 192.8 (-COOH), 166.1 (C-1), 133.5 (C-3,5), 128.7 (C-4), 116.9 (C-2,6), 30.8 ($-CH_3$). These data matched literature values (Qian et al., 2019), and the compound was identified as p-methylbenzoic acid.

Compound 9 was obtained as a light yellow solid. ESI-MS m/z: 195.2 $[M+H]^+$, molecular formula $C_{10}H_{10}O_4$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.52 (1H, d, J = 17.1 Hz, H-7), 7.03 (1H, d, J = 2.2 Hz, H-2), 6.89 (1H, dd, J = 8.7, 2.1 Hz, H-6), 6.81 (1H, d, J = 8.3 Hz, H-5), 6.27 (1H, d, J = 16.5 Hz, H-8), 3.88 (3H, s,

-OCH_3); ^{13}C-NMR (150 MHz, CD_3OD) δ: 169.9 (C-9), 149.6 (C-4), 146.5 (C-3), 146.1 (C-7), 127.9 (C-6), 127.3 (C-1), 116.6 (C-8), 115.2 (C-5), 115.0 (C-2), 53.7 (-OCH_3). These data were consistent with literature values (Hori et al., 2021), and the compound was identified as caffeic acid methyl ester.

Compound 10 was obtained as a white powder. ESI-MS m/z: 187.1 $[M-H]^-$, molecular formula $C_7H_8O_6$. ^1H-NMR (600 MHz, CD_3OD) δ: 6.91 (1H, s, H-4), 3.84 (2H, s, H-2), 3.71 (3H, s, 1-OCH_3); ^{13}C-NMR (150 MHz, CD_3OD) δ: 172.5 (C-1), 168.9 (C-6), 168.3 (C-5), 141.2 (C-3), 130.7 (C-4), 52.5 (1-OCH_3), 33.6 (C-2). These data matched literature values (Xu et al., 2017), and the compound was identified as aconitate A.

Compound 11 was isolated as a white powder. ESI-MS m/z: 225.0 $[M+Na]^+$, molecular formula $C_8H_{10}O_6$. ^1H-NMR (600 MHz, CD_3OD) δ: 6.93 (1H, s, H-4), 3.82 (2H, s, H-2), 3.78 (3H, s, 1-OCH_3), 3.67 (3H, s, 5-OCH_3); ^{13}C-NMR (150 MHz, CD_3OD) δ: 172.5 (C-1), 168.8 (C-6), 167.3 (C-5), 142.1 (C-3), 129.3 (C-4), 52.6 (5-OCH_3), 52.4 (1-OCH_3), 33.6 (C-2). These data were consistent with literature values (Xu et al., 2017), and the compound was identified as aconitate E.

Compound 12 was obtained as a white solid. ESI-MS m/z: 381.1 $[M-H]^-$. TLC on silica gel plates was performed using chloroform/methanol/glacial acetic acid (8:1.5:0.02, Rf = 0.5), petroleum ether/ethyl acetate/glacial acetic acid (6:4:0.02, Rf = 0.55), and petroleum ether/acetone/glacial acetic acid (7:3:0.02, Rf = 0.6) as developing solvents, with visualization by sulfuric acid-ethanol solution heating at 105 °C. The compound showed spots of the same color as the reference standard at corresponding positions, confirming its identity as 5-O-dimethoxycinnamoylquinic acid (Lou et al., 2021).

Compound 13 was isolated as a yellow solid. ESI-MS m/z: 303.2 $[M+H]^+$, molecular formula $C_{15}H_{10}O_7$. ^1H-NMR (600 MHz, CD_3OD) δ: 7.78 (2H, d, J = 2.2 Hz, H-2), 7.59 (1H, dd, J = 8.4, 2.1 Hz, H-6), 6.85 (1H, d, J = 8.2 Hz, H-5), 6.31 (1H, d, J = 2.2 Hz, H-8), 6.22 (1H, d, J = 2.0 Hz, H-6); ^{13}C-NMR (150 MHz, CD_3OD) δ: 177.2 (C-4), 165.1 (C-7), 161.4 (C-5), 155.3 (C-9), 146.9 (C-4), 146.7 (C-2), 144.7 (C-3), 136.7 (C-3), 121.6 (C-1), 118.7 (C-6), 115.6 (C-5), 115.3 (C-2), 98.3 (C-6), 94.1 (C-8). These data matched literature values (Yan et al., 2021), and the compound was identified as quercetin.

