Antihepatotoxic Activity and Chemical Constituents of *Buddleja asiatica* Lour.

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Z. Naturforsch. **64c**, 11–19 (2009); received July 3/September 4, 2008

A new natural compound, named 6-*O*-(3'',4''-dimethoxycinnamoyl) catalpol, was isolated from the defatted alcoholic extract of the flowering parts of *Buddleja asiatica* Lour. (family Scrophulariaceae). Other separated known compounds included steroids (*β*-sitosterol, stigmasterol, stigmasterol-*O*-glucoside, *β*-sitosterol-*O*-glucoside), iridoid glucosides (methyl catalpol, catalpol, aucubin), phenylpropanoids (isoacteoside and acteoside), a triterpene saponin (mimengoside A), flavonoids (diosmin and linarin) in addition to the free sugars mannitol and sucrose. The structures of the isolated compounds were established by ^{1}H and ^{13}C NMR and mass spectrometry. Furthermore, the polar fraction of the flowering parts and the roots showed substantial antihepatotoxic activity comparable to that of the lignan silymarin.

Key words: Buddleja, Iridoid Glycosides, Antihepatotoxic Activity

Introduction

Species belonging to the genus *Buddleja*, known as butterfly bush, are usually trees or shrubs and rarely herbs. The genus comprises about 90 species, native in the tropics of America, Asia and Africa; several are kept as ornamental plants (Bailey, 1957; Zucker, 1968). Formerly placed in the Loganiaceae, this genus is now affiliated with the Scrophulariaceae (Fathy *et al.*, 2006). Many plants of this genus are reported to have antihepatotoxic, anti-inflammatory, analgesic, antipyretic, anticataract, antioxidative, hypotensive, hypoglycemic, neuroprotective, antimicrobial, molluscicidal and amoebicidal activities; they are also utilized in folk medicine for their woundhealing and diuretic effects (Singh *et al.*, 1980; Houghton and Hikino, 1989; Roman *et al.*, 1992; Romo deVivar *et al.*, 1995; El-Nahas, 1998; Rodriguez *et al.*, 1999; Piao *et al.*, 2003; Lu *et al.*, 2005; Fathy *et al.*, 2006). Many constituents of different chemical classes have been reported in the genus *Buddleja*, *viz.* iridoids, flavonoids, phenylpropanoids, lignans, triterpene saponins, diterpenes and sesquiterpenes (Houghton and Hikino, 1989; El-Nahas, 1998; Piao *et al.*, 2003; Lu *et al.*, 2005; Yamamato *et al.*, 1993).

To date, only few phytochemical studies have been reported on *B. asiatica* Lour. by Kapoor *et al.* (1981), Handa *et al.* (1985), Garg and Dengre (1992); these authors recorded the isolation of sitosterol, stigmasterol, an *n*-alkane mixture and some volatile terpenoids. A previous study also afforded 3,23,28-trihydroxyoleanan-11,13(18)diene-3-acetate, euphol-3-*β*-D-glucoside, 7-hydroxy buddledone, 2,6-dimethyl-2,7-octadienoic acid, in addition to four flavonoids: apigenin, apigenin-7-*O*-*β*-D-glucoside, acacetin-7-*O*-*β*-D-glucoside and linarin, and the phenylpropanoid verbascoside (Fathy *et al.*, 2006). In the present study, the isolation of five iridoids (among them a new compound), two flavonoids, two steroidal compounds, a triterpenoidal saponin, sucrose and mannitol are reported. The antihepatotoxic activity of the defatted alcoholic extracts of the flowering parts and roots was also studied.

Results and Discussion

Structure elucidation

Column chromatography of the defatted alcoholic extract of the flowering aerial parts of *B. asiatica* afforded fourteen compounds: Compounds **1**, **2** and **3** were identified as mixtures

0939–5075/2009/0100–0011 $ 06.00

of β-sitosterol and stigmasterol, β-sitosterol and stigmasterol-*O*-glucoside, and β-sitosterol-*O*-glucoside, respectively. The identity of these compounds was established by TLC, co-TLC, melting point and comparison of their IR and MS data with those of authentic samples (Goad and Akihisa, 1997).

Compound **4** was identified as isoacteoside (Zimin and Zhongjian, 1991) and compound **8** as acteoside (Fig. 1) by comparison with an authentic sample, co-TLC, and by reviewing the relevant literature (Piao *et al.*, 2003; Zimin and Zhongjian, 1991).

