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Chemical constituents from *Chorisia chodatii* flowers and their biological activities

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Abstract From the flowers of *Chorisia chodatii* Hassl. (family: Bombacaceae), seventeen compounds were isolated and identified, including: two sterols, β-sitosterol (**1**) and β-sitosterol 3-*O*-β-D-glucopyranoside (**10**); two furanoids, 5-hydroxymethyl furfural (**3**) and (3*R*,4*R*,5*S*)-3,4-dihydroxy-5-methyl-dihydrofuran-2-one (**12**); two coumarins, scopoletin (**8**) and aesculetin (**9**); four phenolic acids and esters, ethyl vanillate (**4**), vanillic acid (**5**), protocatechuic acid ethyl ester (**6**) and *p*-hydroxy benzoic acid (**7**); five flavonoids, kaempferol 3-*O*-β-D-(6″-acetyl)-glucopyranoside (**13**), kaempferol 3-*O*-β-D-(6″-*E*-*p*-coumaroyl)-glucopyranoside (**14**), kaempferol 3-*O*-β-D-glucopyranoside (**15**), luteolin 7-*O*-β-D-glucopyranoside (**16**) and apigenin 7-*O*-neohesperidoside (**17**), in addition to mono-octyl phthalate (**2**) and succinic acid (**11**). All the isolated metabolites were reported for the first time from this plant, and among them, compounds (**3**), (**4**), (**6**), (**7**), (**12**) and (**13**) were isolated for the first time from family Bombacaceae. Besides, this is the first report for isolation of (**2**) in a pure form from a natural source. These phytochemical data revealed important chemotaxonomic value and may broaden the use of this plant in future phytotherapy. Moreover, all of the obtained phytocompounds were evaluated for their DPPH free radical scavenging properties and cytotoxic activities against the human lung cancer cell line A549.

Keywords Bombacaceae · Chorisia · Cytotoxicity · DPPH · Flavonoids · Phenolics

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Introduction

Oxidative stress represents a common and threatening health theme that is associated with pathogenic mechanisms of many degenerative diseases including aging, Alzheimer's, atherosclerosis, neurodegeneration and cancer (Polterait, 1997). Free radicals produced on disturbance of normal redox state in biological systems can harmfully attack several biomolecules like proteins, lipids and DNA, resulting in cell membrane damage, a decrease in membrane fluidity and DNA mutations leading to cancer (Pietta, 2000). Potent scavengers of these free radical species such as plant polyphenols, flavonoids, carotenoids, tocopherols and antioxidant enzymes may serve as a possible preventive intervention for free radical-mediated diseases, suggesting that natural antioxidants could be effective agents for inhibiting the spread of cancers (Ames *et al.*, 1995). Lung cancer is now considered the most common cancer worldwide, comprising 17 % of cancers in men (twice the worldwide rate of the second-most common cancer in men, prostate cancer) and the third-most common cancer in women worldwide, after breast and colorectal cancers (Ancuceanu and Istudor, 2004). Despite some progress in recent decades, lung cancer remains the leading cause of death from cancer in the world (Strassheim *et al.*, 2000).

Chorisia is a genus of deciduous trees, belongs to family Bombacaceae and comprises about twenty species. It occurs naturally throughout the tropical and subtropical regions of the world, especially South America (Huxley, 1992). These plants are traditionally used for many health disorders, e.g., headache, fever, diabetes, diarrhea, parasitic infections, peptic ulcer and rheumatism (Adjanohoun, 1988). *Chorisia* is mainly cultivated for its ornamental brilliant flowers since it blooms best during months of autumn, adding a touch of color at the time when most blooms are fading. It is also cultivated for the silky white fiber that is obtained from the ripened seeds. Economically, this floss has been used to stuff cushions, pillows and vests, and for thermal insulation at times, which explains the common name of this tree "silk floss tree." Moreover, the wood of these trees is employed in packaging, to make canoes, ropes and as wood pulp to make paper. Likewise, the vegetable oil obtained from the seeds is both edible and industrially useful. Some species are also added to some versions of the hallucinogenic drink Ayahuasca (Bailey, 1976; Huxley, 1992). *Chorisia* is characterized by a bottle-shaped trunk generally bulging in its lower third, measuring up to two meters in girth and is studded with thick conical sharp spines as an adaptation for dry climates to store water, so it is commonly named as "bottle tree" (Ravenna, 1998).

Biologically, it was reported that some *Chorisia* species possess wide range of useful anti-inflammatory, hepatoprotective, cytotoxic, antioxidant, hypoglycemic and antimicrobial effects with high safety margins and LD_{50} up to 7–8 g/kg in laboratory animals (Hafez *et al.*, 2003; Hassan, 2009; El-Alfy *et al.*, 2010; Ashmawy *et al.*, 2012). On the other hand, a limited number of *Chorisia* species including *C. speciosa* A. St.-Hil., *C. insignis* H.B.K. and *C. crispiflora* H.B.K. were subjected to some phytochemical analyses that provided a number of flavonoids, anthocyanins, sterols, triterpenes and carbohydrates (Refaat *et al.*, 2013). On the contrary, the searched literature data indicated that neither the chemical profile nor the biological potential of *C. chodatii* was previously investigated. Accordingly, these results provoked us to carry out the first phytochemical visit of this unstudied species along with its chemotaxonomic significance, in addition to biological evaluation of the isolated phytoconstituents for their DPPH free radical scavenging activities and cytotoxic effects toward the human lung cancer cell line (A549) by means of MTT assay.

Materials and methods

Chemicals and reagents

Each of the following chemicals and authentic reference materials, with source indicated in parentheses, was purchased (or obtained) and used as-received: β-sitosterol, β-sitosterol 3-*O*-β-D-glucopyranoside, vanillic acid, and succinic acid (Pharmacognosy Department, Faculty of Pharmacy, Minia University, Minia, Egypt). Silica gel 60 (E. Merck, Darmstardt, Germany; 60-120 mesh) was used for column chromatography (CC), whereas silica gel GF_{254} for thin layer chromatography (TLC; El-Nasr Company for Pharmaceuticals and Chemicals, Egypt) was employed for vacuum liquid chromatography (VLC). Pre-coated silica gel 60 GF_{254} plates (E. Merck, Darmstardt, Germany; 20 × 20 cm, 0.25 mm in thickness) were used for TLC analyses. Spots were visualized by spraying with sulfuric acid (10 % in methanol) and heating at 110 °C on a hot plate (Stahl, 1970). Ammonia vapors and spraying with aluminum chloride reagent (5 % in ethanol) were also used for detection of flavonoids on TLC (Markham, 1982), while ferric chloride reagent (1 % in ethanol) was used for phenolic compounds (Smith, 1960). Sodium methoxide, sodium acetate, aluminum chloride and hydrochloric acid were used for UV analysis of flavonoids (Mabry *et al.*, 1970). All chemicals used for preparation of different spraying and UV reagents were purchased from El-Nasr Company for Pharmaceuticals and Chemicals, Egypt. All of the following chemicals and media, with source indicated in parentheses, were used for the biological studies: Dulbecco's modified Eagle's medium, 2,2-diphenyl-1-picrylhydrazyl (DPPH), trolox (Sigma-Aldrich, St. Louis, MO, USA), fetal bovine serum (FBS; GIBCO Life Technologies, NY, USA), doxorubicin (Wako Pure Chemical Industry Co., Ltd., Japan) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT; Nacalai Tesque, Kyoto, Japan). Solvents used in this work, e.g. light petroleum ether (b.p. 60–80 °C), chloroform, ethyl acetate, methanol and ethanol, were purchased from El-Nasr Company for Pharmaceuticals and Chemicals, Egypt. Deuterated solvents, e.g., $CDCl_3$ (Cica, Japan), CD_3OD (Merck, Germany) and DMSO-d_6 (Isotec, Germany) were used for NMR spectroscopic analysis.

