Egypt. J. Chem. **Vol. 64**, No. 10, pp. 5529- 5536 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Comparative Chemical Investigation of *Brachychiton australis* (Schott & Endl.) A. Terracc. and *Brachychiton discolor* F.Muell.

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Abstract

Brachychiton australis (Schott and Endl.) A. Terrac and *Brachychiton discolor* F.Muell. (Malvaceae) are widely grown in Egypt and observed as important biological species. Based on chromatographic and spectroscopic methods, chemical studies of 70% methanol/water extracts of the leafy branches of both species resulted in the isolation and identification of twelve phenolics. Eight isolated flavonoids (vicenin, schaftoside, violanthin, isoviolanthin, luteolin 7-*O*-*β*-glucuronide, apigenin 7-*O*-*β*-glucuronide, apigenin 7-*O*-*β*-glucopyranoside, and acacetin) were characterized for *B. australis* while three phenolic acid derivatives (*trans*-*p*-coumaric acid, *trans*-*p*-coumaric acid-*O*-glucoside, chlorogenic acid) and one flavonol glycoside (rutin) are considered for *B. discolor*. Except rutin, all compounds were reported for the first time in each species. The LC-ESI-MS assay of both extracts revealed the annotation of additional nineteen compounds including eight flavonoids, five phenolic acids, two organic acids, two fatty acids, one saccharide, and one amino acid. Most of the characterized bioactive compounds could be useful for further pharmaceutical applications. Moreover, this study presents a step forward for approaching chemosystematic revision of the examined species within the genus *Brachychiton*.

Key words: Brachychiton australis; *Brachychiton discolor*; Malvaceae; LC-ESI-MS; Phenolic acids; Flavonoids

1. Introduction

From ancient times, medicinal plants have been used as nutritional supplements, a source of drugs, and to treat many ailments, established on their usage in folk medicine [1]. In the past few years, there has been exponential growth in the field of herbal medicine. Herbal extracts are less toxic, cheaper, and easily reachable as compared to synthetic drugs. Sometimes, they are considered more effective in disease management [2]. Thus, the study of their chemical constituents has become an important aspect for improving general human health.

The genus *Brachychiton* Schott & Endl. (Malvaceae) has been introduced as remedies for skin disorders, inflammations, and digestive problems. Due to the presence of a wide range of secondary products such as phenolics, terpenoids, alkaloids, and others, *Brachychiton* species produced numerous pharmacological activities including antioxidant, antihyperglycemic, anti-inflammatory, and antimicrobial effects. On the other hand, the genus *Brachychiton* was previously classified as a section of the important medicinal genus (*Sterculia* L.). However, botanical and chemical findings supported its splitting as a separate distinct genus [3, 4].

Brachychiton australis (Schott and Endl.) A. Terrac. [Syn. *Sterculia australis* (Schott & Endl.) Druce, *Brachychiton platanoides* R.Br.] and *Brachychiton discolor* F. Muell. [Syn. *Sterculia discolor* (F. Muell.) Benth., *Brachychiton luridum* C.Moore] are ornamental plants native to eastern Australia. They are cultivated and widely planted in Egypt. Some biological activities were reported for both species; hypoglycemic [5], anti-allergic, anti-inflammatory, antimicrobial, and cytotoxic effects [6, 7].

Despite the importance of these species traditionally and biologically, only one publication dealt with the chemical constituents of *B. australis* reporting the isolation of quercetin, quercetin 3-*O*-glucoside, isorhamnetin 3-*O*-rutinoside, scopoletin, *ß*-amyrin acetate, lanosterol, lupeol, and oleanolic acid in addition to the identification of some steroids and fatty acids [5]. For *B. discolor*, their chemical reports characterize quercetin, quercitrin, rhamnetin,

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Receive Date: 09 May 2021, Revise Date: 13 May 2021, Accept Date: 19 May 2021

DOI: 10.21608/EJCHEM.2021.75834.3712

(8Z, 11Z, 14Z)-heptadecatrienal, and palmitic acid [7, 8].

Little reports concerning the chemical contents of both species designed this study to get in-depth and investigate further phytochemical principles responsible for biological effects. The current study was performed on chromatographic and spectroscopic analyses in addition to LC-ESI-MS profiling of *B. australis* and *B. discolor* as in approaches to further biological and chemosystematics studies.