The molecular formula of **5** was determined as $C_{26}H_{32}O_{13}$ by positive FAB-MS. ^{1}H and ^{13}C NMR spectral data (Table I) in combination with 2D-NMR experiments indicated the presence of an iridoid glycoside, acylated with a *trans*-dimethoxycinnamoyl moiety. The iridoid moiety showed two doublets for two vicinally coupled protons at δ 6.38 (d, J = 5.8 Hz, H-3) and δ 4.99 (dd, J = 4.2, 5.8 Hz, H-4), in addition to signals at δ 5.17 (d, J = 8.7 Hz, H-1), δ 2.59 (m, H-5), 5.06 (d, J = 7 Hz, H-6), δ 3.56 (m, H-7), δ 2.65 (t, J = 9 Hz, H-9), as well as two signals at δ 4.19 and 3.94 (both d, J = 13 Hz, H-10). The corresponding ^{13}C NMR spectrum included seven methine signals at δ 95.04, 142.4, 102.91, 36.72, 81.37, 60.23, and 43.14 assignable to C-1, C-3, C-4, C-5, C-6, C-7, and C-9, respectively, in addition to one methylene signal at δ 61.28 (C-10) and one quaternary carbon signal at δ 66.83 (C-8). One β-glucose unit at C-1 was also indicated from the downfield shift of H-1 (δ 5.17, d, J = 8.7 Hz) and C-1 (δ 95.04) in addition to the anomeric proton signal at δ 4.8 (d, J = 6 Hz, H-1') and other sugar proton signals at

Table I. ^{1}H NMR and ^{13}C NMR spectral data of compounds **5**, **6** and **7**.

C	**5**		**6**		**7**	
	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
1	5.17 (d, J = 8.7 Hz)	95.04	4.99 (d, J = 9 Hz)	93.25	4.99 (d, J = 9 Hz)	93.24
2	–	–	–	–	–	–
3	6.38 (d, J = 5.8 Hz)	142.4	6.38 (d, J = 6 Hz)	140.83	6.36 (d, J = 6 Hz)	140.24
4	4.99 (dd, J = 5.8, 4.2 Hz)	102.91	5.03 (d, J = 6 Hz)	103.05	5.01 (d, J = 6 Hz)	103.35
5	2.59 (m)	36.72	2.23 (m)	35.60	2.33 (m)	37.41
6	5.06 (d, J = 7 Hz)	81.37	3.58 (d, J = 6 Hz)	86.37	3.73 (d, J = 6 Hz)	76.38
7	3.56 (m)	60.23	3.60 (m)	57.14	3.87 (m)	58.9
8	–	66.83	–	65.21	–	64.79
9	2.65 (t, J = 9 Hz)	43.14	2.35 (t, J = 9 Hz)	41.85	2.33 (t, J = 9 Hz)	42.11
10	4.19, 3.94 (d, J = 13 Hz)	61.28	3.90, 3.60 (d, J = 12 Hz)	58.95	3.37, 3.41(d, J = 12 Hz)	60.69
1'	4.8 (d, J = 6 Hz)	99.67	4.58 (d, J = 6 Hz)	97.90	4.16 (d, J = 6 Hz)	97.79
2'	3.44 (d, J = 9.3 Hz)	74.83	3.01 (m)	73.51	3.01–3.09 (m)	73.40
3'	3.37 (m)	78.63	3.16–3.20 (m)	77.56	3.11–3.17 (m)	77.42
4'	3.90 (m)	71.75	3.16–3.20 (m)	70.30	3.01–3.09 (m)	70.21
5'	3.30 (m)	77.65	3.16–3.20 (m)	76.46	3.11–3.17 (m)	77.16
6'	3.94 (m)	62.92	3.64/3.39	61.43	3.65 (m)	61.29
1''	–	128.66				
2''	6.96 (d, J = 2.1 Hz)	115.88				
3''	–	146.80				
4''	–	150.75				
5''	6.98 (d, J = 8.7 Hz)	115.44				
6''	7.21 (dd, J = 8.7, 2.1 Hz)	124.21				
7''	7.71 (d, J = 15.6 Hz)	146.80				
8''	6.48 (d, J = 15.6 Hz)	115.53				
9''	–	168.32				
OMe	3.85 (s)	56.4	3.35 (s)	57.14	–	–
OMe	3.82 (s)	55.89				