Apparatus

Melting points were measured using Stuart Scientific (SMPI) melting point apparatus and were uncorrected. UV spectra of different samples were acquired using a Spectronic® Genesys™ 2PC UV spectrophotometer (Shimazdu, Japan) as solutions in methanol and with different diagnostic UV shift and complexing reagents for flavonoids (Mabry *et al.*, 1970). ^1H and ^{13}C-NMR spectra were recorded in $CDCl_3$, CD_3OD or DMSO-d_6 on JEOL JNM α-400 (400 MHz for ^1H and 100 MHz for ^{13}C) and Bruker AVANCE 600 MHz (600 MHz for ^1H and 150 MHz for ^{13}C) spectrometers with tetramethylsilane as an internal standard. HR-ESI-MS spectra were obtained using a LTQ

Orbitrap XL mass spectrometer. Portable Ultraviolet lamp (UVP, LLC, USA) was used for visualization of spots on thin layer chromatograms at 254 and/or 365 nm.

Plant material

Flowers of *C. chodatii* were collected during September–November 2010 from plants cultivated in the campus of Minia University, Minia, Egypt. Authentication of the plant species was established by Prof. Ahmed Abdel-Monem, Department of Horticulture, Faculty of Agriculture, Minia University, Minia, Egypt. A voucher specimen (Mn-Ph-Cog-001) has been deposited in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Minia University, Minia, Egypt.

Extraction and isolation of different constituents from *C. chodatii* flowers

The air-dried powdered flowers (4.75 kg) were exhaustively extracted with 70 % ethanol (8 L × 10) and concentrated under reduced pressure to a syrupy consistency. The solvent-free residue (690 g) was suspended in distilled water (900 ml) and deprived from its major part of rhoifolin (**17**) that precipitated as yellow powder (31.6 g). After filtration, the precipitate was dissolved in methanol and allowed to crystallize. The aqueous filtrate was then transferred to a separating funnel and successively extracted with light petroleum ether, chloroform and ethyl acetate. The organic phase in each step was separately evaporated under reduced pressure to give the corresponding fractions I (94.3 g), II (12.2 g) and III (43.4 g), respectively. The remaining mother liquor was concentrated to provide the aqueous fraction IV (534.6 g).

A part of fraction I (30 g) was subjected to VLC on silica gel column (6.5 × 30 cm, 500 g). Elution was performed initially using petroleum ether and then with petroleum ether–ethyl acetate gradient mixtures in the order of increasing polarities (10, 20, 50, 70 and 100 %) and finally washed with methanol. The effluents were collected in fractions (250 ml each); each fraction was concentrated under reduced pressure and monitored by TLC. Similar fractions were combined together to provide seven subfractions (I_1–I_7). Upon concentration, subfraction I_3 (12 g) yielded greenish yellow crystals that were purified by recrystallization from methanol to give colorless needles (100.2 mg) of compound **1**.

Similarly, the obtained residue of fraction II (12.2 g) was subjected to VLC on silica gel column (6.5 × 30 cm, 85 g). Elution was performed using petroleum ether–ethyl acetate gradient mixtures in the order of increasing polarities (20, 30, 40, 60 and 100 %) and finally with methanol. The effluents were collected in fractions (50 ml each); each fraction was concentrated under reduced pressure and monitored by TLC. Similar fractions were grouped together and then concentrated under reduced pressure to afford six subfractions (II_1–II_6). Subfraction II_1 (0.3 g) was purified on silica gel column using petroleum ether–ethyl acetate gradient mixtures to provide compound **2** (5.4 mg). Subfraction II_2 (1.6 g) was fractionated on silica gel column using petroleum ether–ethyl acetate gradient mixtures to give four subfractions (II_2-F-1: II_2-F-4). II_2-F-2 was further subjected to silica gel CC using chloroform–methanol gradient elution to yield compounds **3** (40.2 mg) and **4** (12.3 mg). Likewise, II_2-F-3 was subjected to silica gel CC using chloroform–methanol gradient elution to provide compounds **5** and **6** that were recrystallized from methanol to give white needles (19.6 mg) and colorless needles (8.9 mg), respectively. CC fractionation of subfraction II_3 (1.3 g) on silica gel using petroleum ether–ethyl acetate gradient mixtures provided four subfractions (II_3-F-1: II_3-F-4). II_3-F-3 was further subjected to silica gel CC employing gradient elution with chloroform–methanol to yield compound **7** as white needles (31 mg) and compound **8** as white powder (10.7 mg). Subfraction II_4 (1.7 g) was chromatographed on silica gel column using petroleum ether–ethyl acetate gradient mixtures to afford five subfractions (II_4-F-1: II_4-F-5). II_4-F-2 was further subjected to silica gel CC employing isocratic elution with chloroform–methanol (98:2) to yield compound **9** (14.9 mg). Upon concentration, subfraction II_5 (2 g) yielded yellowish white precipitate that was thoroughly washed with different petroleum ether–ethyl acetate mixtures to give white amorphous powder of compound **10** (600 mg).

On the other hand, a part of fraction III (40 g) was subjected to VLC on silica gel column (6.5 × 30 cm, 500 g). Elution was performed initially using petroleum ether–ethyl acetate gradient mixtures in the order of increasing polarities (30, 50, 80 and 100 %), then with ethyl acetate–methanol (90:10), and finally with methanol. The effluents were collected in fractions (100 ml each); each fraction was concentrated under reduced pressure and monitored by TLC. Similar fractions were combined together and concentrated under reduced pressure to afford six subfractions (III_1–III_6). Subfraction III_2 (3.2 g) was subjected to silica gel CC using petroleum ether–ethyl acetate gradient elution to give five subfractions (III_2-F-1: III_2-F-5). Compound **11** (54.6 mg) was obtained from subfraction III_2-F-4 after purification on silica gel column using gradient elution with chloroform–methanol. CC fractionation of subfraction III_3 on silica gel using gradient mixtures of petroleum ether–ethyl acetate, ethyl acetate and then with ethyl acetate–methanol provided seven subfractions (III_3-F-1: II_3-F-7). Subfraction III_3-F-4 was further purified on silica gel CC using chloroform–methanol

gradient elution to yield compound **12** by crystallization as colorless needles (8.6 mg) from III_3-F-4-3. Subfraction III_4 (6.2 g) was chromatographed on silica gel column using gradient elution with petroleum ether–ethyl acetate (1:1), ethyl acetate, ethyl acetate–methanol (8:2) and finally with methanol to afford four subfractions (III_4-F-1: III_4-F-4). Subfraction III_4-F-1 was further subjected to silica gel CC using gradient mixtures of chloroform–methanol to give compound **13** (4.5 mg). Upon concentration, subfraction III_4-F-2 yielded compound **14** as yellowish white powder (2.3 g) that was then crystallized from methanol to give yellow needles. Similarly, 11.5 mg of compound **15** was obtained as yellow powder from subfraction III_4-F-3. Additional amount (47.3 mg) of compound **15** was also obtained after silica gel CC fractionation of subfraction III_5 (10 g) using ethyl acetate–methanol gradient mixtures, followed by precipitation from subfraction III_5-F-1-3. Besides, compound **16** (20 mg) was obtained from subfraction III_5-F-1-4 after silica gel CC fractionation of subfraction III_5-F-1. Likewise, further amount (3.1 g) of compound **17** was also obtained by precipitation from subfraction III_5-F-3. On the other hand, fraction IV was kept for future phytochemical investigation.

