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Bignanoside A "A new neolignan glucoside" and bignanoside B "A new iridoid glucoside" from *Bignonia binata* leaves

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ABSTRACT

One new neolignan glucoside; bignanoside A (**1**) and one new iridoid glucoside; bignanoside B (**2**), and one new lignan rhamnoside; (-)-1yoniresinol 3α-*O*-α-L-rhamnopyranoside (**3**) reported for the first time, alongside with seven known compounds were isolated from *Bignonia binata* leaves. The structures of the isolated compounds were elucidated by various spectroscopic methods, including 1D and 2D NMR experiments, as well as HR-ESI-MS. Compound **2** had a potent DPPH radical scavenging activity with IC_{50} value of 18.34 ± 0.81 μM. Likewise, compounds **4** and **9** exhibited antitrypanosomal activity with IC_{50} value of 29.12 ± 1.21 and 17.80 ± 0.32 μM, respectively, against Trypanosoma brucei *brucei* S427.

1. Introduction

Bignonia L. is the fifth largest genus in the tribe Bignonieae (Bignoniaceae), with 31 lianas species distributed from Argentina to USA (Zuntini et al., 2015a, b). Many species were reported to have various secondary metabolites including; phenylethanoids, phenolics, lignans, flavonoids, coumarins and xanthones (Mahmoud et al., 2019; da Rocha et al., 2017; Martin et al., 2008, 2007). Most species are used in folk medicine for treating a wide range of ailments, such as skin ailments like fungal infections, boils, psoriasis and eczema, dysentery, ringworm, tapeworm, malaria, diabetes, pneumonia, venereal diseases and ulcers (Atawodi and Olowoniyi, 2015). *B. binata* Thunb. (syn: *Clytostoma binatum* Thunb.) is a polymorphic and polyphyletic liana and distributed from Mexico to Argentina (Zuntini et al., 2015a), The lack of reports about any phytochemical investigation of *B. binata* encouraged us to investigate the isolation and characterization of active constituents from leaves of *B. binata*. As a result, ten compounds (**1-10**) were isolated and characterized. All of the isolated compounds were reported for the first time from this plant. In addition, the DPPH radical scavenging, cytotoxic and antitrypanosomal activities of the isolated compounds were described.

2. Results and discussion

Extraction, fractionation and isolation of *B. binata* leaves, using various chromatographic procedures led to the identification of ten compounds, including two new compounds

2.1. Structure elucidation of the new compounds

Compound **1** $[\alpha]_D^{24}$ +19.37 (c = 0.16, MeOH), was obtained as yellow powder. Its molecular formula was established as $C_{28}H_{36}O_{12}$ from the positive ion mode HR-ESI-MS. The IR spectrum of **1** displayed absorption bands at 3123 cm^{-1} for hydroxyl groups. The ^{1}H-NMR spectrum of **1** (Table 1), showed the presence of three aromatic signals, including one singlet signal was equivalent to two protons at δ_H 6.72 (2H, H-2,6) and two broad singlet aromatic protons at δ_H 6.96 (1H, H-2') and 7.07 (1H, H-6'), which were attributable to two sets of tetra-substituted benzene rings, besides other three singlet signals corresponding to four methoxyls at δ_H 3.36 (3H, OCH_3-9'), 3.82 (6H, OCH_3-3,5) and 3.89 (3H, OCH_3-3'). In addition to, two *trans* olefinic proton signals at δ_H 6.59 (d, J = 16.0 Hz, H-7') and 6.21 (m, H-8'). Additionally, the ^{1}H-NMR data revealed the presence of three doublet

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Table 1
^{1}H and APT spectral data of compound **1** (400 MHz and 100 MHz, respectively; CD_3OD).