2. Experimental

General

Column chromatography (CC) was achieved on Polyamide 6S (Riedel-De-Haen AG, Seelze Haen AG, Seelze Hanver, Germany) and Sephadex LH-20 (Pharmazia, Uppsala, Sweden). Paper chromatography (PC) No. 1 and 3 MM (Whatman Ltd. Maidstone, Kent, England, 46 x 57 cm) were used for the isolation of pure compounds. Solvents: distilled water, methanol (HPLC grade, Fisher Chemical, UK), acetic acid and petroleum ether (40–60°C) (Adwic, Cairo, Egypt) and n-butanol (St. Louis, MO, USA). Solvent systems: 15% HOAc (H_2O: acetic acid, 85:15) and BAW (n-butanol: acetic acid: H_2O, 4:1:5, upper layer). Acid hydrolysis (2N HCl, 2 hrs, 100 °C). Rotary evaporator (HEIDOLPH 4000, Germany), Water distiller (Hamilton, Westwood industrial Estate) and Water bath (labtech) are used in the laboratory work. ^{1}H-NMR; Jeol EX-500 spectrometer: 500 MHz (^{1}H NMR), 125 MHz (^{13}C NMR) and Bruker 400 MHz (^{1}H NMR), 100 MHz (^{13}C NMR). UV; UV spectrophotometer (Shimadzu UV-240). LC-ESI-MS; Waters Alliance 2695 (HPLC) and Waters 3100 (mass spectrometry). The research group of Phytochemistry and Plant Systematics Department (NRC) supplied the authentic reference samples.

Plant material and extraction

B. australis and *B. discolor* were collected from Orman Botanical Garden, Giza, Egypt in January 2016. The species were kindly authenticated by Dr. Mohamed El Gibaly. Voucher specimens (no. AY116_1 & AY116_2, respectively) were kept in the herbarium of the National Research Centre, Giza, Egypt. Leafy branches of both species were air-dried yielding about 2.1 and 1.7 Kg powder, respectively. They were extracted by repeated maceration with 70% methanol/water (Temp. 40-60°C) till exhaustion affording 93 g *B. australis* (BA) and 82 g *B. discolor* (BD) dried residues.

Phytochemical Profiling

Phenolics isolation

The defatted aqueous methanol extract *B. australis* (89 g) and *B. discolor* (78 g) residues were applied each to a polyamide column. Stepwise gradient elution was started with water then the percentage of methanol was increased to decrease the polarity. Fractions of 200 mL were collected and the similar were combined based on their paper chromatography properties using BAW, 15% AcOH, and distilled water as solvent systems. Six main fractions were yielded for each *B. australis* (BA1- BA6) and *B. discolor* (BD7- BD12). BA1 (100% H_2O; 16 g) was subjected to Sephadex CC using water followed by preparative paper chromatography (PPC) twice using BAW to yield compounds **I** (23 mg) and **II** (19 mg). BA2 (20% MeOH/H_2O; 7 g) & BA3 (40% MeOH/H_2O; 8 g) were separately chromatographed on Sephadex columns using H_2O: MeOH (1:1) then PPC using BAW followed by H_2O yielding compounds **III** (18 mg) and **IV** (15 mg), respectively. BA4 (60% MeOH/H_2O; 7 g) & BA5 (80% MeOH/H_2O; 8 g) were purified by PPC using BAW then 15% HOAc followed by Sephadex CC to give compounds **V** (13 mg), **VI** (22 mg), **VII** (20 mg). BA6 (100% MeOH; 10 g) was rechromatographed over PPC using BAW followed by application on Sephadex CC using methanol as eluent and yielded compounds **VIII** (8 mg). BD7 (100% H_2O; 18 g) was chromatographed over Sephadex CC using methanol followed by PPC using15% HOAc to yield compound **IX** (14 mg). BD8 (80% MeOH/H_2O; 16 g) was subjected to PPC using 15% HOAc followed by BAW to yield compound **X** (8 mg). Compound **XI** was obtained from BD9 (40% MeOH/H_2O; 11 g) though Sephadex CC [H_2O:MeOH (1:1)] while compound **XII** was gained from BD10 (60% MeOH/H_2O; 24 g) by PPC using twice BAW followed by Sephadex CC and methanol as eluent. Structure elucidation was established on the basis of physical properties, R_f values, and color reactions. Chemical degradation methods included acid hydrolysis (2N HCl, 2 hrs, 100 °C) for *O*-glycosides, $FeCl_3$ degradation for *C*-glycoside sugar units (0.2 g $FeCl_3$ in 0.8 ml water, refluxed 6 hrs) and alkaline hydrolysis (2M NaOH, 2 hrs, room temp.) for cinnamic acids. Spectroscopic (UV, ^{1}H- & ^{13}C-NMR) and spectrometric (ESI-MS) techniques were applied. The results were confirmed by comparison of these data with previous literature reports and/or authentic standards [9, 10]. (Mabry et al. 1970; Markham 1982). The spectral data of the isolated compounds were recorded as follow:

*Compound **(I), Apigenin 6, 8-di-C-β-glucopyranoside (vicenin)*** [11]: Yellow amorphous powder, R_f 0.44 (BAW). UV, spectral data, λ_{max} (nm): MeOH: 274, 329. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 7.99 (2H, d, J = 8.8 Hz, H-2′, H-6′),

6.86 (2H, d, *J* = 8.8 Hz, H-3', H-5′), 6.78 (1H, s, H-3), 4.72 (1H, d, *J* = 9.6 Hz, H-1‴), 4.67 (1H, d, *J* = 9.6 Hz, H-1″), 3-4 (10H, m, H-2″- H-6″, H-2‴- H-6‴). ESI-MS [M-H]⁻: 593.2.