DPPH radical scavenging activity

Determination of the free radical scavenging activity was carried out using a quantitative DPPH assay (Matsunami *et al.*, 2011). The absorbance with various concentrations of the test compounds dissolved in methanol (100 µL) in 96-well microtiter plates was measured at 515 nm as A_{blank}. Then, a 200 µM DPPH solution (100 µL) was added to each well, followed by incubation in a dark chamber at room temperature for 30 min. The absorbance was then measured again as A_{sample}. The % inhibition of free radicals was calculated according to the following equation:

$$\% \text{ Inhibition} = \left[1 - \left(A_{sample} - A_{blank}\right)/\left(A_{control} - A_{blank}\right)\right] \times 100$$

where $A_{control}$ is the absorbance of the control reaction mixture containing DMSO and all reagents, except for the test compound. IC_{50} was determined as the sample concentration required to inhibit the formation of the DPPH radical by 50 %. Measurements were carried out in triplicates.

Human cancer cell growth inhibition assay

This assay was performed according to the method described by Phan *et al.* (2006) using the human lung cancer cell line A549 (obtained from the National Institute of Biomedical Innovation JCRB Cell Bank, Japan). The viability of cells was estimated by means of the colorimetric MTT assay. Dulbecco's modified Eagle's medium supplemented with 10 % heat-inactivated FBS and 100 µg/mL of kanamycin was used as the cell culture medium. The test compounds were dissolved in DMSO and then added to the wells of 96-well microtiter plates to the final concentration of 1 %. A549 cells (5×10^3 cells/well) were cultured in a 5 % CO_2 incubator at 37 °C for 72 h, then a MTT solution was added to each well, and the plates were incubated for further 1.5 h. The formed formazan precipitates were then dissolved in DMSO, and the optical density value for each well was measured at 540 nm using a microplate reader. Doxorubicin was used as a positive control. The cell growth inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = \left[1 - \left(A_{sample} - A_{blank}\right)/\left(A_{control} - A_{blank}\right)\right] \times 100$$

where $A_{control}$ is the absorbance of the control reaction mixture containing DMSO and all reagents except for the test compound. IC_{50} was determined as the sample concentration required to inhibit the formation of MTT formazan by 50 %.

Results and discussion

Identification of compounds (**1**–**17**)

Structures of the isolated compounds (Fig. 1) were identified based on their physicochemical and chromatographic properties, UV, HR-ESI-MS, ^{1}H- and ^{13}C-NMR, DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC spectral analyses (Refer to the supplementary files as "Online Resource 1"), as well as comparison with authentic samples.

*β-sitosterol (**1**)* Colorless needles, m.p. 140–142 °C. It was identified by comparison of its physical and chromatographic properties with an authentic sample, and this is the first report for its isolation from this species.

*Mono-octyl phthalate (**2**)* Yellow oil, UV λ_{max} (MeOH) nm (log ε): 264 (4.87) and 227 (4.80). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.64 (1H, dd, $J = 9$, 3 Hz, H-3), 7.63 (1H, dd, $J = 9$, 3.6 Hz, H-6), 7.46 (1H, ddd, $J = 9$, 6, 3.6 Hz, H-4), 7.45 (1H, ddd, $J = 9$, 6, 3 Hz, H-5), 4.23 (2H, t, $J = 6.7$ Hz, H_2-1′), 1.65 (2H, m, H_2-2′), 1.38 (2H, m, H_2-7′), 1.18 (8H, br.s, H_2-3′: H_2-6′), 0.81 (3H, t, $J = 7.2$ Hz, H_3-8′). ^{13}C NMR (150 MHz, $CDCl_3$): δ 132.4 (C-1), 132.3 (C-2), 128.85 (C-3), 130.9 (C-4, C-5), 128.81 (C-6), 163.6 (C-7), 171.9 (C-8), 65.5 (C-1′), 29.6 (C-2′), 29.3 (C-3′), 29.7 (C-4′, C-5′), 31.9 (C-6′), 22.7 (C-7′), 14.1 (C-8′). Positive-ion mode HR-ESI-MS: m\z 301.1412 $[M + Na]^+$

Fig. 1 Structures of the isolated compounds from *Chorisia chodatii* flowers

1 R=H
10 R=Glc

4 R_1= CH_2CH_3, R_2= OCH_3
5 R_1= H, R_2= OCH_3
6 R_1= CH_2CH_3, R_2= OH
7 R_1= H, R_2= H

8 R= CH_3
9 R= H

13 R= Acetyl
14 R= *p*-coumaroyl
15 R= H

16 R_1= OH, R_2= Glc
17 R_1= H, R_2= Glc(2''-1''')Rha

Glc=β-D-Glucopyranose
Rha=α-L-Rhamnopyranose

(calcd. for $C_{16}H_{22}O_4$ Na: 301.1410), Negative-ion mode HR-ESI-MS: *m\z* 277.1452 $[M - H]^-$ (calcd. for $C_{16}H_{21}O_4$: 277.1445).

The spectroscopic data of compound **2** revealed the presence of a long saturated alkyl chain attached to a phthalic acid moiety. The ^1H NMR spectrum showed two overlapping doublet of doublets at δ_H 7.64 and 7.63 for H-3 and H-6, respectively, in addition to another two overlapping doublet of doublet of doublets at δ_H 7.46 and 7.45 for H-4 and H-5, respectively, consistent with an *ortho*-disubstituted benzene ring. The significant ^1H-^1H COSY correlations (Fig. 2) observed between H-3 and H-4 as well as H-5 and H-6 came also in complete harmony with the previous assignment. The two triplets at δ_H 4.23

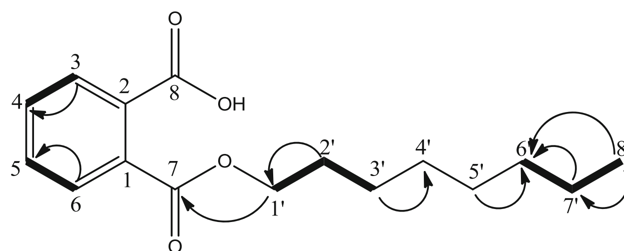

Fig. 2 Important ^1H–^1H COSY and HMBC correlations of compound **2**

($J = 6.7$ Hz) and 0.81 ($J = 7.2$ Hz) were characteristic for the oxymethylene group (CH_2-1′) and the terminal methyl group (CH_3-8′). Moreover, the remaining protons of the

hydrocarbon chain resonated as two two-proton multiplets at δ_H 1.65 and 1.38 for CH_2-2′ and CH_2-7′, respectively, along with a broad singlet at δ_H 1.18 integrating for eight protons due to four further methylene groups. These assignments were also corroborated by the observed correlations in the ^1H–^1H COSY spectrum between H_2-2′ and both H_2-1′ and H_2-3′, in addition to those of H_2-7′ with H_2-6′ and H_3-8′ (Fig. 2).

On the other hand, the ^{13}C NMR spectrum displayed two quaternary carbon signals at δ_C 163.6 and 171.9 characteristic for an ester carbonyl and another free carboxyl group, respectively. Besides, the carbon signal at δ_C 65.5 attributable to the oxygenated methylene (C-1′) indicated acylation at one carboxyl group only, and that compound **2** is a mono-alkyl phthalate ester. The carbon resonances at δ_C 132.4, 132.3, 130.9, 128.85 and 128.81 were characteristic for the aromatic ring, whereas those between δ_C 31.9 and 22.7—all are methylenes in DEPT—were indicative to the long hydrocarbon chain. Different carbons were also distinguished with the aid of HMQC and HMBC experiments. Important HMBC correlations were detected between H-3 and C-4, H-6 and C-5, H_2-1′ and the carbonyl C-7, as well as H_2-2′ and C-1′. Another correlation was observed between the terminal methyl CH_3-8′ and C-6′ and C-7′. The H_2-5′ and H_2-7′ showed two significant HMBC correlations with C-6′ (Fig. 2).