No.	δ_H (m, *J* in Hz)	δ_C
1	–	132.5
2	6.72 (s)	104.5
3	–	149.5
4	–	134.4
5	–	149.5
6	6.72 (s)	104.5
7	5.64 (d, 6.7)	89.9
8	3.73 (m)	52.9
9	3.69 (d, 6.7)	63.0
OCH_3-3,5	3.82 (s)	57.0
1′	–	132.5
2′	6.96 (br s)	112.5
3′	–	145.7
4′	–	150.4
5′	–	130.3
6′	7.07 (br s)	117.0
7′	6.59 (d, 16.0)	134.5
8′	6.21 (m)	124.5
9′	4.07 (d, 6.2)	74.5
OCH_3-3'	3.89 (s)	56.9
OCH_3-9'	3.36 (s)	58.1
1″	4.39 (d, 7.8)	104.4
2"	3.2–4.3 (m)	75.3
3″	3.2–4.3 (m)	78.4
4″	3.2–4.3 (m)	71.8
5″	3.2–4.3 (m)	78.2
6″	3.2–4.3 (m)	62.9

proton signals at δ_H 5.64 (d, *J* = 6.7 Hz, H-7), 4.07 (2H, d, *J* = 6.2 Hz, H-9') and 3.69 (2H, d, *J* = 6.7 Hz, H-9), besides one multiplet proton signal at δ_H 3.73 (H-8). Also, the ^{1}H-NMR spectrum showed the presence of a doublet anomeric proton signal at δ_H 4.39 (H-1") was assignable to glucopyranosyl moiety with *β*-configuration, which was deduced from the coupling constant (7.8 Hz) (Samy et al., 2017). The D-configuration of the *β*-glucopyranosyl unit was deduced by HPLC analysis, using chiral detector. The resonance of C-8 at δ_C 52.9, besides the coupling constant (6.7 Hz) of H-7 at δ_H 5.64, indicating the *trans* isomers of the dihydrobezofuran neolignan (Wang et al., 2010; Li et al., 1997), additionally comparing the NMR spectroscopic data of **1** (Table 1) with those reported in the literature was in agreement with (+)-dehydrodiconiferyl alcohol-4-*O*-*β*-D-glucopyranoside (Changzeng and Zhongjian, 1997). The presence of methoxy groups at δ_H 3.82 (OCH_3-3,5) and 3.36 (OCH_3-9') attached to C-3,5 and C-9', respectively, was confirmed by HMBC correlations. The anomeric proton signal at δ_H 4.39 (H-1") of *β*-D-glucopyranosyl moiety was correlated to C-4 at δ_C 134.4, indicating the glycosylation at C-4 (Fig. 2). All the previous assignments were completely substantiated with the help of APT, ^{1}H-^{1}H COSY, HSQC, and HMBC experiments, therefore compound **1** was characterized as (+)-5, 9'- dimethoxy dehydrodiconiferyl alcohol-4-*O*-*β*-D-glucopyranoside and named as bignanoside A (Fig. 1).

Compound **2** $[\alpha]_D^{23}$ − 6.0 (*c* = 0.1, MeOH), was obtained as an amorphous yellow powder and having the molecular formula $C_{26}H_{30}O_{14}$, which had been determined by HR-ESI-MS positive mode. The IR spectrum of **2** showed absorption bands at 3360 cm^{-1} for hydroxyl, (1696 and 1627 cm^{-1}) for carbonyl groups and (1285, 1164 and 1079 cm^{-1}) for an aromatic system. The ^{1}H-NMR of compound **2** (Table 2) revealed the presence of a singlet signal at δ_H 7.48 of an olefinic proton (H-3) and one hemiacetal proton signal at δ_H 5.27 (H-1, d, *J* = 6.6). This was confirmed from ^{13}C-NMR spectrum which showed an olefinic carbon signal at δ_C 155.2 (C3), one quaternary carbon at δ_C 113.1 (C-4) and one hemiacetal carbon at δ_C 97.2 (C-1), in addition to the presence of one carbonyl carbon signal at δ_C 168.2 (C-11), which were characteristic signals of the iridoid skeleton (Ye et al., 2010). Additionally, the downfield shift of C-7 at δ_C 72.4, indicating the presence of hydroxyl group, besides the resonance of quaternary carbon

