β-glucopyranoside (**BNL10**), ethyl α-D-glucopyranoside (**BNL11**), hydroxytyrosol 4-β-D-glucoside (**BNL16**), and cimidahurinine (**BNL17**). Among them, **BNL3**, **BNL9**, **BNL10**, **BNL11**, **BNL16**, and **BNL17** were first isolated from the *Boehmeria*.

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MEGASTIGMANE, ALKALOID, FLAVONOID, AND PHENOLIC COMPOUNDS FROM *CISSUS TRILOBUS* (LOUR.) MERR.

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Summary

Megastigmane, Alkaloid, Flavonoid, and Phenolic Compounds from *Cissus trilobus* (Lour.) Merr.

From the dichloromethane and ethyl acetate extracts of *Cissus trilobus* seven compounds were isolated and identified as blumenol B (**1**), blumenol A (**2**), indole-3-carboxylic acid (**3**), vitexin (**4**), cytisoside (**5**), acacetin-8-*C*-neohesperidoside (**6**), and 4-hydroxybenzoic acid (**7**). These structures were determined through the analysis of NMR and MS spectroscopic data and comparison with the reported literature. These compounds were isolated for the first time from *C.trilobus*.

Keywords: *C.trilobus, Megastismane, Alkaloid, Flavonoid.*

1. Introduction

Cissus trilobus (Lour.) Merr. (synonym: *Cissus modeccoides* Planch.) is a vine belonging to the Vitaceae family and is naturally distributed throughout many regions of Vietnam, Cambodia, Thailand, and China. In folk medicine, the tuberous root and the aerial part of this plant have been used for the treatment of bone and joint pain, swelling, snakebite… [1]. Although this plant has been used commonly, there are very few studies on its chemical composition. A previous study revealed the presence of three anthraquinones (chrysophanol, emodin, and physcione) and two phenolic compounds (lasiodiplodin and *trans*-4-hydroxymellein) from the aerial part of *C. trilobus* [2]. In a pharmacological study, the hot aqueous extract of *C. trilobus* did not have a hypoglycemic effect on

alloxan-induced diabetic rats, however, it showed toxicity through alterations in biochemical parameters [3]. In this study, we described the isolation and structural elucidation of isolated compounds from the dichloromethane and ethyl acetate fractions of *C. trilobus* grown in Vietnam.

2. Experimental

2.1. Plant material

The aerial part of *C.trilobus* was collected in Cau Ke, Tra Vinh province in November 2018 and authenticated by Dr. Tran Thi Van Anh (Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh city - UMP). The voucher specimen (Cissus.M112018) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, UMP.

2.2. General experimental procedures

Column chromatography was performed with *silica gel* (40 - 63 µm, Scharlau) and Sephadex LH-20 (GE Healthcare Life). Thin layer chromatography (TLC) was carried out on *silica gel* 60 F_{254} plates (Merck). Compounds were visualized by UV lamp and spraying with the vanillin-sulfuric acid reagent followed by heating to 105 °C. ESI-MS spectra were recorded on a Quattro micro-API mass spectrometer. NMR spectra were recorded by a Bruker Avance NEO 400 MHz using tetramethylsilane as an internal standard.

2.3. Extraction and isolation

The sample was prepared by chopping, air-drying, and grinding to a raw powder. The powder of *C. trilobus* (5 kg) was percolated with 96% EtOH at room temperature. The solvent was evaporated under reduced pressure to obtain the concentrated EtOH extract (900 mL). The EtOH extract was suspended in water and successively partitioned with petroleum ether (0.5 L x 5 times), dichloromethane (0.4 L x 5 times), ethyl acetate (0.3 L x 6 times), and saturated *n*-butanol (0.1 L x 3 times). After removing the solvents, five corresponding fractions were obtained, including petroleum ether (128.05 g), dichloromethane (17.72 g), ethyl acetate (22.86 g), *n*-butanol (10.21 g), and the remaining water (209.0 g) fractions.