Furthermore, positive- and negative-ion mode HR-ESI-MS spectra showed pseudomolecular ion peaks at *m/z* 301.1412 $[M + Na]^+$ and 277.1452 $[M - H]^-$, respectively, in agreement with the molecular formula $C_{16}H_{22}O_4$. Therefore, compound **2** was identified as mono-octyl phthalate. This compound was only formerly identified in the gel of *Aloe vera* leaves by GC–MS (Yamaguchi *et al.*, 1993), while this is the first report for its isolation in a pure form from a natural source along with its spectroscopic data.

*5-hydroxymethyl furfural [5-(hydroxymethyl) furan-2-carbaldehyde] (**3**)* Yellow oil, UV λ_{max} (MeOH) nm (log ε): 280 (4.55). ^1H NMR (600 MHz, $CDCl_3$): δ 9.54 (1H, s, CHO), 7.22 (1H, d, $J = 3.5$ Hz, H-3), 7.50 (1H, d, $J = 3.5$ Hz, H-4), 4.69 (2H, s, H_2-6); ^{13}C NMR (150 MHz, $CDCl_3$): δ 152.2 (C-2), 123.2 (C-3), 110.0 (C-4), 161.0 (C-5), 57.4 (C-6), 177.8 (C-7). The physical and spectral data were in accordance with those reported in the literature (Li *et al.*, 2009). This is the first report for its isolation from family Bombacaceae.

*Ethyl vanillate (**4**)* Colorless needles, m.p. 41–43 °C, UV λ_{max} (MeOH) nm (log ε): 264 (4.71) and 295 (4.76). ^1H NMR (600 MHz, DMSO-d_6): δ 7.36 (1H, d, $J = 2.4$ Hz, H-2), 7.31 (1H, dd, $J = 8.4$, 2.4 Hz, H-6), 6.80 (1H, d, $J = 8.4$ Hz, H-5), 3.47 (2H, q, $J = 7.2$ Hz, H_2-8), 1.09 (3H, t, $J = 7.2$ Hz, H_3-9), 3.67 (3H, s, OCH_3-3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 121.7 (C-1), 115.1 (C-2), 149.9 (C-3), 144.8 (C-4), 116.5 (C-5), 121.8 (C-6), 167.3 (C-7), 66.3 (C-8), 18.5 (C-9), 55.9 (OCH_3-3). It was previously identified during characterization of aroma compounds in apple cider by GC–MS (Xu *et al.*, 2007), while this is the first report for its isolation from family Bombacaceae.

*Vanillic acid (**5**)* White needles, m.p. 208–210 °C, UV λ_{max} (MeOH) nm (log ε): 260 (4.64) and 284 (4.68). ^1H NMR (600 MHz, CD_3OD): δ 7.55 (1H, d, $J = 1.8$ Hz, H-2), 7.54 (1H, dd, $J = 9$, 1.8 Hz, H-6), 6.82 (1H, d, $J = 9$ Hz, H-5), 3.89 (3H, s, OCH_3-3). ^{13}C NMR (150 MHz, CD_3OD): δ 123.2 (C-1), 113.8 (C-2), 152.6 (C-3), 148.6 (C-4), 115.8 (C-5), 125.2 (C-6), 170.1 (C-7), 56.4 (OCH_3-3). The physical and spectral data were in accordance with those reported in the literature (Chen *et al.*, 2010; Yu-Bo *et al.*, 2011). It was also confirmed by comparison with an authentic sample, and this is the first report for its isolation from the genus *Chorisia*.

*Protocatechuic acid ethyl ester (**6**)* Colorless needles, m.p. 127–129 °C, UV λ_{max} (MeOH) nm (log ε): 254 (4.67) and 290 (4.72). ^1H NMR (600 MHz, CD_3OD): δ 7.43 (1H, s, H-2), 7.41 (1H, d, $J = 8.4$ Hz, H-6), 6.78 (1H, d, $J = 8.4$ Hz, H-5), 4.11 (2H, q, $J = 7.2$ Hz, H_2-8), 1.25 (3H, t, $J = 7.2$ Hz, H_3-9). ^{13}C NMR (150 MHz, CD_3OD): δ 122.8 (C-1), 115.7 (C-2), 146.0 (C-3), 151.4 (C-4), 117.7 (C-5), 123.8 (C-6), 169.1 (C-7), 61.4 (C-8), 14.5 (C-9). The physical and spectral data were in accordance with those reported in the literature (De-Wu *et al.*, 2010). This compound was previously isolated from some Leguminous plants, e.g., peanut seeds (Huang *et al.*, 2003) and vines of *Pueraria lobata* (Willd.) Ohwi (De-Wu *et al.*, 2010). This is the first report for its isolation from family Bombacaceae.

*p-hydroxy benzoic acid (**7**)* White needles, m.p. 213–215 °C, UV λ_{max} (MeOH) nm (log ε): 252 (4.54). ^1H NMR (600 MHz, CD_3OD): δ 7.87 (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 (150 MHz, CD_3OD): δ 122.8 (C-1), 133.0 (C-2, C-6), 116.0 (C-3, C-5), 163.3 (C-4), 170.5 (C-7). The physical and spectral data were in accordance with those reported in the literature (Chen *et al.*, 2010; De-Wu *et al.*, 2010). This is the first report for its isolation from family Bombacaceae.

*Scopoletin (**8**)* White powder, m.p. 204–206 °C, UV λ_{max} (MeOH) nm (log ε): 296 (4.75), 344 (4.82); +NaOAc 242 (4.67), 392 (increase intensity) (4.88). ^1H NMR (600 MHz, CD_3OD): δ 6.19 (1H, d, $J = 9$ Hz, H-3), 7.85 (1H, d, $J = 9$ Hz, H-4), 6.77 (1H, s, H-5), 7.11 (1H, s, H-8), 3.90 (3H, s, OCH_3-7). ^{13}C NMR (150 MHz, CD_3OD): δ 164.1 (C-2), 110.0 (C-3), 146.1 (C-4), 112.5 (C-5), 147.1 (C-6), 153.0 (C-7), 104.0 (C-8), 151.4 (C-9), 112.6 (C-10), 56.8

(OCH_3-7). The physical and spectral data were in accordance with those reported in the literature (El-Demerdash *et al.*, 2009). In addition, the observed bathochromic shift in the UV spectrum from 344 to 392 nm after addition of NaOAc—with increasing the intensity of this band—differentiated it from isoscopoletin and confirmed the scopoletin skeleton (El-Demerdash *et al.*, 2009). This is the first report for its isolation from the genus *Chorisia*.

*Aesculetin (**9**)* Yellow oil, UV λ_{max} (MeOH) nm (log ε): 290 (4.71) and 344 (4.79). ^{1}H NMR (600 MHz, DMSO-d_6): δ 6.15 (1H, d, $J = 9.6$ Hz, H-3), 7.85 (1H, d, $J = 9.6$ Hz, H-4), 6.74 (1H, s, H-5), 6.98 (1H, s, H-8). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.7 (C-2), 110.6 (C-3), 144.3 (C-4), 112.2 (C-5), 142.8 (C-6), 150.4 (C-7), 102.5 (C-8), 148.4 (C-9), 111.4 (C-10). The physical and spectral data were in accordance with those reported in the literature (Liu *et al.*, 2005). This is the first report for its isolation from the genus *Chorisia*.

*β-sitosterol 3-O-β-D-glucopyranoside (**10**)* White powder, m.p. 290–292 °C. It was identified by comparison of its physical and chromatographic properties with an authentic sample, and this is the first report for its isolation from this species.

*Succinic acid (**11**)* Colorless needles, m.p. 180–182 °C. ^{1}H NMR (600 MHz, CD_3OD): δ 2.56 (4H, s, H-2, H-3). ^{13}C NMR (150 MHz, CD_3OD): δ 176.2 (C-1, C-4) and 29.9 (C-2, C-3). The physical and spectral data were in accordance with those reported in the literature (Parker, 1997; Chatwal and Anand, 2001). It was also confirmed by comparison with an authentic sample, and this is the first report for its isolation from the genus *Chorisia*.