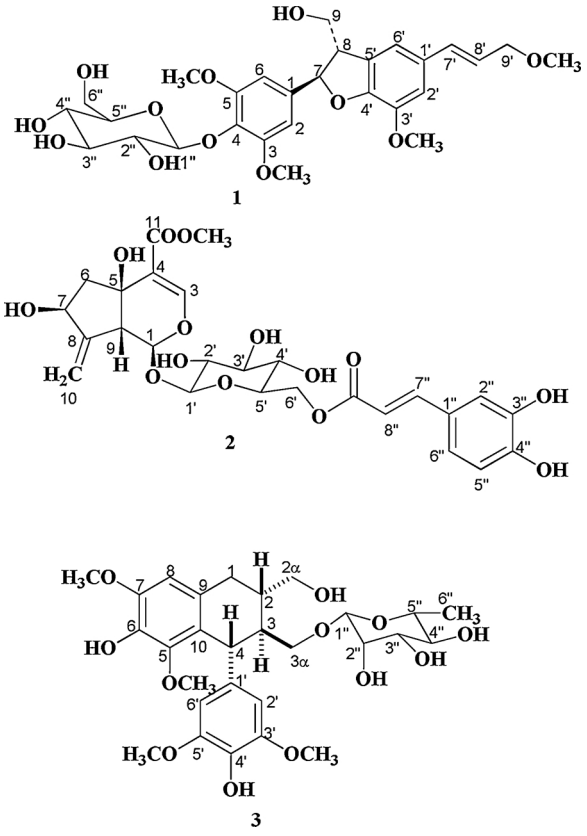

Fig. 1. Structures of the isolated compounds **1–3** from *B. binata* leaves.

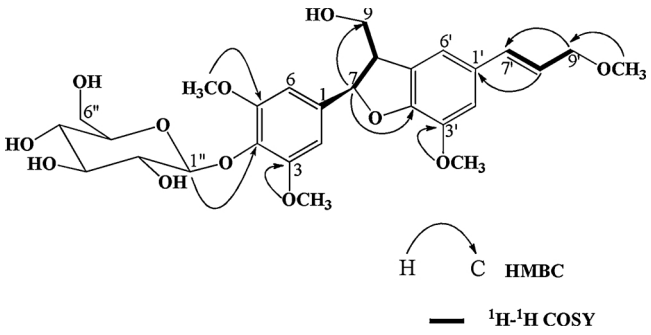

Fig. 2. Significant COSY and HMBC correlations of **1**.

signal at δ_C 151.9 (C-8) and one triplet carbon signal at δ_C 111.9 (C-10), revealing the presence of an exocyclic methylene carbon in ^{13}C-NMR, which was confirmed by the resonance of the two methylene proton signals of H-10 at δ_H 5.10 (1H, t, *J* = 2.5 Hz, H-10a) and 5.22 (1H, t, *J* = 2.7 Hz, H-10b) in ^{1}H-NMR. The ^{1}H-NMR showed an anomeric proton signal 4.26 (d, *J* = 7.9 Hz, H-1'), assignable to *β*-D-glucopyranosyl moiety, in which the *β*- configuration was concluded from the coupling constant (7.9 Hz) and the D-configuration of the *β*-glucopyranosyl unit was deduced by HPLC analysis, using chiral detector. All previously mentioned NMR data was in agreement with those reported in the literature for strictoloside (Ye et al., 2010; Boros and Stermitz, 1990), in addition to the presence of an additional 9 carbon signals. Six of them were attributed for trisubstituted benzene ring, concluded from three aromatic proton signals at δ_H 6.76 (1H, d, *J* = 8.2 Hz, H-5"), 6.93 (1H, dd, *J* = 8.2, 2.0 Hz, H-6") and 7.02 (1H, d, *J* = 2.0 Hz, H-2") in ^{1}H-NMR spectrum. Additionally, two olefinic carbons at δ_C 147.8 (C-7") and 115.3 (C-8"), resonated at δ_H 7.58 (1H, d, *J* = 15.9 Hz, H-7") and 6.30 (1H, d, *J* = 15.9 Hz, H-8") in ^{1}H-NMR and one carbonyl carbon signal at δ_C 169.5 that were characteristic for a caffeoyl moiety (Xiong

Table 2
^{1}H and ^{13}C -NMR spectral data of compound **2** (500 MHz and 125 MHz, respectively; CD_3OD).