Compound 14 was obtained as a yellow solid. ESI-MS m/z: 433.4 $[M-H]^-$, molecular formula $C_{20}H_{18}O_{11}$. ^1H-NMR (600 MHz, CD_3OD) δ: 8.68 (2H, d, J = 2.1 Hz, H-2), 8.12 (1H, d, J = 8.4, 1.8 Hz, H-6), 7.41 (1H, d, J = 8.2 Hz, H-5), 6.92 (1H, d, J = 1.8 Hz, H-8), 6.75 (1H, d, J = 1.6 Hz, H-6), 5.60 (1H, d, J = 7.1 Hz, H-1); ^{13}C-NMR (150 MHz, CD_3OD) δ: 177.9 (C-4), 163.6 (C-7), 161.5 (C-5), 156.9 (C-9), 156.5 (C-2), 148.7 (C-4), 147.0 (C-3), 135.1 (C-3), 121.3 (C-1), 116.9 (C-5), 116.5 (C-2), 106.3 (C-10), 102.0 (C-1), 99.5 (C-6), 94.3 (C-8), 74.3 (C-3), 72.2 (C-2), 69.0 (C-4), 67.1 (C-5). These data were consistent with literature values (Han et al., 2020), and the compound was identified as quercetin-3-O-α-L-arabinoside.

Compound 15 was isolated as a light yellow solid. ESI-MS m/z: 463.4 $[M-H]^-$, molecular formula $C_{21}H_{20}O_{12}$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 8.43 (1H, d, J = 2.0 Hz, H-2), 8.10 (1H, dd, J = 8.2, 2.1 Hz, H-6), 7.22 (1H, d, J = 8.5 Hz, H-5), 6.67 (1H, d, J = 2.3 Hz, H-8), 6.64 (1H, d, J = 2.2 Hz, H-6), 6.13 (1H, d, J = 7.9 Hz, H-1); ^{13}C-NMR (150 MHz, CD_3OD) δ: 178.7 (C-4), 166.4 (C-7), 162.9 (C-5), 157.9 (C-9), 157.5 (C-2), 150.6 (C-4), 146.5 (C-3), 135.3 (C-3), 122.6 (C-1), 122.2 (C-6), 117.6 (C-2), 116.0 (C-5), 105.5 (C-10), 105.1 (C-1), 99.7 (C-6), 94.5 (C-8), 77.9 (C-5), 75.3 (C-3), 73.1 (C-2), 69.4 (C-4), 62.6 (C-6). These data matched literature values (Li et al., 2019), and the compound was identified as quercetin-3-O-β-D-galactopyranoside.

Compound 16 was isolated as transparent needle-like crystals. ESI-MS m/z: 531.5 $[M+H]^+$, molecular formula $C_{30}H_{58}O_5S$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 4.12 (2H, t, J = 7.1 Hz, H-1 ,1), 3.08 (1H, m, H-2), 2.93 (1H, m, H-2), 2.83 (2H, m, H-3,3), 0.87 (2H, tt, J = 7.7, 5.6 Hz, H-12 ,12); ^{13}C-NMR (150 MHz, CD_3OD) δ: 171.3 (C-1,1), 65.5 (C-1 ,1), 47.2 (C-2,2), 27.1 (C-3,3), 31.9 (C-11 ,11), 29.7 (C-10 ,10), 29.6 (C-9 ,9), 29.5 (C-8 ,8), 29.4 (C-7 ,7), 29.3 (C-6 ,6), 29.2 (C-5 ,5), 28.5 (C-4 ,4), 25.9 (C-3 ,3), 22.7 (C-2 ,2), 14.1 (C-12 ,12). These data were consistent with literature values (Malak et al., 2013), and the compound was identified as didodecyl thiodipropionate (propionic acid, 3,3-sulfinyl di-1,1 -didodecyl ester).