Compound ***(II)*****, *Apigenin 6-C-β-glucopyranoside 8-C-α-arabinopyranoside (schaftoside)*** [12]: Faint yellow powder, R_f 0.23 (BAW). UV, spectral data, λ_{max} (nm): MeOH: 272, 333. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 7.83 (2H, d, *J* = 8.6 Hz, H-2′, H-6′), 6.84 (2H, d, *J* = 8.6 Hz, H-3', H-5′), 6.35 (1H, s, H-3), 4.57 (1H, d, *J* = 8.6 Hz, H-1‴), 4.51 (1H, d, *J* = 9.15 Hz, H-1″), 3-4 (9H, m, H-2″- H-6″, H-2‴- H-5‴). ESI-MS [M-H]⁻: 563.1.

Compound ***(III)*****, *Apigenin 6-C-β-glucopyranoside 8-C-α-rhamnopyranoside (violanthin)*** [13]: Yellow powder, R_f 0.34 (BAW). UV, spectral data, λ_{max} (nm): MeOH: 273, 334. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 7.7 (2H, d, *J* = 8.5 Hz, H-2′, H-6′), 6.9 (2H, d, *J* = 8.5 Hz, H-3', H-5′), 6.4 (1H, s, H-3), 4.9 (1H, d, *J* = 2.0 Hz, H-1‴), 4.6 (1H, d, *J* = 9.2 Hz, H-1″), 3-4 (9H, m, H-2″- H-6″, H-2‴- H-5‴), 1.1 (1H, d, *J* = 6.0 Hz, H-6‴). ESI-MS [M-H]⁻: 577.3.

Compound ***(IV)*****, *Apigenin 6-C-α-rhamnopyranoside 8-C-β-glucopyranoside (isoviolanthin)*** [13]: Yellow powder, R_f. 0.39 (BAW). UV, spectral data, λ_{max} (nm): MeOH: 272, 334. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 7.95 (2H, d, *J* = 8.8 Hz, H-2′, H-6′), 6.85 (2H, d, *J* = 8.8 Hz, H-3',5′), 6.68 (1H, s, H-3), 4.93 (1H, d, *J* = 1.8 Hz, H-1″), 4.72 (1H, d, *J* = 9.6 Hz, H-1‴), 3-4 (9H, m, H-2″- H-5″, H-2‴- H-6‴), 0.81 (1H, d, *J* = 6.0 Hz, H-6″). ^{13}C-NMR (125 MHz in DMSO-d_6, δ, ppm): δ 163.9 (C-2), 102.7 (C-3), 182.1 (C-4), 158.1 (C-5), 108.8 (C-6), 160.1 (C-7), 105.6 (C-8), 155.8 (C-9), 102.9 (C-10), 122.3 (C-1′), 129.7 (C-2′), 116.3 (C-3′), 161.6 (C-4′), 116.3 (C-5′), 129.7 (C-6′),77.7 (C-1″), 74.7 (C-2″), 74.6 (C-3″), 72.7 (C-4″), 72.8 (C-5″), 18.8 (C-6″), 73.9 (C-1‴), 71.4 (C-2‴), 79.4 (C-3‴), 71.2 (C-4‴), 82.4 (C-5‴), 61.9 (C-6‴). ESI-MS [M-H]⁻: 577.

Compound ***(V)*****, *Luteolin 7-O-β-glucuronide.*** [14]: Yellow powder, R_f. 0.13 (BAW). UV spectral data, λ_{max} (nm): MeOH: 255sh, 268, 347. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): δ 7.43 (1H, d, *J* = 1.8 Hz, H2′), 7.41 (1H, dd, *J* = 1.8, 8.5 Hz, H-6′), 6.9 (1H, d, *J*= 8.5 Hz, H-5′), 6.76 (1H, d, *J*=2.0 Hz, H-8), 6.71 (1H, s, H3), 6.41 (1H, d, *J* = 2.0 Hz, H-6), 5.16 (1H, d, *J*= 7.6 Hz, H-1″), 3-4 (5H, m, H-2″- H-6″). ESI-MS [M-H]⁻: 461.1.