The dichloromethane fraction (17.0 g) was subjected to a *silica gel* column using a gradient solvent system of DCM-MeOH (100:0 to 90:10, v/v) to yield 10 fractions (B.1-B.10). Fraction B.7 (818.0 mg) was separated by a Sephadex LH-20 column, eluting with MeOH to obtain 5 sub-fractions (B7.1-B7.5). Fraction B7.2 (276.4 mg) was purified through a *silica gel* column with *n*-hexane-EtOAc (6:4, v/v) and then a Sephadex LH-20 column, eluting with DCM-MeOH (9:1, v/v) to obtain compound **1** (11.6 mg) and compound **2** (12.3 mg). Fraction B7.4 (106.1 mg) was chromatographed by a *silica gel* column eluted with $CHCl_3$-EtOAc (7:3, v/v) to get compound **3** (6.0 mg). Fraction B.9 (580 mg) was separated by a Sephadex LH-20 column, using MeOH as the mobile phase to get 4 subfractions (B.9.2-B.9.4). Subfraction B.9.2 (99.2 mg) was purified through a *silica gel* column using the mobile phase of DCM-MeOH (98:2, v/v) to yield compound **7** (12.8 mg).

The ethyl acetate fraction (20.1 mg) was subjected to a *silica gel* column using a solvent system of EtOAc-MeOH-H_2O (95:3:2, v/v/v) to get 11 fractions (C.1-C.11). The precipitate (37.0 mg) was filtrated from fraction C.1 and then purified through a *silica gel* column, eluted with $CHCl_3$-MeOH (8:2, v/v) to obtain compound **4** (21.0 mg) and compound **5** (4.3 mg). Fraction C.8 (786.2 mg) was separated by a Sephadex LH-20 column with MeOH as the mobile phase to get 6 subfractions. Subfraction C8.2 (186.0 mg) was purified through a *silica gel* column, eluting with $CHCl_3$-MeOH (8:2, v/v) to get compound **6** (52.3 mg).

Blumenol B (1): pale yellow oil; ESI-MS *m/z* 209.33 $[M-H_2O+H]^+$, 249.32 $[M+Na]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 5.84 (1H, brs, H-4), 3.77 (1H, dqd, J = 12.3, 6.1, 3.6 Hz, H-9), 2.50 (d, J = 18.2 Hz, H-2a), 2.24 (d, J = 18.2 Hz, H-2b), 2.05 (3H, d, J = 1.4 Hz, H-11), 2.02 (1H, m, H-7a), 1.86 (1H, m, H-7b), 1.64 (1H, m, H-8a), 1.52 (1H, m, H-8b), 1.22 (3H, d, J = 6.1 Hz, H-10), 1.09 (3H, s, H-13), 1.06 (3H, s, H-12); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) 198.2 (C-3), 168.5 (C-5), 126.0 (C-4), 68.7 (C-9), 50.0 (C-2), 41.8 (C-1), 34.8 (C-7), 33.5 (C-8), 24.2 (C-10), 23.9 (C-12), 23.7 (C-13), 21.8 (C-11).

Blumenol A (2): pale yellow oil; ESI-MS *m/z* 207.31 $[M-H_2O+H]^+$, 247.29 $[M+Na]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 7.26 (1H, s, -OH), 5.92 (1H, brs, H-4), 5.88 (1H, d, J = 15.6, 5.1 Hz, H-7a), 5.80 (1H, d, J = 15.6, Hz, H-7b), 4.43 (1H, dt, J = 12.1, 6.3 Hz, H-9), 2.46 (1H, d, J = 17.1 Hz, H-2a), 2.26 (d, J = 17.1 Hz, H-2b), 1.91 (3H, d, J = 1.4 Hz, H-11), 1.32 (1H, d, J = 4.4 Hz, H-10), 1.10 (3H, s, H-13), 1.03 (3H, s, H-12); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) 198.1 (C-3), 162.8 (C-5), 135.7 (C-8), 129.0 (C-7), 126.9 (C-4), 79.1 (C-6), 68.1 (C-9), 49.7 (C-2), 41.2 (C-1), 24.1 (C-12), 23.8 (C-10), 22.9 (C-13), 18.9 (C-11).

Indole-3-carboxylic acid (3): colorless needles; ESI-MS *m/z* 160.02 [M-H]$^-$; ^{1}H-NMR (400 MHz, MeOD) δ (ppm) 8.07 (1H, m, H-7), 7.94 (1H, s, H-2), 7.43 (1H, s, H-4), 7.18 (2H, m, H-5, H-6); ^{13}C-NMR (100 MHz, CD_3OD) δ (ppm) 169.2 (-COOH), 138.2 (C-7a), 133.40 (C-2), 127.6 (C-4a), 123.6 (C-5), 122.4 (C-6), 122.0 (C-7), 112.8 (C-4), 108.7 (C-3).