Compound 17 was obtained as a white powder. ESI-MS m/z: 357.3 $[M+H]^+$, molecular formula $C_{20}H_{20}O_6$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.28 (1H, d, J = 8.2 Hz, H-2), 6.99 (1H, d, J = 2.1 Hz, H-5), 6.78 (1H, d, J = 1.8 Hz, H-2), 6.75 (1H, dd, J = 1.8, 8.4 Hz, H-6), 6.72 (1H, d, J = 1.8 Hz, H-5), 3.86 (3H, s, 3-OCH_3), 3.65 (3H, s, 3 -OCH_3), 2.65 (2H, t, J = 7.5 Hz, H-7); ^{13}C-NMR (150 MHz, CD_3OD) δ: 178.7 (C-9), 148.5 (C-3), 147.3 (C-3), 146.2 (C-4), 145.9 (C-4), 136.7 (C-1), 133.6 (C-6), 127.6 (C-1), 123.2 (C-6), 117.6 (C-5), 115.9 (C-5), 114.1 (C-2), 112.9 (C-2), 72.7 (C-9), 56.4 (4-OCH_3), 56.3 (4 -OCH_3), 50.2 (C-8), 47.0 (C-7), 41.6 (C-8), 30.7 (C-7). These data matched literature values (Fedorova et al., 2016), and the compound was identified as α-conidendrin.

Compound 18 was isolated as a colorless solid. ESI-MS m/z: 361.4 $[M+H]^+$, molecular formula $C_{20}H_{24}O_6$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 6.99 (1H, d, J = 1.8 Hz, H-2), 6.87 (1H, dd, J = 1.8, 8.3 Hz, H-6), 6.77 (1H, d, J = 1.8 Hz, H-5), 6.71 (1H, s, H-6), 6.70 (1H, brs, H-4), 5.47 (1H, d, J = 6.6 Hz, H-2), 3.88 (3H, s, 7-OCH_3), 3.83/3.74 (2H, m, H-3a/3a), 3.66 (3H, s, 3 -OCH_3), 3.58 (2H, t, J = 6.5 Hz, H-5c), 3.49 (1H, dt, J = 6.4, 6.3 Hz, H-3), 2.65 (2H, t, J = 7.5 Hz, H-5a), 1.80 (2H, tt, J = 6.6, 7.7 Hz, H-5b); ^{13}C-NMR (150 MHz, CD_3OD) δ: 149.0 (C-3), 147.4 (C-4), 147.4 (C-7a), 145.1 (C-7), 136.9 (C-5), 134.8 (C-1), 129.8 (C-4a), 119.7 (C-6), 117.9 (C-4), 116.1 (C-5), 114.0 (C-6), 110.5 (C-2), 89.0 (C-2), 64.9 (C-3a/a), 62.2 (C-5c), 56.7 (7-OCH_3), 56.3 (3 -OCH_3), 55.4 (C-3), 35.8 (C-5b), 32.9 (C-5a). These data were consistent with literature values (Jia et al., 2017), and the compound was identified as rel-(2α,3β)-7-O-methylcedrusin.

Compound 19 was obtained as a white powder. ESI-MS m/z: 383.2 $[M+H]^+$, molecular formula $C_{18}H_{22}O_9$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.55 (1H, d, J =

15.8 Hz, H-7), 7.28 (1H, d, J = 1.3 Hz, H-2), 7.12 (1H, dd, J = 1.4, 8.1 Hz, H-6), 6.76 (1H, d, J = 8.2 Hz, H-5), 6.43 (1H, d, J = 15.8 Hz, H-8), 5.15 (1H, m, H-3), 3.84 (4H, s, 3-OCH_3, H-4), 3.60 (1H, m, H-5), 3.55 (3H, s, $COOCH_3$), 2.05 (1H, dd, J = 4.2, 12.6 Hz, H-2 b), 2.04 (1H, dd, J = 5.3, 13.5 Hz, H-6 b), 1.87 (1H, dd, J = 1.5, 12.6 Hz, H-2 a), 1.83 (1H, dd, J = 2.6, 13.4 Hz, H-6 a); ^{13}C-NMR (150 MHz, CD_3OD) δ: 174.2 (CO), 166.1 (C-9), 149.1 (C-4), 147.9 (C-3), 144.5 (C-7), 125.7 (C-1), 123.0 (C-6), 115.5 (C-5), 115.3 (C-8), 110.9 (C-2), 72.6 (C-1), 70.3 (C-3), 70.0 (C-4), 67.8 (C-5), 55.7 (3-OCH_3), 51.5 ($COOCH_3$), 39.1 (C-6), 34.8 (C-2). These data matched literature values (Yan et al., 2017), and the compound was identified as 3-O-Ferulylquinic acid methyl ester.