No.	δ_H (*J* in Hz)	δ_C
1	5.27 (d, 6.6)	97.2
3	7.48 (s)	155.2
4	–	113.1
5	–	70.3
6	1.88 (dd,12.2, 10.4)	46.6
	2.88 (dd, 12.2, 6.8)	
7	4.09 (m)	72.4
8	–	151.9
9	3.10 (m)	54.0
10	5.10 (t, 2.5)	111.9
	5.22 (t, 2.7)	
11	–	168.2
OCH_3-11	3.71 (s)	52.2
1′	4.26 (d,7.9)	100.6
2′	overlapped	74.8
3′	3.79 (m)	77.9
4′	3.77 (m)	72.2
5′	3.21 (m)	76.4
6′	4.42 (dd, 5.9, 12.1)	64.8
1″	–	128.1
2"	7.02 (d, 2.0)	115.7
3″	–	147.3
4″	–	150.2
5″	6.76 (d, 8.2)	123.6
6″	6.93 (dd, 8.2, 2.0)	117.0
7″	7.58 (d, 15.9)	147.8
8″	6.30 (d, 15.9)	115.3
9″	–	169.5

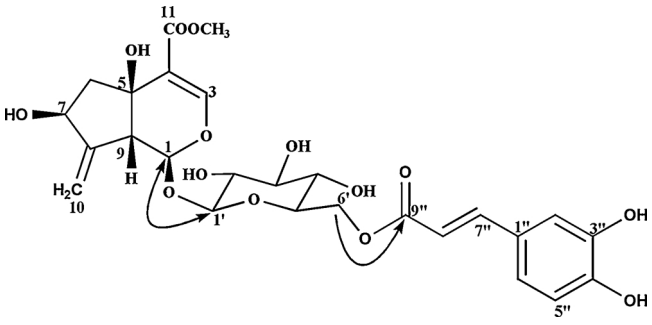

Fig. 3. Significant HMBC correlations of **2**.

et al., 1996). The downfield resonance of the of C-6' of the *β*-D-glucopyranosyl moiety at δ_C 64.8 and the upfield shift of C-5' at δ_C 76.4, indicated the attachment of the caffeoyl moiety at C-6', which was confirmed from the long range correlation between H-6' of the glucopyranosyl moiety at δ_H 4.42 and the carbonyl carbon (C-9") at δ_C 169.5 in HMBC (Fig. 3). Thus, the structure of **2** was confirmed as 6'-caffeoyl strictoloside, and named as bignanoside B.

Compound **3** $[\alpha]_D^{26}$ −33.3 (*c* = 0.27, MeOH), was obtained as yellow residue, its absolute configuration was found to be levorotatory based on its negative optical rotation. The ^{1}H-NMR and DEPT-Q spectral data of **3** (Table 3), were similar to those of (-)-1yoniresinol 3*α*-*O*-*β*-D-glucopyranoside (Ohashi et al., 1994; Tian et al., 2012), except for the presence of one anomeric proton signal at δ_H 4.68 (1H, br s, H-1") and one doublet methyl signal at δ_H 1.33 (3H, d, J = 6.0 Hz, H_3-6") in ^{1}H-NMR, suggesting the presence of rhamnose instead of glucose. Reviewing the available literature, only (+)-1yoniresinol 3*α*-*O*-*α*-L-rhamnopyranoside was previously isolated from *Eucalyptus maideni* (Tian et al., 2012) and *Ulmus thomasii* (Hostettler and Seikel, 1969). It is the first time for isolation of this compound from natural source. Consequently, it was identified as (-)-1yoniresinol 3*α*-*O*-*α*-L-rhamnopyranoside.

Table 3
^{1}H and ^{13}C -NMR spectral spectral data of compound **3** (400 and 100 MHz, respectively CD_3OD).

No.	δ_H (*J* in Hz)	δ_C
1	2.63 (dd, 14.9, 11.7)	33.7
2	1.69 (m)	40.8
3	2.12 (m)	46.7
4	4.34 (d, 5.5)	43.1
5	–	147.7
6	–	139.0
7	–	148.8
8	6.61 (s)	107.9
9	–	130.3
10	–	126.3
2*α*	3.64 (dd, 10.6, 4.4)	66.5
	3.48 (dd, 10.6, 6.9)	
3*α*	3.72 (dd, 11.2, 3.5)	70.2
	3.36 (m)	
OCH_3-5	3.38 (s)	60.3
OCH_3-7	3.88 (s)	56.7
1′	–	134.7
2′	6.38 (s)	107.0
3′	–	149.2
4′	–	139.4
5′	–	149.2
6′	6.38 (s)	107.0
OCH_3-3', 5'	3.76 (s)	57.0
1″	4.68 (br s)	102.5
2"		72.7
3″		72.4
4″		74.1
5″		70.4
6″	1.33 (d, 6.2)	18.3

m; multiplet or overlapped.