Vitexin (4): amorphous yellow powder; ESI-MS *m/z* 431.29 [M-H]$^-$; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm) 13.16 (1H, s, 5-OH); 8.01 (2H, d, J = 8.4 Hz, H-2′ and H6′), 6.89 (2H, d, J = 8.4 Hz, H-3′ and H-5′), 6.75 (1H, s, H-3), 6.24 (1H, s, H-6), 4.72 (1H, d, J = 9.8 Hz, H-1″), 3.85 (1H, t, J = 9.4 Hz, H-2″), 3.76 (1H, d, J = 11.6 Hz, H-6a″), 3.52 (1H, dd, J = 11.6, 5.3 Hz, H-6b″), 3.28 (2H, m, H-3″ and H-4″), 3.26 (1H, m, H-5″); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm) 182.4 (C-4), 164.3 (C-2), 164.1 (C-7), 161.7 (C-4′), 160.9 (C-5), 156.5 (C-9), 129.4 (C-2′ and C-6′), 122.1 (C-1′), 116.3 (C-3′ and C-5′), 105.1 (C-8), 104.5 (C-10), 102.8 (C-3), 98.8 (C-6), 82.3 (C-5″), 79.2 (C-3″), 73.9 (C-1″), 71.4 (C-2″), 71.0 (C-4″), 61.8 (C-6″).

Cytisoside (5): amorphous yellow powder; ESI-MS *m/z* 445.35 [M-H]$^-$, ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm) 13.12 (1H, s, 5-OH), 8.13 (2H, d, J = 8.5 Hz, H-2′ and H6′), 7.08 (2H, d, J = 8.5 Hz, H-3′ and H-5′), 6.85 (1H, s, H-3), 6.24 (1H, s, H-6), 4.72 (1H, d, J = 9.8 Hz, H-1″), 3.87 (3H, s, 4′-OCH_3), 3.83 (1H, m, H-2″), 3.75 (1H, m, H-6a″), 3.55 (1H, dd, J = 11.6, 5.3 Hz, H-6b″), 3.31 (1H, m, H-4″), 3.27 (1H, m, H-3″), 3.25 (1H, m, H-5″); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm) 182.4 (C-4), 163.8 (C-7), 162.8 (C-4′), 160.9 (C-5), 156.5 (C-9), 129.2 (C-2′), 123.8 (C-1′), 114.9 (C-3′), 105.1 (C-8), 104.2 (C-10), 103.6 (C-3), 82.3 (C-5″), 79.2 (C-3″), 74.0 (C-1″), 71.4 (C-2″), 70.9 (C-4″), 61.7 (C-6″), 56.1 (4′-OCH_3).

Acacetin-8-*C*-neohesperidoside (6): amorphous yellow power; ESI-MS *m/z* 591.36 [M-H]$^-$; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm) 13.1 (1H, s, 5-OH), 8.18 (2H, d, J = 9.0 Hz, H-2′ and H-6′), 7.11 (2H, d, J = 9.0 Hz, H-3′ and H-5′), 6.88 (1H, s, H-3), 6.32 (1H, s, H-6), 4.98 (1H, d, J = 1.6 Hz, H-1‴), 4.77 (1H, d, J = 10 Hz, H-1″), 4.06 (1H, m, H-2″), 3.87 (3H, s, 4′-OCH_3), 3.75 (1H, ddd, J = 12.0, 6.0 Hz, C-6″), 3.58 (1H, m, H-2‴), 3.47 (2H, m, H-3″ and H-4″), 3.24 (1H, ddt, J = 7.4, 5.5, 2.0 Hz, C-5″), 3.10 (1H, ddd, J = 9.0, 5.6, 4.0 Hz, C-3‴), 2.92 (1H, dt, J = 9.2, 4.0 Hz, C-4‴), 2.12 (1H, dd, J = 9.2, 6.0 Hz, C-5‴), 0.48 (3H, J = 6.0 Hz, H-6‴); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm) 182.6 (C-4), 164.0 (C-2), 163.0 (C-7), 162.9 (C-4′), 161.1 (C-5), 156.3 (C-9), 129.4 (C-6′), 123.6 (C-1′), 115.0 (C-3′/C-5′), 105.0 (C-8), 104.7 (C-10), 103.6 (C-3), 100.8 (C-1‴), 98.9 (C-6), 82.3 (C-5″). 80.3 (C3″), 75.5 (C-2″), 72.1 (C-1″), 72.0 (C-4‴), 70.9 (C-4″/C-2‴), 70.7 (C-3‴), 68.7 (C-5‴), 61.5 (C-6″), 56.10 (-OCH_3), 18.2 (C-6‴)