Compound ***(VI)*****, *Apigenin 7-O-β-glucuronide*** [15]: Yellow powder, R_f 0.19 (BAW). UV spectral data, λ_{max} (nm): MeOH: 269, 332. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 7.93 (2H, d, *J* = 8.7 Hz, H-2′, H-6′), 6.91 (2H, d, *J* = 8.7 Hz, H-3′, H-5′), 6.84 (1H, s, H-3), 6.76 (1H, d, *J* = 2.0 Hz, H-8), 6.41 (1H, d, *J* = 2.0 Hz, H-6), 5.12 (1H, d, *J* = 7.5 Hz, H-1″), 3-4 (5H, m, H-2″- H-6″). ESI-MS [M-H]⁻: 445.

Compound ***(VII)*****, *Apigenin 7-O-β-glucopyranoside*** [13]: Yellow powder, R_f. 0.51 (BAW). UV spectral data, λ_{max} (nm): MeOH: 267, 332. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 7.82 (2H, d, *J* = 8.7 Hz, H-2′,H-6′), 6.85 (2H, d, *J* = 8.7 Hz, H-3′, H-5′), 6.75 (1H, s, H-3), 6.72 (1H, d, *J* = 2.0 Hz, H-8), 6.36 (1H, d, *J* = 2.0 Hz, H-6), 5.39 (1H, d, *J* = 7.7 Hz, H-1″), 3-4 (5H, m, H-2″- H-6″). ESI-MS [M-H]⁻: 431.

Compound ***(VIII)*****, *Apigenin 4'-methylether (acacetin)*** [16]: Yellow amorphous powder, R_f. 0.7 (BAW). UV spectral data, λmax (nm): MeOH: 269, 303,326. ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): δ 8.01 (2H, d, *J* = 8.5 Hz, H2′, H-6′), 7.09 (2H, d, *J* = 8.5 Hz, H-3′, H-5′), 6.8 (1H, s, H-3), 6.41 (1H, d, *J* = 2.0 Hz, H-8), 6.12 (1H, d, *J* = 2.0 Hz, H-6), 3.85 (1H, s, OCH_3). ESI-MS [M-H]⁻: 283.1.

Compound ***(IX)*****, *trans-p-Coumaric acid-O-glucoside*** [17]: White amorphous powder, R_f. 0.51 (BAW). UV spectral data, λ_{max} (nm): MeOH: 267, 332. ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm, *J*/Hz): 7.57 (2H, d, *J* = 8.44 Hz, H-2, H-6), 7.37 (1H, d, *J* = 15.84 Hz, H-7), 6.66 (2H, d, *J* = 8.32 Hz, H-3, H-5), 6.24 (1H, d, *J* = 15.92 Hz, H-8), 5.09 (1H, d, *J* = 7.8 Hz, H-1′), 3.5-4.0 (5H, overlapped with OH groups, H-2′-6′). ESI-MS [M-H]⁻: 325.2.

Compound ***(X)*****, *trans-p-Coumaric acid*** [18]: White crystals, R_f. 0.3 (BAW). UV spectral data, λ_{max} (nm): MeOH: 284. ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm, *J*/Hz): 7.5 (2H, d, *J* = 8.4 Hz, H-2, H-6), 7.3 (1H, d, *J* = 15.8 Hz, H-7), 6.6 (2H, d, *J* = 8.3 Hz, H-3, H-5), 6.2 (1H, d, *J* = 15.9 Hz, H-8). ESI-MS [M-H]⁻: 163.

Compound ***(XI)*****, *trans-5-O-Caffeoylquinic acid*** [19]: White amorphous powder, R_f. 0.6 (BAW). UV spectral data, λ_{max} (nm): MeOH: 278. ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm, *J*/Hz): Caffeoyl moiety; 7.36 (1H, d, *J* = 15.82 Hz, H-7′), 6.99 (1H, s, H-2′), 6.94 (1H, d, *J* = 8.6 Hz, H-6′), 6.72 (1H, d, *J* = 8.6 Hz, H-5′), 6.09 (1H, d, *J* = 15.3 Hz, H-8′). Quinic acid moiety; 5.03 (1H, m, H-5), 3.88 (1H, m, H-3), 3.53 (1H, m, H-4), 1.97 (2H, m, H-2), 1.76 (2H, m, H-6).^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): δ 175.4 (C-7), 166.3 (C-9′), 148.9 (C-4′), 146.1 (C-3′), 145.4 (C-7′), 126.2 (C-1′), 121.9 (C-6′) , 116.3 (C-5′), 115.3 (C-2′), 114.8 (C-8′), 74.1 (C-1), 71.4 (C-4), 71 (C-5), 68.7 (C-3), 38.5 (C-6), 37.1 (C-2). ESI-MS [M-H]⁻: 353.