Compound 20 was obtained as a yellow powder. ESI-MS m/z: 285.1 $[M-H]^-$, molecular formula $C_{15}H_{10}O_6$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 12.23 (1H, s, 5-OH), 7.42 (1H, dd, J = 8.6, 0.8 Hz, H-6), 7.40 (1H, d, J = 1.0 Hz, H-2), 6.96 (1H, d, J = 8.4 Hz, H-3), 6.60 (1H, s, H-3), 6.52 (1H, d, J = 0.8 Hz, H-8), 6.20 (1H, d, J = 0.8 Hz, H-6); ^{13}C-NMR (150 MHz, CD_3OD) δ: 183.8 (C-4), 166.4 (C-7), 166.1 (C-2), 163.2 (C-9), 159.4 (C-5), 151.2 (C-4), 147.1 (C-3), 123.1 (C-1), 120.3 (C-3), 116.8 (C-5), 114.1 (C-6), 105.3 (C-2), 103.8 (C-10), 100.1 (C-6), 95.0 (C-8). These data were consistent with literature values (Cao et al., 2021), and the compound was identified as luteolin.

Compound 21 was obtained as a yellow amorphous powder. ESI-MS m/z: 509.3 $[M+H]^+$, molecular formula $C_{27}H_{24}O_{10}$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.48 (1H, s, H-6), 6.70 (1H, d, J = 2.2 Hz, H-15), 6.55 (1H, d, J = 8.0 Hz, H-18), 6.44 (1H, d, J = 2.2 Hz, H-8), 6.33 (1H, d, J = 8.0 Hz, H-19), 6.18 (1H, d, J = 2.2 Hz, H-6), 3.56 (3H, s, 16-OCH_3), 3.50 (1H, m, H-12), 3.24 (1H, m, H-11b), 4.82 (1H, s, H-13), 4.05 (3H, s, 3 -OCH_3), 3.72 (3H, s, 5 -OCH_3), 3.61 (1H, m, H-11a), 3.20 (1H, m, H-11b); ^{13}C-NMR (150 MHz, CD_3OD) δ: 182.0 (C-4), 165.7 (C-7), 163.3 (C-5), 160.8 (C-2), 158.7 (C-9), 149.7 (C-5), 148.9 (C-16), 147.7 (C-3), 146.0 (C-17), 145.5 (C-4), 136.6 (C-14), 128.8 (C-2), 120.8 (C-19), 119.1 (C-1), 115.9 (C-18), 112.4 (C-15), 112.0 (C-3), 105.3 (C-10), 103.8 (C-6), 100.0 (C-6), 94.9 (C-8), 62.8 (C-11), 60.9 (3 -OCH_3), 56.9 (5 -OCH_3), 56.3 (16-OCH_3), 42.3 (C-12), 37.8 (C-13). These data were consistent with literature values (Xiao et al., 2020), and the compound was identified as (5S,6S)-5,6-dihydro-3,8,10-trihydroxy-5-(4-hydroxy-3-methoxyphenyl)-6-hydroxymethyl-2,4-dimethoxy-7H-benzo[c]xanthen-7-one.

Compound 22 was obtained as a white powder. ESI-MS m/z: 381.4 $[M-H]^-$, molecular formula $C_{18}H_{22}O_9$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 9.59 (1H, brs, 7 -OH), 7.50 (1H, d, J = 15.6 Hz, H-3), 7.27 (1H, d, J = 1.6 Hz, H-5), 7.01 (1H, dd, J = 8.2, 1.6 Hz, H-9), 6.81 (1H, d, J = 8.0 Hz, H-8), 6.40 (1H, d, J = 15.6 Hz, H-2), 5.49 (1H, brs, 1-OH), 5.13 (1H, dt, J = 9.2, 3.8 Hz, H-5), 4.91 (1H, brs, 4-OH), 4.83 (1H, brs, 3-OH), 3.85 (1H, overlapped, H-3), 3.84 (3H, s, 6 -OCH_3), 3.68 (1H, m, H-4), 3.55 (3H, s, 7-OCH_3), 2.05 (1H, dd, J = 12.7, 3.9 Hz, H-6b), 1.95 (1H, dd, J = 13.0, 5.9 Hz, H-2b), 1.86 (1H, dd, J = 12.3, 9.6 Hz, H-6a), 1.84 (1H, dd, J = 13.3, 3.1 Hz, H-2a); ^{13}C-NMR (150 MHz, CD_3OD) δ: 176.5 (C-7), 168.9 (C-1), 150.4 (C-7), 149.4 (C-6), 146.8