4-Hydroxybenzoic acid (7): colorless needle, ESI-MS *m/z* 137.18 [M-H]$^-$; ^{1}H-NMR (400 MHz, CD_3OD) δ (ppm) 7.88 (2H, d, J = 8.8 Hz, H-2, H-6), 6.81 (2H, d, J = 8.8 Hz, H-3, H-5). ^{13}C-NMR (100 MHz, CD_3OD) δ (ppm) 170.4 (-COOH), 163.4 (C-4), 133.0 (C-2 and C-6), 116.1 (C-3 and C-5), 123.0 (C-1).

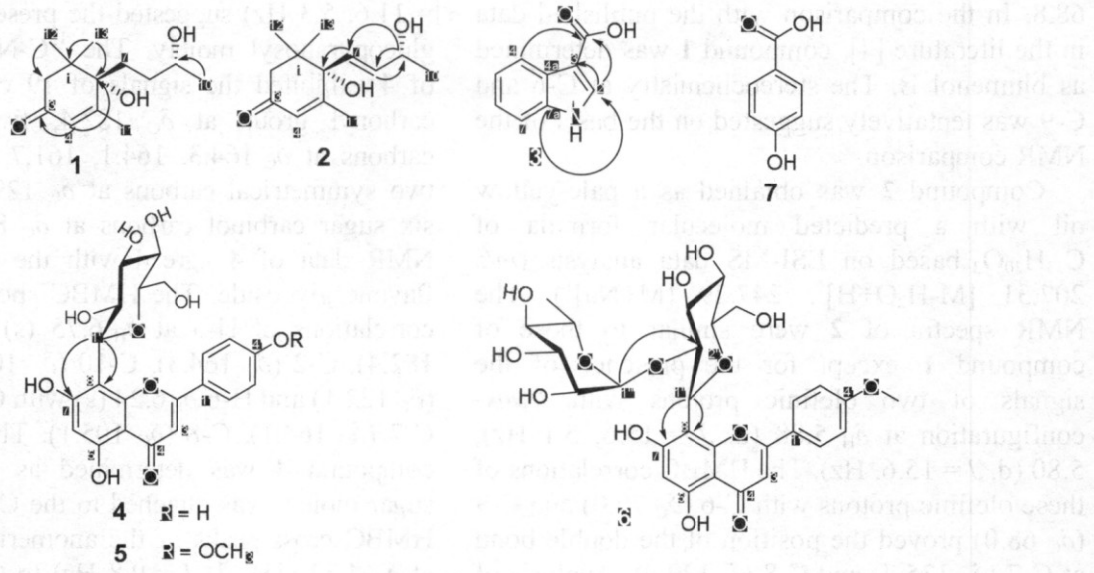

Fig. 1. Chemical structures and key HMBC correlations of the isolated compounds (**1 - 7**)