*(3R,4R,5S)-3,4-dihydroxy-5-methyl-dihydrofuran-2-one (**12**)* Colorless needles, ^{1}H (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD): see Tables 1 and 2. The ^{1}H NMR spectrum of compound **12** showed two doublet proton signals at δ_H 1.44 (3H, $J = 6$ Hz) and 4.30 (1H, $J = 9$ Hz) assignable to one methyl and one oxymethine proton, respectively. In addition to one doublet of doublets proton signals at δ_H 3.78 (1H, $J = 9$, 7.8 Hz) and one doublet of quartets at 4.18 (1H, $J = 8.2$, 6.4 Hz) attributable to two oxymethine protons. The ^{13}C NMR spectrum exhibited three oxymethine carbons at δ_C 75.6, 78.6, and 80.7 for C-3, C-4, and C-5, respectively, along with a methyl carbon signal at δ_C 18.1 and another carbonyl carbon at δ_C 176.4 in coincidence with a 3, 4-dihydroxy-5-methyl-dihydrofuran-2-one structure. The stereochemistry at C-3, C-4 and C-5 was explicitly determined by comparison with the ^{1}H and ^{13}C NMR spectral data of some related stereoisomers (Fernandez *et al.*, 1997; Ley *et al.*, 2004) as mentioned in Tables 1 and 2. The stereocenter C-3 in compound **12** was assigned as (*R*) due to the considerable upfield shift (~2–4 ppm) of H-3 of the (3*R*,4*R*,5*S*)-isomer, together with its large coupling constant ($J = 9$ Hz) compared to the other stereoisomers (Ley *et al.*, 2004). Furthermore, the downfield shift (~2–5 ppm) of C-3 was also in complete agreement with the previously deduced stereochemistry. In contrast to its (3*R*,4*S*,5*R*) and (3*S*,4*R*,5*S*) counterparts, H-4 of the (3*R*,4*R*,5*S*)-isomer resonated as a doublet of doublets with a characteristic upfield shift (~0.4–0.5 ppm) and larger coupling constants ($J = 9$ and 8 Hz). Moreover, the methine carbon C-4 of compound **12** was further downfield shifted (~1.5–2.2 ppm) compared to that of the (3*R*,4*S*,5*R*)- and (3*R*,4*S*,5*S*)-isomers, indicating the (*R*) configuration at C-4 (Fernandez *et al.*, 1997; Ley *et al.*, 2004).

On the other hand, the stereochemistry at the neighboring asymmetric carbon C-5 could be unambiguously determined as (*S*) from the characteristic splitting pattern of H-5—that resonated as a doublet of quartets—along with its strong coupling ($J = 8$ Hz) with H-4. Conversely, a much weaker coupling ($J = 2.6$ Hz) was witnessed between H-5 (qd) and H-4 (dd) in the (3*R*,4*S*,5*S*)-isomer, whereas they showed no coupling in the (3*S*,4*R*,5*S*)-isomer (H-5 appeared as a quartet only). Both protons resonated also as a two-proton multiplet at δ_H 4.19–4.21 in case of the (3*R*,4*S*,5*R*)-configurations. Additionally, H-5 of compound **12** suffered an upfield shift (~0.4–0.5 ppm) compared to that of the remaining isomers. It is also worth mentioning that the characteristic downfield shift (~3.5–6 ppm) observed for C-5 in compound **12** was in complete accordance with the (3*R*,4*R*,5*S*)-configuration. Besides, the marked downfield shift (~1–4 ppm) of the methyl group CH_3-6 relative to other stereoisomers was also typical for that stereochemistry (Fernandez *et al.*, 1997; Ley *et al.*, 2004). This lactone was formerly naturally obtained from flowers of *Pterospermum acerifolium* L. (family Sterculiaceae; Dixit *et al.*, 2011), while this is the first report for its isolation from its closely related family; Bombacaceae.

*Kaempferol 3-O-β-D-(6″-acetyl)-glucopyranoside (**13**)* Yellow oil, UV λ_{max} (MeOH) nm (log ε): 266 (5.11), 350 (5.23); +NaOMe 268 (5.12), 398 (5.29); +NaOAc 272 (5.13), 356 (5.24); +$AlCl_3$ 272 (5.13), 392 (5.28), 302 sh (5.17); +$AlCl_3$/HCl 272 (5.13), 392 (5.28), 302 sh (5.17). ^{1}H NMR (600 MHz, CD_3OD): δ 8.01 (2H, d, $J = 8.9$ Hz, H-2′, H-6′), 6.85 (2H, d, $J = 8.9$ Hz, H-3′, H-5′), 6.40 (1H, br.d, $J = 2$ Hz, H-8), 6.20 (1H, d, $J = 2.0$ Hz, H-6), 5.14 (1H, d, $J = 7.3$ Hz, H-1″), 3.30 (1H, m, H-2″), 3.41 (1H, m, H-3″), 3.18 (1H, m, H-4″), 3.43 (1H, m, H-5″), 4.15 (1H, dd, $J = 12$, 1.8 Hz, H-6″a), 4.03 (1H, dd, $J = 12$, 6.6 Hz, H-6″b), 1.82 (3H, s, H_3-8″); ^{13}C NMR (150 MHz, CD_3OD): δ 158.6 (C-2), 135.8 (C-3), 179.7 (C-4), 163.5 (C-5), 98.6 (C-6), 166.5 (C-7), 94.9 (C-8), 159.6 (C-9), 104.3 (C-10), 123.1 (C-1′), 132.2 (C-2′, C-6′), 116.0 (C-3′, C-5′), 161.6 (C-4′), 102.7 (C-1″), 75.7 (C-2″), 78.5 (C-3″),

Table 1 ^{1}H NMR spectral data of compound **12** and some related stereoisomers

H	Compound **12** (CD_3OD, 600 MHz)	(3*R*,4*R*,5*S*)-3,4-dihydroxy-5-methyl-dihydrofuran-2-one (CD_3OD, 400 MHz)	(3*R*,4*S*,5*R*)-3,4-dihydroxy-5-methyl-dihydrofuran-2-one (CD_3OD, 400 MHz)	(3*R*,4*S*,5*S*)-3,4-dihydroxy-5-methyl-dihydrofuran-2-one (CD_3OD, 400 MHz)	(3*S*,4*R*,5*S*)-3,4-dihydroxy-5-methyl-dihydrofuran-2-one (D_2O, 400 MHz)
H-3	4.30 (d, 1H, $J = 9$ Hz)	4.33 (d, 1H, $J = 8.8$ Hz)	4.65–4.78 (m, 1H)	4.55 (d, 1H, $J = 4.8$ Hz)	4.76 (d, 1H, $J = 5.1$ Hz)
H-4	3.78 (dd, 1H, $J = 9$, 8 Hz)	3.78 (dd, 1H, $J = 8.8$, 8.7 Hz)	4.19–4.21 (m, 2H)	4.25 (dd, 1H, $J = 4.7$, 2.8 Hz)	4.25 (d, 1H, $J = 5.1$ Hz)
H-5	4.18 (dq, 1H, $J = 6.2$, 8 Hz)	4.19 (dq, 1H, $J = 6.3$, 8.3 Hz)		4.57 (qd, 1H, $J = 6.6$, 2.6 Hz)	4.64 (q, 1H, $J = 7.0$ Hz)
H-6	1.44 (d, 3H, $J = 6$ Hz)	1.46 (d, 3H, $J = 6.3$ Hz)	1.34 (d, 3H, $J = 6.7$ Hz)	1.41 (d, 3H, $J = 6.5$ Hz)	1.36 (d, 3H, $J = 7.0$ Hz)

Table 2 ^{13}C NMR spectral data of compound **12** and some related stereoisomers