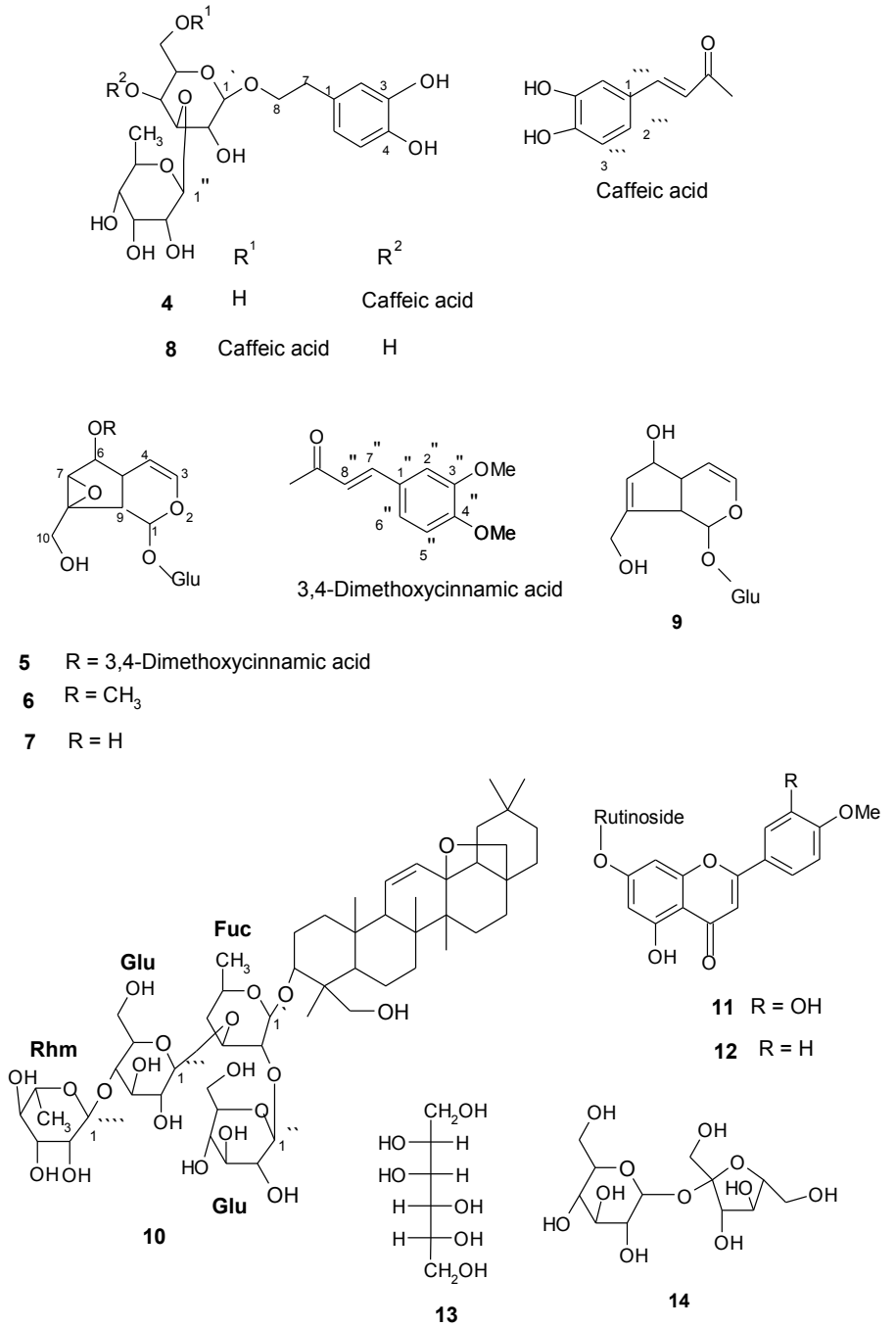

Fig. 1. Chemical structures of the compounds isolated from *Buddleja asiatica* Lour.