2.2. Identification of known compounds

Compounds **4** and **5** were identified by comparison of their physical and chromatographic properties with an authentic samples and were identified as ursolic acid (**4**) and *β*-sitosterol 3-*O*-*β*-D-glucopyranoside (**5**), while the structures of the other isolated compounds were elucidated by various spectroscopic methods, including 1D and 2D NMR experiments (^{1}H-NMR, ^{13}C-NMR, DEPT, APT, COSY, HSQC, and HMBC), as well as HR-ESI-MS analysis as acteoside (**6**) (Pereira et al., 2008), isoacteoside (**7**) (Chen et al., 2013), adenosine (**8**) (Ciuffreda et al., 2007), theviridoside (**9**) (Martin et al., 2007) and (+)-lyoniresinol 3*α*-*O*-*β*-D-glucopyranoside (**10**) (Ohashi et al., 1994) (Fig. 1).

2.3. Biological assay of the isolated compounds

Compounds **1–10** were tested for their 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity and tumor cell growth inhibitory activities toward A549 by means of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. While, compounds (**3-10**) were evaluated for their antitrypanosomal activity against *Trypanosoma brucei brucei* S427 (Table 4).

Compounds **2, 3, 6** and **10** displayed DPPH radical scavenging activity with IC_{50} of 18.34 ± 0.81, 89.40 ± 4.80, 37.00 ± 1.20 and 72.10 ± 20.30 *μ*M, respectively, comparable with that of the standard trolox (16.60 ± 2.20 *μ*M). Only compound **4** showed weak cytotoxic activity toward A549 with IC_{50} of 45.00 ± 5.80 *μ*M. On the other hand, the antitrypanosomal activity evaluation revealed that compounds **4** and **9** exhibited the highest activity against *T. brucei brucei* S427, with IC_{50} values of 29.12 ± 1.21 and 17.80 ± 0.32 *μ*M, respectively, as shown in Table 4. Whereas, the antitrypanosomal activity of theviridoside (**9**) is reported for the first time.

Table 4
DPPH radical scavenging, cytotoxic and antitrypanosomal activities of the isolated compounds.

No.	IC_{50} (μM)		
	DPPH	A549	Trypanosoma brucei *brucei* S427
1	> 100	> 100	NT
2	18.34 ± 0.81	> 100	NT
3	89.40 ± 4.80	> 100	> 100
4	> 100	45.00 ± 5.80	29.12 ± 1.21
5	> 100	> 100	> 100
6	37.00 ± 1.20	> 100	> 100
7	> 100	> 100	> 100
8	> 100	> 100	> 100
9	> 100	> 100	17.80 ± 0.32
10	72.10 ± 20.30	> 100	> 100
Trolox	16.60 ± 2.20	> 100	–
Doxorubicin	–	0.90 ± 0.02	–
α-Difluoromethyl ornithine	–	–	34.59 ± 0.08

NT: Not tested. Data are presented as mean ± SD (n = 3).

2.4. Chemotaxonomic significance

Ten secondary metabolites were isolated from leaves of *B. binata,* including one neolignan (**1**), two iridoids (**2** and **9**), two lignans (**3** and **10**), one triterpene (**4**), one sterol (**5**), two phenylethanoids (**6** and **7**) and one nucleoside (**8**). Among them, only compound (**3**) was reported in the family Bignoniaceae for the first time and compound (**8**) was new from the genus *Bignonia*. The obtained results reflected the chemical diversity of *B. binata* and provide evidence for further chemotaxonomic studies. This is the first comprehensive chemical investigation of leaves of *B. binate,* where all the isolated compounds were reported for the first time from this plant.

In this study two phenylethanoids (**6** and **7**) were previously obtained from genus *Bignonia*. Among them, acteoside (**6**) was previously reported in *Tecoma stans* (Syn. *B. stans*) (Ramirez et al., 2016) and *Pithecoctenium crucigerum* (Syn. *B. crucigera*) (Martin et al., 2007), while isoacteoside (**7**) was previously isolated from *P. crucigera* (Martin et al., 2007).

Theviridoside (**9**) was previously gained from *B. crucigera* (Martin et al., 2007). Additionally, (+)-lyoniresinol 3*α*-*O*-*β*-D-glucopyranoside (**10**), and *β*-sitosterol 3-*O*-*β*-D-glucopyranoside (**5**) were isolated from *Macfadyena unguis-cati* (Syn. *B. unguis-cati*) (Chen et al., 2017; Duarte et al., 2000).