C	Compound **12** (CD_3OD, 150 MHz)	(3*R*,4*R*,5*S*)-3,4-dihydroxy-5-methyl-dihydrofuran-2-one (CD_3OD, 100 MHz)	(3*R*,4*S*,5*R*)-3,4-dihydroxy-5-methyl-dihydrofuran-2-one (CD_3OD, 100 MHz)	(3*R*,4*S*,5*S*)-3,4-dihydroxy-5-methyl-dihydrofuran-2-one (CD_3OD, 100 MHz)	(3*S*,4*R*,5*S*)-3,4-dihydroxy-5-methyl-dihydrofuran-2-one (D_2O, 100 MHz)	DEPT
C-2	176.4	176.5	177.2	178.6	178.7	C
C-3	75.6	75.7	74.0	72.3	69.0	CH
C-4	80.7	80.8	79.2	78.5	83.7	CH
C-5	78.6	78.7	75.0	72.6	72.8	CH
C-6	18.1	18.2	14.8	14.2	17.4	CH_3

71.1 (C-4″), 77.9 (C-5″), 64.2 (C-6″), 20.4 (CO$\underline{CH_3}$), 168.2 ($\underline{C}OCH_3$). The physical and spectral data were in accordance with those reported in the literature (Slimestad *et al.*, 1995). This is the first report for its isolation from family Bombacaceae.

*Tiliroside [kaempferol 3-O-β-D-(6″-E-p-coumaroyl)-glucopyranoside] (**14**)* Yellow needles, m.p. 259–260 °C, UV λ_{max} (MeOH) nm (log ε): 266 (5.20), 314 (5.27); +NaOMe 272 (5.21), 368 (5.34); +NaOAc 278 (5.22), 314 (5.27); +$AlCl_3$ 308 (5.26), 362 (5.33); +$AlCl_3$/HCl 310 (5.26), 362 (5.33). ^{1}H NMR (400 MHz, CD_3OD): δ 7.96 (2H, d, $J = 8.8$ Hz, H-2′, H-6′), 7.37 (1H, d, $J = 16.0$ Hz, H-7‴), 7.29 (2H, d, $J = 8.6$ Hz, H-2‴, H-6‴), 6.80 (2H, d, $J = 8.8$ Hz, H-3′, H-5′), 6.78 (2H, d, $J = 8.8$ Hz, H-3‴, H-5‴), 6.29 (1H, d, $J = 2.2$ Hz, H-8), 6.12 (1H, d, $J = 2.2$ Hz, H-6), 6.04 (1H, d, $J = 16.0$ Hz, H-8‴), 5.21 (1H, d, $J = 6.9$ Hz, H-1″), 3.30 (1H, m, H-2″), 3.44 (1H, m, H-3″), 3.28 (1H, m, H-4″), 3.45 (1H, m, H-5″), 4.27 (1H, dd, $J = 11.7$, 2 Hz, H-6″a), 4.15 (1H, dd, $J = 11.7$, 6.6 Hz, H-6″b); ^{13}C NMR (100 MHz, CD_3OD): δ 158.4 (C-2), 135.2 (C-3), 179.4 (C-4), 162.9 (C-5), 100.1 (C-6), 166.2 (C-7), 94.9 (C-8), 159.3 (C-9), 105.5 (C-10), 122.7 (C-1′), 131.1 (C-2′, C-6′), 116.0 (C-3′, C-5′), 161.4 (C-4′), 104.3 (C-1″), 75.7 (C-2″), 78.1 (C-3″), 71.7 (C-4″), 75.8 (C-5″), 64.3 (C-6″), 127.1 (C-1‴), 132.1 (C-2‴, C-6‴), 116.8 (C-3‴, C-5‴), 161.1 (C-4‴), 146.5 (C-7‴), 114.7 (C-8‴), 168.7 (C-9‴). The physical and spectral data were in accordance with those reported in the literature (Costa *et al.*, 2007). This is the first report for its isolation from this species.

*Astragalin [kaempferol 3-O-β-D-glucopyranoside] (**15**)* Yellow powder, m.p. 198–200 °C, UV λ_{max} (MeOH) nm (log ε): 266 (5.08), 344 (5.18); +NaOMe 272 (5.08), 398 (5.25), 302 sh (5.13); +NaOAc 274 (5.09), 348 (5.19), 302 sh (5.13); +$AlCl_3$ 272 (5.08), 392 (5.24); 302 sh (5.13); +$AlCl_3$/HCl 272 (5.08), 392 (5.24), 306 sh (5.14). ^{1}H NMR (600 MHz, CD_3OD): δ 8.04 (2H, d, $J = 8.4$ Hz, H-2′, H-6′), 6.88 (2H, d, $J = 8.4$ Hz, H-3′, H-5′), 6.40 (1H, br s, H-8), 6.20 (1H, br s, H-6), 5.23 (1H, d, $J = 7.2$ Hz, H-1″), 3.30 (1H, m, H-2″), 3.39 (1H, m, H-3″), 3.19 (1H, m, H-4″), 3.41 (1H, m, H-5″), 3.67 (1H, dd, $J = 11.4$, 1.8 Hz, H-6″a), 3.51 (1H, dd, $J = 11.4$, 6.6 Hz, H-6″b); ^{13}C NMR (150 MHz, CD_3OD): δ 158.5 (C-2), 135.5 (C-3), 179.5 (C-4), 163.1 (C-5), 99.9 (C-6), 166.0 (C-7), 94.7 (C-8), 159.1 (C-9), 105.7 (C-10), 122.8 (C-1′), 132.3 (C-2′, C-6′), 116.1 (C-3′, C-5′), 161.6 (C-4′), 104.1 (C-1″), 75.7 (C-2″), 78.4 (C-3″), 71.4 (C-4″), 78.0 (C-5″), 62.6 (C-6″). The physical and spectral data were in accordance with those reported in the literature (Lee *et al.*, 2007). This is the first report for its isolation from the genus *Chorisia*.

*Cynaroside [luteolin 7-O-β-D-glucopyranoside] (**16**)* Yellow powder, m.p. 262–264 °C, UV λ_{max} (MeOH) nm (log ε): 266 (5.08), 332 (5.17); +NaOMe 266 (5.08), 386 (5.23); +NaOAc 266 (5.08), 332 (5.17); +$AlCl_3$ 272 (5.09), 388 (5.24); +$AlCl_3$/HCl 272 (5.09), 344 (5.19). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.41 (1H, d, $J = 8.4$ Hz, H-6′), 7.40 (1H, s, H-2′), 6.88 (1H, d, $J = 8.4$ Hz, H-5′), 6.77 (1H, br s, H-8), 6.71 (1H, br s, H-6), 6.43 (1H, s, H-3), 5.05

(1H, d, $J = 7.1$ Hz, H-1″), 3.26 (1H, m, H-2″), 3.42 (1H, m, H-3″), 3.17 (1H, m, H-4″), 3.44 (1H, m, H-5″), 3.69 (1H, d, $J = 10.4$ Hz, H-6″a), 3.44 (1H, d, $J = 10.4$ Hz, H-6″b), 12.9 (1H, s, H-bonded OH-5); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.4 (C-2), 103.1 (C-3), 181.7 (C-4), 161.0 (C-5), 99.4 (C-6), 162.8 (C-7), 94.6 (C-8), 156.8 (C-9), 105.2 (C-10), 121.3 (C-1′), 113.5 (C-2′), 145.7 (C-3′), 149.8 (C-4′), 115.9 (C-5′), 119.0 (C-6′), 99.9 (C-1″), 73.0 (C-2″), 76.3 (C-3″), 69.5 (C-4″), 77.1 (C-5″), 60.5 (C-6″). The physical and spectral data were in accordance with those reported in the literature (Gohari *et al.*, 2011). This is the first report for its isolation from this species.