(C-3), 127.9 (C-4), 124.1 (C-9), 116.5 (C-8), 116.1 (C-2), 111.7 (C-5), 75.3 (C-1), 73.9 (C-4), 72.7 (C-5), 68.6 (C-3), 56.4 (6 -OCH_3), 52.9 (7-OCH_3), 40.8 (C-2), 36.4 (C-6). These data matched literature values (Li et al., 2020), and the compound was identified as 5-O-Ferulylquinic acid methyl ester.

3.2 Results of DPPH Free Radical Scavenging Assay

As shown in Table 1 , among the fifteen tested compounds, only compound **12** (5-O-dimethoxycinnamoylquinic acid) exhibited good antioxidant activity with an IC_{50} value of 49.58 $\mu g \cdot mL^{-1}$. The other compounds showed no significant antioxidant activity ($IC_{50} > 1000\ \mu g \cdot mL^{-1}$).

4. Discussion and Conclusion

The reuse of crop waste represents a strategic approach to addressing current shortages of traditional Chinese medicine resources and achieving sustainable development (Deng, 2010). Sugarcane, a plant used both as food and medicine, is produced in enormous quantities primarily for sugar extraction. The resulting bagasse and leaves are mainly discarded or burned as waste. How to scientifically and rationally reuse these byproducts remains a major challenge, and chemical composition analysis offers a primary methodology for addressing this issue. Based on this premise, we conducted systematic chemical investigations of sugarcane bagasse and leaves, isolating and identifying 22 compounds from methanol extracts. The structural types encompass flavonoids, phenolic acids, esters, lignans, and phenylpropanoids, including five flavonoids and six phenolic acids, revealing that flavonoids and phenolic acids constitute the core chemical components of sugarcane bagasse and leaves and clarifying their chemical composition profile.

Flavonoids and phenolic acids often exhibit significant biological activities. For example, *Erigeron breviscapus*, rich in flavonoids, has been developed into breviscapine tablets and injections for clinical treatment of cardiovascular and cerebrovascular diseases (Zhang, 2020). Willow bark, containing the phenolic acid salicylic acid, has been formulated into salicylic acid creams for treating seborrheic dermatitis and psoriasis (Hu et al., 2021). This study provides a pathway for obtaining flavonoid and phenolic acid components and establishes a chemical foundation for the development and utilization of sugarcane bagasse and leaves.

Among the identified compounds, compound **4** (vanillin) represents a major high-content core component. Vanillin is an important aromatic substance with extensive applications in the pharmaceutical field, commonly serving as a raw material for drug synthesis. It is used to synthesize berberine for treating diarrhea, L-methyldopa preparations (trade name Aldomet) for hypertension, methoxyaminopyridine for upper respiratory tract infections and preventing sexually transmitted bacterial strains, and papaverine for heart disease (Yang et al., 2010). Global demand for vanillin is substantial, approximately 12,000 tons, with market supply falling short of demand. Currently, vanillin is obtained from plants or chemically synthesized from lignin precursors. Sugarcane is rich in vanillin, with abundant resources and relatively low production costs, making it a promising raw material for vanillin production and an excellent pathway for waste valorization.

Free radicals are the root cause of numerous diseases, and identifying safe and effective antioxidant substances from plants represents a current research hotspot. Studies have shown that flavonoids and phenolic acids possess multiple physiological activities including antioxidant, anti-inflammatory, lipid-lowering, hypoglycemic, and anti-proliferative effects, and are considered potential exogenous antioxidant raw materials (Wei et al., 2016). To explore related active components, we evaluated the free radical scavenging capacity of 15 high-content compounds (1–9, 11–16) from sugarcane using the DPPH method. The results demonstrated that compound **12** (5-O-dimethoxycinnamoylquinic acid) possesses good antioxidant activity. In addition to its excellent antioxidant properties, compound **12** occurs in relatively high content. Through further investigation of its antioxidant activity and scientific evaluation, compound **12** may become a promising antioxidant candidate molecule, providing both chemical and pharmacological foundations for the deep development of waste sugarcane bagasse and leaves.

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