Likewise, ursolic acid (**4**) was previously isolated from *B. unguis-cati* (Chen et al., 2017), in addition to *Arrabidaea triplinervia* (Syn. *B. triplinervia*) (Leite et al., 2006) and *Arrabidaea samydoides* (Syn. *B. samydoides*) (Pauletti et al., 2003), which were various species of the same genus, revealing the close phylogenetic relationship between these *Bignonia* species.

On the other hand, adenosine (**8**) was reported in the genus *Bignonia* for the first time and previously isolated from other genus in the family Bignoniaceae, including *Oroxylum indicum* (Wei et al., 2013), indicating chemotaxonomic homogeneity between the genera *Bignonia* and *Oroxylum*.

It is noteworthy that compounds **1–3** have not been isolated from any other plant, which enhances the chemical diversity of natural compounds and gives evidence for the value of chemotaxonomic studies of *B. binata*.

3. Materials and methods

3.1. General experimental procedures

Optical rotation of the compounds was obtained on a JASCO P-1030 polarimeter. IR and UV spectra were measured on Horiba FT-710 Fourier transform infrared and JASCO V-520 UV/Vis spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 400 MHz, 500 MHz and 600 MHz instruments in DMSO-d_6 and CD_3OD. HR-ESI mass spectrum was taken on a LTQ Orbitrap XL mass spectrometer. Solvents used in this work, e.g., petroleum ether (pet. ether; B.p. 60–80 °C), dichloromethane (DCM), ethyl acetate (EtOAc), methanol (MeOH), and ethanol (EtOH), were purchased from El-Nasr Company for Pharmaceuticals and Chemicals, Egypt and were distilled before use. Acetonitrile (CH_3CN) and MeOH of high performance liquid chromatography (HPLC) grade were used for HPLC separations and purifications and were obtained from SDFCL sd Fine-Chem Limited, India. Deuterated solvents (Sigma-Aldrich, Germany), including methanol (CD_3OD) and dimethyl sulfoxide (DMSO-d_6), were used for nuclear magnetic resonance (NMR) spectroscopic analyses. Column chromatography (CC) was performed using silica gel 60 (El-Nasr Company for Pharmaceuticals and Chemicals, Egypt; 60–120 mesh) or sephadex LH-20 (0.25–0.1 mm, GE Healthcare, Sweden), while silica gel GF254 for thin layer chromatography (TLC) (El-Nasr Company for Pharmaceuticals and Chemicals, Egypt) was employed for vacuum liquid chromatography (VLC)

HPLC purifications were performed on KNAUER HPLC (smart line pump 1000, degasser, diode array detector) with UV Detector, using semi-prep RP-18 column (5 μm, 10 × 250 mm; Waters XBridge, Germany), while an analytical Gemini-NX RP-18 column (5 μm, 4.60 × 100 mm; Phenomenex, Germany) was used for analytical purposes. Ultraviolet lamp (UVP, LLC, USA) was used for visualization of spots on thin layer chromatograms at 254 and/or 365 nm. Thin layer chromatography (TLC) analyses were carried out using pre-coated silica gel 60 GF254 plates (E. Merck, Darmstadt, Germany; 20 × 20 cm, 0.25 mm in thickness). Spots were visualized by spraying with 10 % sulfuric acid in methanol followed by heating at 110 °C on a hot plate. The vacuum liquid chromatography (VLC) technique was performed on dry silica gel for TLC packed column (6 × 30 cm, 90 g) in the room temperature and then the sample was loaded as solute and the elution produced by the aid of water vacuum pump.

3.2. Plant material

The leaves of *B. binata* were collected in December 2015, from El-Zohria botanical garden, Giza, Egypt. Authentication of the plant was identified by Prof. Dr. Nasser Barakat, Professor of Botany, Faculty of Science, Minia University. A voucher specimen (Mn-ph-Cog-033) has been deposited in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Minia University, Minia, Egypt.

3.3. Extraction and isolation

The air dried, powdered leaves (2.7 kg) of *B. binata* were extracted by maceration with 95 % ethanol and concentrated under reduced pressure to give solvent-free residue (420 g), which was then suspended in 400 ml of distilled water, and defatted with pet. ether, followed by partitioning with EtOAc (300 ml each × 6) and then the solvents were separately evaporated under vacuum, affording pet. ether (75 g) and EtOAc fractions (125 g). Finally, the remaining mother liquor was concentrated under reduced pressure to afford aqueous fraction (200 g).