δ 3.44 (d, J = 9.3 Hz, H-2'), δ 3.94 (m, H-6') and δ 3.30–3.90 (m, H-3', H-4' and H-5'). The previous data was in agreement with those reported for catalpol derivatives (El-Naggar and Beal, 1980; Houghton and Hikino, 1989; Arnold *et al.*, 2002; Akdemir *et al.*, 2004). The acyl moiety was established to be *trans*-3,4-dimethoxy cinnamate by ^{1}H NMR data showing two vicinal olefinic protons at δ 6.48 and 7.71 (each d, J = 15.6 Hz), protons of the aromatic ring (ABX system) at δ 6.96 (d, J = 2.1 Hz, H-2''), 6.98 (d, J = 8.7 Hz, H-5'') and 7.21 (dd, J = 2.1, 8.7 Hz, H-6''), in addition to the arise of two singlets at δ 3.85 and 3.82 (two methoxy groups). The acylation at C-6 was indicated by the downfield shift of H-6 (δ 5.06) and C-6 (δ 81.37) and by comparison with the previously reported free and acylated catalpol derivatives (Damtoft *et al.*, 1985; Lahloub *et al.*, 1989; Tatli *et al.*, 2003). Thus compound **5** was identified as 6-*O*-(3'',4''-dimethoxycinnamoyl) catalpol (Fig. 1). This compound has not been previously isolated from the genus *Buddleja*. Furthermore, to our knowledge, this is the first report on the isolation of compound **5** from a natural source.

FAB-MS of **6** showed a molecular ion peak at *m/z* 377 $[M+1]^+$ which was in accordance with the molecular formula $C_{16}H_{24}O_{10}$. ^{1}H and ^{13}C NMR spectral data of compound **6** (Table I) showed close resemblance to those of compound **5** indicating a catalpol derivative. However, the ^{1}H and ^{13}C NMR spectral data of **6** showed the presence of one methoxy group through the arise of a methoxy singlet at δ 3.35 in the ^{1}H NMR and δ 57.14 in the ^{13}C NMR spectra instead of the signals of the acyl moiety in compound **5**. Taking into account the upfield shift of H-6 and results from 2D NMR experiments, as well as the relevant literature data (El-Naggar and Beal, 1980; Houghton and Hikino, 1989; Arnold *et al.*, 2002; Akdemir *et al.*, 2004), compound **6** was identified as methylcatalpol (Fig. 1).

Compound **7** showed ^{1}H NMR and ^{13}C NMR data similar to those of compound **6** (Table I) except for the absence of the methoxy signal and field shift of the ^{1}H NMR signal at δ 3.73 for H-6 and the ^{13}C NMR signal at δ 76.38 for C-6 (cf. δ 3.58, δ 86.37 for H-6 and C-6, respectively, in methylcatalpol), which was confirmed to be in accordance with catalpol (Fig. 1) (El-Naggar and Beal, 1980; Houghton and Hikino, 1989; Arnold *et al.*, 2002; Akdemir *et al.*, 2004).

The iridoid aucubin (**9**) and the triterpenoid mimengoside A (**10**) (Fig. 1) were identified through comparison with the data reported in the literature (El-Naggar and Beal, 1980; Davini *et al.*, 1986; Houghton and Hikino, 1989; Arnold *et al.*, 2002; Akdemir *et al.*, 2004; Yamamoto *et al.*, 1991; Tatli *et al.*, 2004).

The flavonoids diosmin (**11**) and linarin (**12**) were (Fig. 1) unambiguously identified in this plant (Harborne *et al.*, 1975, 1982; El-Domiaty *et al.*, 1996). The presence of the free sugars mannitol (**13**) and sucrose (**14**) (Fig. 1) were also confirmed through comparison with authentic samples and published data (Fex, 1982; Fukuyama *et al.*, 1983; Bashir *et al.*, 1993). The occurrence of diosmin, mannitol, and 6-*O*-(3'',4''-dimethoxycinnamoyl) catalpol, which were not previously published from other species of *Buddleja*, can unambiguously considered as a chemotaxonomical marker to differentiate *B. asiatica* from other species of *Buddleja*.

Antihepatotoxic activity

Effect on ALT and AST levels

The results presented in Table II and Fig. 2 illustrate that intraperitoneal injection of CCl_4 in

Table II. Effect of oral treatment with silymarin (100 mg/kg), extract 1 (200 mg/kg) and extract 2 (200 mg/kg) for 30 d on ALT, AST, total protein and albumin levels in adult male cirrhotic rats.