Compound ***(XII)*****, *Quercetin 3-O-β-rutinoside (rutin)*** [16]: Yellow powder, R_f. 0.45 (BAW). UV spectral data, λ_{max} (nm): MeOH: 257, 358. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): δ 7.50 (2H, multiplet, H-2′, H-6′), 6.80 (1H, d, *J* = 8.6 Hz, H-5′), 6.34 (1H, d, *J* = 2.0 Hz, H-8), 6.15 (1H, d, *J* = 2.0 Hz, H-6), 5.11 (1H, d, *J* = 7.5 Hz, H-1″), 4.33 (1H, d, *J* = 2.0 Hz, H-1‴), 3-4 (9H, m, H-2″- H-6″, H-2‴- H-6‴), 0.95 (3H, d, *J* = 5.6 Hz, H-6‴). ESI-MS [M-H]⁻: 609.2.

LC-ESI-MS Assay

The chemical constituents of *B. australis* and *B. discolor* were investigated through LC-ESI-MS by the method of Farid et al. [20]. The isolated compounds in previous studies by the members of the Phytochemistry and Plant Systematics department in addition to the isolated compounds in the present study were used as authentic samples. Comparing their retention times and mass fragmentations confirmed the known peaks. The mass fragmentation values of the remaining peaks were checked with the previously published works.

3. Results and discussion

Twelve phenolic compounds were isolated and identified from *B. australis* and *B. discolor* after the chromatographic examination (Figure 1). Their physical (R_f, color reactions) and chemical properties (acid & alkaline hydrolysis, $FeCl_3$ oxidation) beside the spectral data (UV, ^{1}H- & ^{13}C-NMR, ESI-MS) confirmed the elucidated structures. In addition, they were co-chromatographed with authentic compounds and the resultant spectral values were matched with previous publication reports. They were specified as eight flavonoids namely; apigenin 6, 8-di-*C*-*β*-glucopyranoside (vicenin) (**I**), apigenin 6-*C*-*β*-glucopyranoside 8-*C*-*α*-arabinopyranoside (schaftoside) (**II**), apigenin 6-*C*-*β*-glucopyranoside 8-*C*-*α*-rhamnopyranoside (violanthin) (**III**), apigenin 6-*C*-α-rhamnopyranoside 8-*C*-*β*-glucopyranoside (isoviolanthin) (**IV**), luteolin 7-*O*-*β*-glucuronide (**V**), apigenin 7-*O*-*β*-glucuronide (**VI**), apigenin 7-*O*-*β*-glucopyranoside (**VII**) and apigenin 4'-methylether (acacetin) (**VIII**) for *B. australis*. In addition to three phenolic acid derivatives; *trans*-*p*-coumaric acid-*O*-glucoside (**IX**), *trans*-*p*-coumaric acid (**X**), and chlorogenic acid (5-*O*-*trans* caffeoylquinic acid) (**XI**) as well as one flavonol glycoside namely; quercetin 3-*O*-*β*-rutinoside (rutin) (**XII**) were isolated from *B. discolor*. Excluding rutin, this is the first report for these compounds in *B. australis* and *B. discolor*.

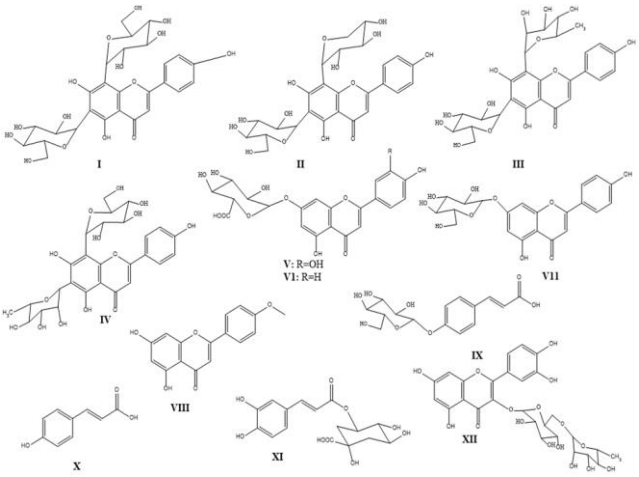

Figure 1: Structures of the compounds isolated from *B. australis* and *B. discolor*

Analysis of *B. australis* and *B. discolor* through LC-ESI-MS (negative ion mode) revealed tentative identification of thirty one compounds, eight of which (peaks 1-5, 7, 31, and 32) were common for both species (Table 1, Figure 2). The twelve isolated compounds were represented as peaks 10, 13, 14, 16-18, 20, 22, 26-28, and 30. Additional nineteen peaks were tentatively characterized (eight flavonoids, five phenolic acids, two organic acids, two fatty acids, one saccharide, and one amino acid), all of them were detected for the first time in *B. australis* and *B. discolor* extracts.