An aliquot of the EtOAc fraction of *B. binata* leaves was subjected to fractionation using VLC technique. In which, it is eluted initially with EtOAc and then the polarity was increased gradually in 10 % by MeOH till EtOAc-MeOH 70:30. Each polarity was collected and concentrated under reduced pressure affording four subfractions. The first subfraction E-I (2.0 g) was rechromatographed on a silica gel column chromatography (CC) (Φ = 20 mm, L = 40 cm) using gradient mixtures of pet. ether-EtOAc gradient mixtures affording compounds **4** (50.0 mg) and **5** (40.0 mg).

The second subfraction E-II (30.0 g) was further fractionated on

silica gel CC (Φ = 60 mm, L = 77 cm), using EtOAc-MeOH gradient mixtures to afford five subfractions. Subfraction E-II-2 was chromatographed on silica gel VLC (Φ = 40 mm, L = 40 cm), which was gradiently eluted with DCM-MeOH to give eight subractions, of which subraction E-II-2-3 was subjected to silica gel CC using DCM-MeOH (92:8) isocratic elution to give three subfractions, of which the second subfraction E-II-2-3-2 (0.53 g) was subjected to sephadex LH-20 CC using MeOH, followed by HPLC purification using H_2O-MeOH (95:5) for 5 min, followed by a linear gradient to 100% MeOH within 55 min and finally with a further isocratic condition of MeOH for 5 min at a flow rate of 2 ml/min to yield compounds **1** (5.0 mg; R_t = 21.69), **3** (15.0 mg; R_t = 21.69).

Additionally, subraction E-II-2-6 (2.0 g) was rechromatographed on sephadex LH-20 CC using MeOH, followed by HPLC using H_2O-MeOH-CH_3CN (95:4:1) for 5 min, followed by a linear gradient to H_2O-MeOH-CH_3CN (10:65:25) within 60 min at a flow rate of 2 ml/min, resulted in the isolation of compounds **6** (60.0 mg; R_t = 23.07) and **7** (10.0 mg; R_t = 25.05). While, subraction E-II-2-7 (2.9 g) was subjected to sephadex LH-20 CC using MeOH, afforded two subractions. Subfraction E-II-2-7-a (0.43 g) was recrystallized by MeOH to afford compound **8** (19.5 mg), while E-II-2-7-b (1.9 g) was rechromatographed on reversed phase (RP_{18}) CC, followed by HPLC purification using H_2O–MeOH (90:10) for 5 min, followed by a linear gradient to 100 % MeOH within 60 min at a flow rate of 2 ml/min., yielded compounds **9** (100.0 mg; R_t = 27.09), **2** (5.0 mg; R_t = 25.02) and **10** (25.0 mg; R_t = 20.27).

3.3.1. Bignanoside A

Yellow powder. $[\alpha]_D^{24}$ +19.37 (*c* = 0.16, MeOH); IR ν_{max} (film) cm^{-1}: 3123, 2885, 1557, 1540, 1508, 1455, 1314, 1200, 1081, 1043; UV λ_{max} (MeOH) nm (log ε): 213 (4.16), 255 (3.94), 350 (3.94), 365 (4.06); ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) *m/z*: 587.2123 $[M + Na]^+$ (Calcd for $C_{28}H_{36}O_{12}Na$: 587.2104).

3.3.2. Bignanoside B

Pale yellow amorphous powder. $[\alpha]_D^{23}$ − 6.0 (*c* = 0.1, MeOH); IR ν_{max} (film) cm^{-1}: 3360, 1696 and 1627, 1285, 1164, 1079; UV λ_{max} (MeOH) nm (log ε): 197 (0.73), 220 (0.11), 242 (0.07) and 332 (0.06); ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD): Table 2; HR-ESI-MS (positive-ion mode) *m/z*: 589.1525 $[M + Na]^+$ (Calcd for $C_{26}H_{30}O_{14}Na$: 589.1533).