3. Result and discussion

Compound **1** was obtained as a pale-yellow oil. The ESI-MS showed the positive ion peak with *m/z* 209.33 ($[M-H_2O+H]^+$ and 249.32 $[M+Na]^+$); predicting the molecular formula of $C_{13}H_{22}O_3$. The ^{1}H-NMR of **1** showed a broad singlet of an olefinic proton at δ_H 5.84, a hydroxymethine proton at δ_H 3.77 (1H, dqd, J = 12.3, 6.1, 3.6 Hz), two singlet signals of tertiary methyl groups at δ_H 1.09 and 1.06, and two doublet signals of methyl groups at δ_H 1.22 (3H, d, J = 6.1 Hz) and 2.05 (3H, d, J = 1.4 Hz). The ^{13}C-NMR revealed 13 signals of a carbonyl carbon at δ_C 198.2, two olefinic carbons at δ_C 168.5 and 126.0, one oxymethine carbon at δ_C 68.8, three methylene carbons at δ_C 50.0, 34.8, and 33.5, two non-protonated carbons at δ_C 77.8, 41.8, and four methyl carbons at δ_C 24.2, 23.9, 23.7 and 21.8. The NMR data suggested compound **1** was a megastigmane derivative. In the HMBC spectrum, the correlations of two singlet methyl protons at δ_H 1.09 and 1.06 with non-protonated carbons at δ_C 41.8 and 77.8 and methylene carbon at δ_C 50.2; as well as the cross-peak of the olefinic proton at δ_H 5.84 with carbons at δ_C 198.3, 77.8 and 21.8 revealed the location of the carbonyl group at C-3 (δ_C 198.3), two tertiary methyl groups at C-1 (δ_C 41.8), a double bond at C-4/C-5 and a hydroxyl group at C-6 (δ_C 77.8). The hydroxyl group at C-9 of the side chain was determined through the correlation of the methyl proton at δ_H 1.22 (3H, d, J = 6.1 Hz) with the oxymethine carbon at δ_C 68.8. In the comparison with the published data in the literature [4], compound **1** was determined as blumenol B. The stereochemistry at C-6 and C-9 was tentatively suggested on the basis of the NMR comparison.

Compound **2** was obtained as a pale-yellow oil with a predicted molecular formula of $C_{13}H_{20}O_3$ based on ESI-MS data analysis (*m/z* 207.31 $[M-H_2O+H]^+$, 247.29 $[M+Na]^+$). The NMR spectra of **2** were similar to those of compound **1** except for the presence of the signals of two olefinic protons with *trans*-configuration at δ_H 5.88 (d, J = 15.6, 5.1 Hz), 5.80 (d, J = 15.6, Hz). The HMBC correlations of these olefinic protons with C-6 (δ_C 79.0) and C-9 (δ_C 68.0) proved the position of the double bond at C-7 (δ_C 135.7) and C-8 (δ_C 129.0). Analysis of a complete set of the NMR spectroscopic data and comparison with those in the literature [4] led to the identification of **2** as blumenol A (vomifoliol). The stereochemistry at C-6 and C-9 was tentatively suggested on the basis of the NMR comparison.

Compound **3** was obtained as colorless needles with a molecular formula of $C_9H_7NO_2$, predicted by ESI-MS spectral data (*m/z* 160.02 $[M-H]^-$). The ^{1}H-NMR spectrum of **3** exhibited five signals of olefinic protons at δ_H 8.07-7.18. The ^{13}C-NMR spectrum showed the presence of nine carbons, including one carbonyl at δ_C 169.2 and eight aromatic carbons at δ_C 138.2-108.7. The correlations in the COSY spectrum of δ_H 7.43 (1H, m) - δ_H 7.18 (2H, m) - 8.07 (1H, m) and the cross-peaks in the HMBC spectrum between H-2 (δ_H 7.94 s) with carbons at δ_C 169.2, 138.2, 127.6, and 108.7 revealed an indole structure with the carbonyl group at C-3. Compound **3** was identified as indole-3-carboxylic acid by analyzing spectral data and comparing them with the previously published data [5].

Compound **4** was isolated as an amorphous yellow powder. The ESI-MS negative ion peak with *m/z* 431.29 $[M-H]^-$ predicted the molecular formula of $C_{21}H_{20}O_{10}$. The ^{1}H-NMR spectrum of **4** showed a singlet signal of a hydroxyl proton at δ_H 13.16, two singlets of olefinic protons at δ_H 6.75 and 6.24, two doublet signals at δ_H 8.01 (2H, d, J = 8.4 Hz) and 6.89 (2H, d, J = 8.4 Hz). Additionally, signals of five hydroxymethine protons at δ_H 4.72-3.26 and two signals of hydroxymethylene protons at δ_H 3.76 (1H, d, J = 11.6 Hz) and 3.52 (1H, dd, J = 11.6, 5.3 Hz) suggested the presence of a β-D-glucopyranosyl moiety. The ^{13}C-NMR spectrum of **4** exhibited the signals of 19 carbons with a carbonyl group at δ_C 182.4, five oxygenated carbons at δ_C 164.3, 164.1, 161,7, 160.9, 156.5, two symmetrical carbons at δ_C 129.4 and 116.3, six sugar carbinol carbons at δ_C 82.3-61.8. The NMR data of **4** agreed with the structure of a flavone glycoside. The HMBC spectrum showed correlations of H-3 at δ_H 6.75 (s) with C-4 (δ_C 182.4), C-2 (δ_C 164.3), C-10 (δ_C 104.1) and C-1′ (δ_C 122.1) and H-6 δ_H 6.24 (s) with C-5 (δ_C 160.9), C-7 (δ_C 164.1), C-8 (δ_C 105.1). The aglycone of compound **4** was determined as apigenin. The sugar moiety was attached to the C-8 through the HMBC cross-peaks of the anomeric proton H-1″ at δ_H 4.72 (1H, d, J = 9.8 Hz) to C-8 (δ_C 105.1) and C-7 (δ_C 164.1). Compound **4** was identified as