Table 1: Tentative identification of chemical compounds by LC-ESI-MS in *B. australis* (BA) and *B. discolor* (BD)

Peak No.	*Rt* (min)	$[M-H]^-$	Fragments *m/z*	Tentative identification	BA	BD	Ref
1	2.25	131	113, 87,70	Asparagine	+	+	[21]
2	2.75	341	179, 129,113	Dihexoside	+	+	[22]
3	3.42	191	111	Citric/Isocitric acid	+	+	[23]
4	3.75	317	165, 79, 62	Unknown	+	+	
5	4.59	191	129, 111, 93, 85	Quinic acid	+	+	[23]
6	13.03	315	153, 152, 109, 108	Dihydroxy benzoic acid-*O*-hexoside	-	+	[24]
7	14.11	153	109, 108	Dihydroxy benzoic acid	+	+	[24]
8	18.45	163	119	Coumaric acid isomer	-	+	[25]
9	20.04	401	341, 311, 163, 119, 93	Apigenin-*C*-pentoside	+	-	[26]
10	21.96	325	163, 119	*trans*-*p*-Coumaric acid-*O*-glucoside*	-	+	[27]
11	22.12	417	357, 327	Luteolin-*C*-pentoside	+	-	[26]
12	25.38	405	243,163,119	Coumaric acid-*O*-sulfate-*O*-glucose	+	-	[25]
13	27.38	593	503, 473	Apigenin 6,8-di-*C*-*β*- glucopyranoside (Vicenin)*	+	-	[28]
14	28.39	577	457, 487, 473, 341, 311	Apigenin 6-*C*-rhamnose -8-*C*- glucose (Isoviolanthin)*	+	-	[26]
15	29.39	563	503, 489, 473, 459	Luteolin-*C*-pentoside-*C*-deoxyhexoside	+	-	[26]
16	29.89	353	191, 119	5-*O*-*trans* Caffeoylquinic acid *	-	+	[23]

17	30.72	163	327, 119	*trans-P*-Coumaric acid*	-	+	[25]
18	32.23	563	503, 473, 443	Apigenin 6-*C*-*β*-glucopyranoside 8-*C*-α-arabinopyranoside (Schaftoside)*	+	-	[28]
19	32.73	563	549, 503, 473, 399, 369	Luteolin di-*C*-pentoside methyl ether	+	-	[29]
20	33.65	577	503, 487, 473, 457	Apigenin 6-*C*-*β*-glucopyranoside 8-*C*-*α*-rhamnopyranoside (Violanthin)*	+	-	[28]
21	34.73	533	447, 285	Luteolin-malonyl-hexoside	+	-	[30]
22	35.65	461	285	Luteolin 7-*O*-*β*-glucuronide*	+	-	[31]
23	36.57	503	371, 357, 283	Pentamethoxy flavone-*O*-pentoside	+	-	[32]
24	37.49	515	353, 191, 179, 173, 147, 105	Dicaffeoyl quinic acid	+	-	[33]
25	38.41	591	269, 163	Apigenin 7-*O*-coumaroyl glucuronide	+	-	
26	38.57	609	301, 225, 209	Rutin*	-	+	[31]
27	39.12	431	269	Apigenin 7-*O*-*β*-glucopyranoside *	+	-	[31]
28	39.49	445	269	Apigenin 7-*O*-*β*-glucuronide *	+	-	[31]
29	42.58	489	343, 299	Dihydroxy trimethoxy flavone-*O*-deoxyhexoside	+	-	[32]
30	43.58	283	299	Acacetin*	+	-	[32]
31	53.94	329	311, 229, 211, 171, 137	Trihydoxy octadecenoic acid (isomer I)	+	+	[34]
32	54.78	329	311, 229, 211, 171, 137	Trihydoxy octadecenoic acid (isomer II)	+	+	[34]

* Compounds identified by comparing their retention times and mass spectrum with the authentic

Amino acid, disaccharide and fatty acids

Peak 1 was tentatively identified as asparagine, based on the molecular ion peak *m/z* 131 $[M-H]^-$ and product ion at *m/z* 113 $[M-H-18\ (H_2O)]^-$ and 87 $[M-H-18\ (H_2O)-26\ (CN)]^-$. Peak (2) was recognized as a saccharide residue (*m/z* 341) based on fragment ions *m/z* 179, 129, and 113. Two fatty acids were noticed as peak 31 and 32 (*m/z* 329) and proposed to be trihydroxy octadecenoic acid isomers, illustrated by the presence of product ion at *m/z* 171 $[OOC(CH_2)_7CH\text{-}OH]^-$ and the neutral loss of 100 amu $(HO\text{-}CH{=}CH(CH_2)_3CH_3)$ (*m/z* 329-229) and (*m/z* 311-211). The compounds are common in both species.