3.3.3. (-)-Lyoniresinol 3α-O-α-L-rhamnopyranoside

Pale yellow amorphous powder. $[\alpha]_D^{26}$ −33.3 (*c* = 0.27, MeOH); IR ν_{max} (film) cm^{-1}: 3340, 2800, 1612, 1517, 1500, 1460, 1320, 1208, 1105, 1048, 980, 752; UV λ_{max} (MeOH) nm (log ε): 225 (1.83), 272 (4.54), 283 (4.65); ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD): Table 3; HR-ESI-MS (positive-ion mode) *m/z*: 589.2261 $[M + Na]^+$ (Calcd for $C_{28}H_{38}O_{12}Na$: 589.2255).

3.4. Analysis of the sugar moiety

About 1 mg of compounds **1–3** were hydrolyzed with 1 M HCl (1.0 ml) at 80 °C for 2 h. The reaction mixtures were neutralized with Amberlite IRA96SB (OH^-), then partitioned with an equal amount of EtOAc (1.0 ml), and then water layers were analyzed for their sugar components. The sugars were evaluated by HPLC on an amino column [Shodex Asahipak NH_2P-50 4E (4.6 mm × 250 mm), CH_3CN-H_2O (3:1), 1 ml/min], using chiral detector (JASCO OR-2090*plus*), in comparison with an authentic sugars (D-glucose and L-rhamnose). Compounds **1** and **2** presented the peak for D-(+)-glucose at retention time of 8.32 min, while compound **3** provided the peak for L-(−)-rhamnose at retention time of 5.38 min.

3.5. Evaluation of antioxidant activity

The antioxidant potential of the isolated compounds (**3-10**) was evaluated by using DPPH radical scavenging method. Whereas, the absorbance with various concentrations of the test compounds were dissolved in MeOH (100 µl) in a 96-well microtiter plate and was measured at 515 nm as A_{blank}. Then, 200 µM DPPH solution (100 µl) was added to each well, followed by incubation in the dark at room temperature for 30 min. The absorbance was measured again as A_{sample}. Trolox was used as positive control with IC_{50} of 16.6 ± 2.2 µM.

The % inhibition was calculated using the following equation:

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

Where $A_{control}$ is the absorbance of the control reaction mixture (containing DMSO and all reagents except for the test compounds and compounds.

IC_{50} was determined as the concentration of sample required to inhibit the formation of the DPPH radical by 50 % (Samy et al., 2015).

3.6. Human cancer cell growth inhibition assay

This assay was performed using a human lung cancer cell line (A549) and the viability was estimated by means of the colorimetric MTT assay. Dulbecco's modified Eagle 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 at concentration of 10 mM 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 and then a MTT solution was added to each well and the plates were incubated for a further 1.5 h. Then the formazan precipitates were dissolved in DMSO and the optical density value for each well was measured at 540 nm with a microplate reader. Doxorubicin was used as a positive control (IC_{50}: 0.90 ± 0.02 µM).

The cell growth inhibition was calculated using the following equation:

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

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 concentration of sample required to inhibit the formation of MTT formazan by 50 % (Samy et al., 2014).

3.7. Evaluation of antitrypanosomal activity

The isolated compounds were tested against *Trypanosoma brucei brucei* S427 in the blood stream form. Whereas, samples were initially screened at a single concentration of 20 µM (n = 3). The pure compounds were prepared as 10 mM stock solution in 100 % DMSO and diluted with HMI-9 (10 % FBS) medium. Controls included a sterility control, 0.2 % DMSO and a concentration range of suramin as a positive control. Suramin gave an antitrypanosomal activity against *T. brucei* brucei at an MIC of 0.11 µM. Trypanosomes were counted using a haemocytometer and diluted to a concentration of 3×10^4 trypanosomes/mL (HMI-9 (10 % FBS)), 100 µL of this suspension was added to each well. The assay plate was incubated at 37 °C with a humidified atmosphere containing 5 % CO2 for 48 h. Then 20 µL of Alamar blue was added and the incubation continued for a further 24 h. Fluorescence was then determined using a Wallac Victor 2 microplate reader (Excitation 530 nm Emission 590 nm). The results were calculated as % of the DMSO control values (Raheem et al., 2019).

4. Conclusion

The present study revealed the phytochemical analysis of *B. binata*

leaves, led to the isolation and identification of ten compounds, including two new compounds with eight known ones, revealing the diverse classes of secondary metabolites in the plant. Besides, evaluation of DPPH radical scavenging, cytotoxic and antitrypanosomal activities of the isolated compounds.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2019.12.009.

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