*Rhoifolin [apigenin 7-O-neohesperidoside] (**17**)* Yellow needles, m.p. 243–245 °C, UV λ_{max} (MeOH) nm (log ε): 266 (5.18), 338 (5.29); +NaOMe 242 (5.15), 386 (5.35), +NaOAc 266 (5.18), 338 (5.29); +$AlCl_3$ 272 (5.20), 380 (5.34); +$AlCl_3$/HCl 272 (5.20), 344 (5.30). ^{1}H NMR (400 MHz, CD_3OD): δ 7.82 (2H, d, $J = 8.4$ Hz, H-2′, H-6′), 6.89 (2H, d, $J = 8.4$ Hz, H-3′, H-5′), 6.73 (1H, br s, H-8), 6.60 (1H, br s, H-6), 6.42 (1H, s, H-3), 5.16 (1H, d, $J = 7.6$ Hz, H-1″), 3.29-3.73 (4H, m, H-2″, H-3″, H-4″, H-5″), 3.69 (1H, dd, $J = 11.3$, 2.4 Hz, H-6″a), 3.58 (1H, dd, $J = 11.3$, 6.7 Hz, H-6″b), 5.28 (1H, s, H-1‴), 3.40-3.95 (4H, m, H-2‴, H-3‴, H-4‴, H-5‴), 1.30 (3H, d, $J = 6.2$ Hz, H_3-6‴); ^{13}C NMR (100 MHz, CD_3OD): δ 166.7 (C-2), 104.1 (C-3), 184.0 (C-4), 162.8 (C-5), 101.0 (C-6), 164.3 (C-7), 95.9 (C-8), 158.9 (C-9), 107.0 (C-10), 123.0 (C-1′), 129.6 (C-2′), 117.0 (C-3′), 162.8 (C-4′), 117.0 (C-5′), 129.6 (C-6′), 99.8 (C-1″), 79.0 (C-2″), 78.3 (C-3″), 71.4 (C-4″), 79.1 (C-5″), 62.4 (C-6″), 102.5 (C-1‴), 72.2 (C-2‴), 72.3 (C-3‴), 74.0 (C-4‴), 70.0 (C-5‴), 18.2 (C-6‴). The physical and spectral data were in accordance with those reported in the literature (Eldahshan, 2013). This is the first report for its isolation from this species.

DPPH free radical scavenging activity

Compounds **1–17** were examined for their anti-radical activities using the DPPH free radical scavenging test. Compound **4** showed moderate scavenging activity (IC_{50}: 37.17 ± 2.35), while compounds **9** and **6** showed weak activities (IC_{50}: 56.5 ± 6.60 and 73.2 ± 4.60 μM, respectively) comparable with the standard trolox (IC_{50}:16.6 ± 2.2 μM). The remaining compounds demonstrated much weaker effects with $IC_{50} > 100$ μM.

Cytotoxic activity

Compounds **1–17** were tested for their tumor cell growth inhibitory effects toward A549 by means of MTT assay. Compounds **7**, **2**, **6**, **8**, **5** and **3** exhibited weak inhibitory activities toward A549 (IC_{50}: 64.2 ± 6.45, 80.1 ± 2.30, 85.6 ± 1.65, 86.3 ± 4.23, 91.0 ± 6.43 and 91.8 ± 6.87 μM, respectively). The results were compared with those of doxorubicin as a positive control (IC_{50}: 1.12 ± 0.02 μM). IC_{50} values of the remaining compounds exceeded 100 μM.

Chemotaxonomic significance

Investigation of secondary metabolites of the unstudied species *C. chodatii* drew an important image concerning the chemotaxonomic situation within the genus *Chorisia* and family Bombacaceae. The isolation of compounds (**1–17**) reflected important chemical homogeneity in terms of the metabolic profile of plants of the genus *Chorisia*. Previous phytochemical analyses of other *Chorisia* species have shown that their phenolic pool comprises mostly flavonoids and phenolic acids, with rhoifolin and tiliroside are two major phenolic metabolites produced by all species (Coussio, 1964; Ashmawy *et al.*, 2012; Eldahshan, 2013). Up to now, *Chorisia* plants are considered the exclusive Bombacaceous source of these two glycosides (Refaat *et al.*, 2013). Besides, cynaroside and protocatechuic acid were also previously obtained from *Chorisia crispiflora* flowers and leaves, respectively (Hassan, 2009; Ashmawy *et al.*, 2012). In the same way, *Bombax ceiba* L. flowers have recently provided astragalin, protocatechuic acid and vanillic acid (Joshi *et al.*, 2013), whereas succinic acid was earlier detected in the fruit pulp of *Adansonia digitata* L. (De Caluwé *et al.*, 2010).

On the other hand, among various Bombacaceous plants, coumarins have been only identified in *Ochroma* (Paula *et al.*, 1996) and lately from *Bombax* (Joshi *et al.*, 2013). However, based on our findings, they can now be added to the phenolic pool of *Chorisia*. Moreover, it is worth mentioning that kaempferol 3-*O*-β-D-(6″-acetyl)-glucopyranoside represents the second example of acylated flavonoidal glycosides in Bombacaceae after isolation of tiliroside. This may refer to the availability of a divergent group of acylating acids within the biosynthetic system of *Chorisia* plants. Recently, a phthalate ester was isolated from *Ceiba pentandra* L. Gaertn. leaves and identified as di-*n*-octyl phthalate (Ibrahim *et al.*, 2012), whereas the present study described the isolation and identification of the mono-octyl ester for the first time from Bombacaceae. On the other hand, some members of Bombacaceae yielded a number of furanoids and lactones including *Quararibea funebris* Llave., *Chorisia crispiflora* and *Bombax ceiba* (Raffauf *et al.*, 1984; Matsuda *et al.*, 1994; Wu *et al.*, 2008; Faizi *et al.*, 2011), and in harmony with these former findings, the current study provided 5-hydroxymethyl furfural and 3,4-dihydroxy-5-methyl-dihydrofuran-2-one from *C. chodatii* flowers for the first time in the family.

As a final point, it is noteworthy that so far, different phytochemical investigations of *Chorisia* plants revealed their lacking of isoflavones, naphthoquinones, sesquiterpenes and sesquiterpene lactones that were previously isolated from the genus *Ceiba*. This fact may oppose the recent botanical opinion to incorporate *Chorisia* within *Ceiba* as a single genus (Ravenna, 1998; Gibbs and Semir, 2003) on a strong chemotaxonomic basis and in turn supports their separation. Consequently, the current phytochemical work intensely complements our chemotaxonomic knowledge about the genus *Chorisia*.

Conclusion

In this study, the chemical composition of *C. chodatii* flowers was studied for the first time and resulted in characterization of seventeen compounds belonging to different structural classes. All the isolated compounds were reported for the first time from this plant, and phenolics were found to prevail among the identified phytoconstituents. Compounds **4**, **9** and **6** were the most active among the tested compounds as DPPH radical scavengers, respectively, whereas compounds **7**, **2**, **6**, **8**, **5** and **3** exhibited weak inhibitory activities toward the human lung cancer cell line A549. On the other hand, the phytochemical results of this study enlighten the chemotaxonomic situation of this plant species and may also broaden its use in future phytotherapy.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard The manuscript does not contain clinical studies or patient data.