a *C*-glycoside based on the HMBC correlation and the chemical shift of the anomeric carbon C-1″ at δ_C 73.9. In comparison with the spectroscopic data in the published paper [6], the structure of **4** was determined as vitexin (apigenin-8-*C*-glucoside).

Compound **5** was isolated as a yellow amorphous powder. The ESI-MS negative ion peak with *m/z* 445.35 $[M-H]^-$ predicted the molecular formula of $C_{22}H_{23}O_{10}$. The NMR data of compound **5** were similar to those of compound **4**, except for the presence of a methoxy group at δ_H 3.87 (3H, s) and δ_C 56.1. The position of the methoxy group was determined through the HMBC interaction between the methyl proton at δ_H 3.87 with C-4′ (δ_C 162.8). The aglycon of compound **5** was identified as acacetin (4′-methoxyapigenin). By comparing the spectroscopic data with the literature [7], the structure of compound **5** was determined as cytisoside (acacetin-8-*C*-*β*-D-glucopyranoside).

Compound **6** was isolated as a yellow amorphous powder with a molecular formula of $C_{28}H_{32}O_{14}$, predicted by ESI-MS analysis (*m/z* 591.36 $[M-H]^-$). The 1D-NMR spectra of **6** showed the characteristics of a flavone with two sugar moieties. The ^{1}H- and ^{13}C-NMR signals of the aglycon were similar to those of acacetin. The signals of the sugar moieties had two anomeric protons at δ_H 4.77 (d, J = 10.0 Hz) and 4.98 (d, J = 1.6 Hz), two oxymethylene protons at δ_H 3.75 and 3.58, eight oxymethine protons at δ_H from 4.06 to 2.12, one methyl protons at δ_H 0.48 (3H, d, J = 6.0 Hz) that suggested the presence a *β*-D-glucopyranose and a *α*-L-rhamnopyranose units. The HMBC spectrum showed a correlation between the anomeric proton at δ_H 4.77 (d, J = 10.0 Hz) and C-8 (δ_H 105.0) confirming the *C*-glycosidic linkage of the *β*-D-glucopyranosyl moiety with the aglycon (acacetin) at C-8. The HMBC correlation between H-1‴ 4.98 (d, J = 1.6 Hz) to C-2″ of the glucose unit (δ_C 75.5) indicated a (1→2) linkage between two sugar moieties. Analysis of the spectral data in comparison with the published data [8] concluded that compound **6** was acacetin-8-*C*-*α*-D-rhamnopyranosyl (1→2)-*β*-D-glucopyranoside (acacetin-8-*C*-neohesperidoside).

Compound **7** was obtained as colorless needles with a predicted molecular formula of $C_7H_6O_3$ based on ESI-MS analysis. The ^{1}H-NMR of **7** showed two doublet signals of aromatic protons at δ_H 7.88 (2H, d, J = 8.8 Hz) and 6.81 (2H, d, J = 8.8 Hz). The ^{13}C-NMR showed five signals including one carbonyl at δ_C 170.4, two signals of four symmetrical aromatic carbons at δ_C 133.0 and 116.1, as well as two aromatic carbons at 123.0 and 163.4. The NMR data suggested compound **7** was 4-hydroxybenzoic acid [9].

4. Conclusion

Seven compounds were isolated from the aerial part of *Cissus trilobus* by chromatographic techniques. The isolated substances were structurally identified, including 2 megastigmanes (blumenol A and blumenol B), one alkaloid (indole-3-carboxylic acid), 3 flavonoids (vitexin, cytisoside, and acacetin-8-*C*-neohesperidoside), and a phenolic compound (4-hydroxybenzoic acid). All of these compounds were isolated for the first time from this species.

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