Organic acids

Two similar molecular ion peaks (*m/z* 191) representative for peak 3 (citric/isocitric acid) and peak 5 (quinic acid) were observed in both extracts. They have been distinguished through the fragment signals at *m/z* 111 $[M-H-CO_2-2H_2O]^-$ and 129 $[M-H-CO_2-H_2O]^-$, respectively.

Phenolic acid derivatives

Together with the three isolated phenolic acid derivatives, five additional products were distinguished and characterized for the base peak chromatogram of *B. discolor* (Figure 2B). Dihydroxy benzoic acid-*O*-hexoside (6) was predicted at 315 $[M-H]^-$ with ion at *m/z* 153 $[M-H-162]^-$ corresponded to the loss of a hexose moiety and *m/z* 109 $[M-H-162-44]^-$, as well as the dihydroxybenzoic acid (*m/z* 153) was presented as peak 7. The pseudomolecular ion of peak 8 indicated a coumaric acid isomer at *m/z* 163 $[M-H]^-$ and *m/z* 119 $[M-H-CO_2]^-$. Peak 12 (*m/z* 405) produced daughter ions at *m/z* 243 (loss of hexose unit) and 163 (loss of hexose unit and sulphate group) to produce coumaric acid. Thus, it was tentatively identified as coumaric acid-*O*-sulfate-*O*-glucose. Peak 24 (*m/z* 515) was identified as dicaffeoylquinic acid, confirmed by the presence of fragment ions 353 $[M\text{-caffeic acid-H}]^-$, 191 $[\text{quinic acid-H}]^-$, 179 $[\text{caffeic acid-H}]^-$ and 173 $[\text{quinic acid-}H_2O\text{-H}]^-$.

Flavonoid glycosides

In addition to the four isolated *C*-glycoside flavones (compounds **I**-**IV**), another four derivatives were observed in the base peak chromatogram of *B. australis* (Figure 2A) and tentatively identified as apigenin-*C*-pentoside (9), luteolin-*C*-pentoside (11), luteolin-*C*-pentoside-*C*-deoxyhexoside (15), and luteolin-di-*C*-pentoside methyl ether (19). The saccharide nature of the *C*-pentoside and *C*-deoxyhexoside moieties were illustrated through the fragment ions of $[M-H-90/60]^-$ and $[M-H-104/74]^-$, respectively. In addition, the sugar parts kept linked to aglycones demonstrating peaks $[\text{Aglycone}+71/41]^-$ and $[\text{Aglycone}+113/83]^-$ for mono- and di-*C*-glycosides, respectively. Peak 9 (*m/z* 401) has been constructed fragment peaks at *m/z* 341 ($[\text{Apigenin}+71]^-$, $[M-H-60]^-$) and 311 ($[\text{Apigenin}+41]^-$, $[M-H-60]^-$), while peak 11 (*m/z* 417) exhibited fragment ions at 357 ($[\text{Luteolin}+71]^-$, $[M-H-60]^-$) and 327 ($[\text{Luteolin}+41]^-$, $[M-H-90]^-$). The daughter ions of peak 15 (*m/z* 563) appeared at 503 $[M-H-60]^-$, 473 $[M-H-90]^-$ indicating *C*-pentoside attachment and further fragments at *m/z* 489 $[M-H-74]^-$, 459 $[M-H-104]^-$ specifying the loss of *C*-deoxyhexose unit. Peak 19 (*m/z* 563) showed the matching fragmentation pattern (503 and 473) with an extra fragment at *m/z* 549 $[M-H-14]^-$ in addition to the fragment ions at 399 [luteolin+113] and 369 [luteolin+83] fit with di-*C*-pentosyl luteolin methyl ether.

Other four molecular ion peaks constituted flavone *O*-glycosides (peaks 21, 23, 25 & 29) were also characterized for *B. australis* (Figure 2A). Peak 21 (luteolin-malonyl-hexoside: *m/z* 533) yielded fragment ion at *m/z* 447 $[M-H-86]^-$ after losing the malonyl moiety and then *m/z* 285 $[M-H-86-162]^-$ due to loss of a hexose unit. Peak 25 (*m/z* 591) showed deprotonated apigenin aglycone fragment at *m/z* 269 $[M-H-146-176]^-$ after cleavage of coumaroyl glucuronide moiety and could be identified as apigenin 7-*O*-coumaroyl glucuronide (acyl derivative of compound **VI**). Furthermore, methoxylated flavones; peaks 23 (*m/z* 503) and 29 (*m/z* 489) lost the glycosyl moieties giving fragment ions *m/z* 371 $[M-H-132]^-$ (pentosyl) and *m/z* 343 $[M-H-146]^-$ (deoxyhexosyl), respectively. Based on characteristic ions generated by successive losing CH_2 (-14 Da) from their methoxy groups, they were tentatively identified as pentamethoxy flavone-*O*-pentoside and dihydroxy trimethoxy flavone-*O*- deoxyhexoside, respectively.