References

Adjanohoun EJ (1988) Contribution aux etudes ethnobotaniques et floristiques en Republique Populaire du Congo. ACCT, Paris, p 605
Ames BN, Gold LS, Willet WC (1995) The causes and prevention of cancer. Proc Natl Acad Sci USA 92:5258–5265
Ancuceanu RV, Istudor V (2004) Pharmacologically active natural compounds for lung cancer. Altern Med Rev 9:402–419
Ashmawy AM, Azab SS, Eldahshan OA (2012) Effects of *Chorisia crispiflora* ethyl acetate extract on P21 and NF-κB in breast cancer cells. J Am Sci 8:965–972
Bailey LH (1976) Hortus third: a concise dictionary of plants cultivated in the United States and Canada. Staff of the L.H. Bailey Hortorium, Cornell University, Ithaca
Chatwal GR, Anand SK (2001) Spectroscopy (atomic and molecular), 5th edn. Himalaya Publishing House, Mumbai, pp 213–214
Chen G, Dai C, Wang T, Jiang C, Han C, Song X (2010) A new flavonol from the stem-bark of *Premna fulva*. ARKIVOC 2:179–185
Costa DA, Silva DA, Cavalcanti AC, De Medeiros MAA, De Lima JT, Cavalcante JMS, Da Silva BA, Agra MD, De Souza MDF (2007) Chemical constituents from Bakeridesia pickelii Monteiro (Malvaceae) and the relaxant activity of kaempferol-3-O-β-D-(6″-E-p-coumaroyl)-glucopyranoside on guinea-pig ileum. Quím Nova 30:901–903
Coussio JD (1964) Isolation of rhoifolin from *Chorisia* species (Bombacaceae). Experientia 20:562
De Caluwé E, Halamova K, Van Damme P (2010) *Adansonia digitata* L. A review of traditional uses, phytochemistry and pharmacology. Afrika focus 23:11–51
De-Wu Z, Sheng-Jun D, Wan-Hui L, Gui-Hai L (2010) Chemical constituents from the vines of *Pueraria lobata*. Chin J Nat Med 8:196–198
Dixit P, Khan MP, Swarnkar G, Chattopadhyay N, Maurya R (2011) Osteogenic constituents from *Pterospermum acerifolium* Willd. flowers. Bioorg Med Chem Lett 21:4617–4621
El-Alfy TS, El-Sawi SA, Sleem A, Moawad DM (2010) Investigation of flavonoidal content and biological activities of *Chorisia insignis* H.B.K. leaves. Aust J Basic Appl Sci 4:1334–1348
Eldahshan OA (2013) Rhoifolin; a potent antiproliferative effect on cancer cell lines. Br J Pharm Res 3:46–53
El-Demerdash A, Dawidar AM, Keshk EM, Abdel-Mogib M (2009) Coumarins from *Cynanchum actum*. Rev Latinoamer Quím 37:65–69
Fernandez A-M, Plaquevent J-C, Duhamel L (1997) Optically pure dihydroxy γ-alkylated γ-butyrolactones starting from L-tartaric acid: application to formal and total syntheses of natural products. J Org Chem 62:4007–4014
Gibbs P, Semir J (2003) A taxonomic revision of the genus *Ceiba* Mill. (Bombacaceae). Anales Jard Bot Madrid 60:259–300
Gohari AR, Ebrahimi H, Saeidnia S, Foruzani M, Ebrahimi P, Ajani Y (2011) Flavones and flavone glycosides from *Salvia macrosiphon* Boiss. Iran J Pharm Res 10:247–251
Hafez SS, Abdel-Ghani AE, El-Shazly AM (2003) Pharmacognostical and antibacterial studies of *Chorisia speciosa* St. Hill. flower (Bombacaeae). Mans J Pharm Sci 19:40–43
Hassan AA (2009) Phytochemical and biological investigation of certain plants containing pigments. Dissertation, Mansoura University, Egypt
Huang SC, Yen GC, Chang LW, Yen WJ, Duh PD (2003) Identification of an antioxidant, ethyl protocatechuate, in peanut seed testa. J Agric Food Chem 51:2380–2383
Huxley A (1992) Dictionary of gardening: the New Royal Horticultural Society. The Macmillan Press Limited, London
Ibrahim S, Nok JA, Abubakar MS, Sarkiyayi S (2012) Efficacy of di-n-octyl phthalate anti-venom isolated from *Ceiba pentandra* leaves extract in neutralization of *Echis ocellatus* venom. Res J Appl Sci Eng Technol 4:2382–2387
Joshi KR, Devkota HP, Yahara S (2013) Chemical analysis of flowers of *Bombax ceiba* from Nepal. Nat Prod Commun 8:583–584
Lee D, Lyu H, Kwak H, Jung L, Lee L, Kim D, Chung I, Kim S, Baek N (2007) Isolation of flavonoids from the fruits of *Cornus kousa* Burg. J Appl Biol Chem 50:144–147
Ley SV, Dixon DJ, Guy RT, Palomero MA, Polara A, Rodriguez F, Sheppard TD (2004) Studies on the generation of enolate anions from butane-2,3-diacetal protected glycolic acid derivatives and subsequent highly diastereoselective coupling reactions with aldehydes and acid chlorides. Org Biomol Chem 2:3618–3627

Li C, Zhang Z, Zhao ZK (2009) Direct conversion of glucose and cellulose to 5-hydroxymethyl furfural in ionic liquid under microwave irradiation. Tetrahedron Lett 50:5403–5405

Liu R, Sun Q, Sun A, Cui J (2005) Isolation and purification of coumarin compounds from *Cortex fraxinus* by high-speed counter-current chromatography. J Chromatogr A 1072:195–199

Mabry TJ, Markham KR, Thomas MB (1970) The systematic identification of flavonoids. Springer, New York, pp 4–35

Markham KR (1982) Techniques of flavonoids identification. Academic press, London

Matsunami K, Otsuka H, Takeda Y (2011) Myrseguinosides A-E, five new glycosides from the fruits of *Myrsine seguinii*. Chem Pharm Bull 59:1274–1280

Parker SP (1997) Dictionary of chemistry, 1st edn. McGraw-Hill, New York, p 372

Paula VF, Barbosa LC, Demuner AJ, Howarth OW, Pilo-Veloso D (1996) Chemical constituents of *Ochroma lagopus* Swartz. Quim Nova 19:225–229

Phan MG, Phan TS, Matsunami K, Otsuka H (2006) Chemical and biological evaluation on scopadulane type diterpenoids from *Scoparia dulcis* of Vietnamese origin. Chem Pharm Bull 54:546–549

Pietta PG (2000) Flavonoids as antioxidant. J Nat Prod 63:1035–1042

Polterait O (1997) Antioxidants and free-radical scavengers of natural origin. Curr Org Chem 1:415–422

Ravenna P (1998) On the identity, validity and actual placement in *Ceiba* of several *Chorisia species* (Bombacaceae) and description of two new South American species. Oniro 3:42–51

Refaat J, Desoky SY, Ramadan MA, Kamel MS (2013) Bombacaceae: a phytochemical review. Pharm Biol 51:100–130

Slimestad R, Andersen QM, Francis GW, Marston A, Hostettmann K (1995) Syringetin, kaempferol 3-*O*-β-D-(6″-acetyl)-glucopyranoside and other flavonols from needles of Norway spruce, *Picea abies*. Phytochemistry 40:1537–1542

Smith I (1960) Chromatographic and electrophoretic techniques. Heinman, London, pp 1–246

Stahl E (1970) Thin layer chromatography, 2nd edn. Springer, New York, p 810

Strassheim D, Shafer SH, Phelps SH, Williams CL (2000) Small cell lung carcinoma exhibits greater phospholipase c-beta1 expression and edelfosine resistance compared with non-small cell lung carcinoma. Cancer Res 60:2730–2736

Xu Y, Fan W, Qian M (2007) Characterization of aroma compounds in apple cider using solvent-assisted flavor evaporation and headspace solid-phase microextraction. J Agric Food Chem 55:3051–3057

Yamaguchi I, Mega N, Sanada H (1993) Components of the gel of *Aloe vera* (L.) Burm. F. Biosci Biotech Biochem 57:1350–1352

Yu-Bo L, Xiang-Rong C, Jiang-Jiang Q, Shi-Kai Y, Hui-Zi J, Wei-Dong Z (2011) Chemical constituents of *Toona ciliata* var. *pubescens*. Chin J Nat Med 9:115–119