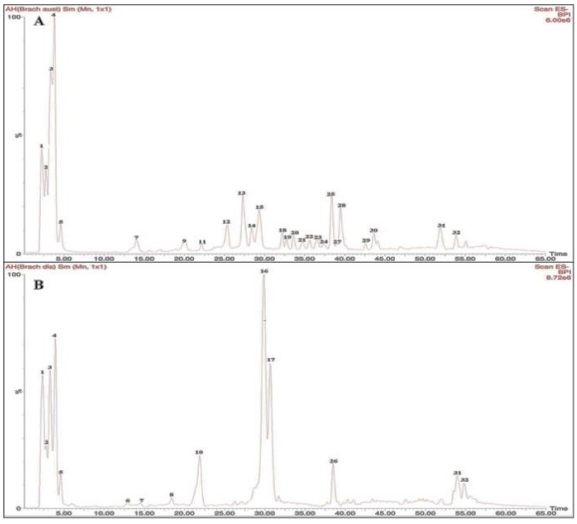

Figure 2: Base Peak Chromatogram of LC-ESI-MS (negative ion mode) of A: *B. australis*, B: *B. discolor*

B. australis discriminated by a relatively high concentration of *C*-glycosyl flavones. The stability of these compounds elevated from the resistant *C–C* glycosidic bonds to acidic hydrolytic conditions achieving additional bioavailability and numerous pharmacological activities, *viz.*, antioxidant, anticancer, antihyperglycemic, antihyperlipidemic, anti-inflammatory, antimicrobial, and others [35]. Vicenin assessed significant anti-inflammatory, antiseptic, anticoagulation, and marked anticancer properties [36], as well as schaftoside exhibited antioxidative stress, antiobesity, antiviral, and pronounced anti-inflammatory effects. Also, violanthin exerted antioxidant, anticholinesterase and suggested as a strong antiviral compound that could inhibit COVID-19 compared to the standard drug [37], whereas isoviolanthin verified as an anticancer agent [38].

However, some findings supported that *C*-glycosylation of the ring-A of the flavonoids reduced their antioxidant activity compared to their corresponding flavonoid *O*-glycosides and aglycones due to the effect of the sugar unit. Moreover, glycosylation of C-6 and -8 inhibited α-glucosidase enzyme. Other researches supported the enhanced scavenging activity of flavonoids by *C*-glycosylation. Investigations quantified that *O*- or *C*-glycosylation as well as type, number, and position of the glycosidic moieties affect the stability and activity of glycosides [39].

Conversely, various phenolic acid derivatives were determined in *B. discolor* which displayed a potential antioxidant effect and afforded antitumor and anti-inflammatory effects as they act as hydrogen-donating compounds scavenging the reactive oxygen and nitrogen species. Besides, A wide range of pathological activities possessed by chlorogenic acids (antihyperglycemic, antiobesity, antimicrobial, and hepatoprotective) [40] and coumaric acid isomers (atherosclerosis, antiulcer, anti-platelet, and skin protection) [41].

On the other hand, from a chemotaxonomic point of view, biochemical compounds produced by a plant could serve as markers and their pattern could help in comparison and significance of the species. For example, the abundance of *C*-glycoside flavonoids in *B. australis* extract provides additional information to further knowledge on its intergeneric relationship within the genus *Brachychiton*. Our previous chemotaxonomic investigation going on the five *Brachychiton* species growing cultivated in Egypt reported the absence of such compounds in *B. populneus* [4]. Besides, the richness of phenolic acid derivatives in the present study was characterized for *B. discolor* among the *Brachychiton* species.

4. Conclusions

Eight flavonoids were isolated and identified for the first time, from the aqueous methanol leaves extract of *B. australis*. Moreover, three phenolic compounds and rutin were isolated from *B. discolor* extract, the latter compound was reported before from *B. discolor*. In addition, LC-ESI-MS assay characterized extra eighteen chemical compounds. It is the first record providing a full fingerprint of chemical metabolites distributed within the two species which in turn afforded considerable chemical variations between them. However, more detailed biological studies have to be followed to explore those promising plants as phytoceutical agents to manage and handling human diseases. Furthermore, the

currently reported compounds could be valuable for upcoming chemosystematic reconsideration of the two species within the genus *Brachychiton*.

5. Conflicts of interest

There are no conflicts to declare.

6. Acknowledgments

The work is funded by National Research Centre, Cairo, Egypt, project number 11010328.

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