Treatment	Control	Cirrhotic	Silymarin	Aerial parts (ext. 1)	Roots (ext. 2)
ALT [IU/ml]	25.9 ± 7.2	350.8 ± 62.08[a]	25.73 ± 6.3[b]	29.26 ± 6.56[b]	37.9 ± 6.7[b]
AST [IU/ml]	103.85 ±10.88	1083.6 ± 95.9[a]	522.2 ± 63.16[b]	594.73 ± 13.9[b]	611.9 ± 12.2[b]
Total protein [mg/ml]	8.05 ± 0.39	7.92 ± 0.21	9.11 ± 0.62[b]	8.81 ± 0.96	9.7 ± 0.65[b]
Albumin [mg/ml]	3.4 ± 0.4	3.75 ± 0.5	3.35 ± 0.43	3.6 ± 0.08	3.39 ± 0.13

Results are presented as the mean ± S.E.M.
[a] Significantly different from the corresponding mean value of normal control group at $P < 0.05$.
[b] Statistics: ANOVA and post ANOVA test.

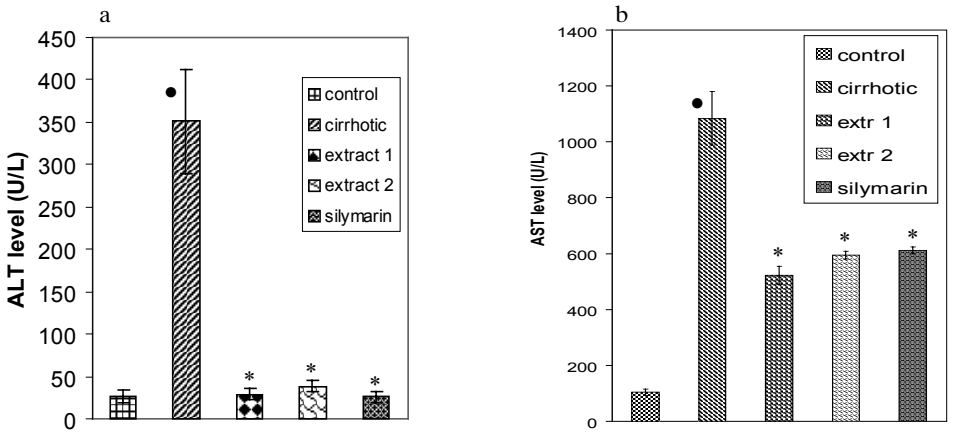

Fig. 2. Effect of oral treatment with silymarin (100 mg/kg), extract 1 (200 mg/kg) and extract 2 (200 mg/kg) for 30 days on (a) ALT and (b) AST levels in adult male cirrhotic rats. Results are presented as the mean ± S.E.M. * Statistics: ANOVA and post ANOVA test. ● Significantly different from the corresponding mean value of normal control group at $P < 0.05$.

a dose of 25 μl/100 g resulted in a significant elevation in the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. On the other hand, administration of silymarin in a dose of 100 mg/kg caused a significant reduction in the ALT level, which was similar to the effect caused by the polar fractions of the aerial parts and roots of *B. asiatica.* In addition, administration of polar fractions of the aerial parts and roots caused a significant reduction in the AST level comparable to that of silymarin.

Effect on total protein and albumin levels

As listed in Table II and graphically presented in Fig. 3, intraperitoneal injection of CCl_4 does not show any significant change in the total protein or albumin levels. On the other hand oral treatment of cirrhotic rats with silymarin exhibited a significant elevation in the total protein level, which is similar to the effect of the roots extract, but it does not show any significant change in the albumin level. In addition, oral treatment of cirrhotic rats with extracts of the flowering aerial parts does not produce any significant change in the total protein or albumin levels.

The results presented in this study (Table II) and (Figs. 2 and 3) showed that the polar fractions of the flowering parts and roots of *B. asiatica* reduced the levels of ALT and AST, thus demonstrating an antihepatotoxic activity. This finding suggests that these fractions might be useful to treat chronic liver diseases. The pronounced antihepatotoxic effect of this plant (as compared with the lignan silymarin) is in agreement with that reported from other *Buddleja* species whose extracts are rich in flavonoids (especially linarin), iridoids (aucubin and catalpol derivatives) and phenylpropanoid glycosides (Houghton and Hikino, 1989). On the other hand, the phenyl-

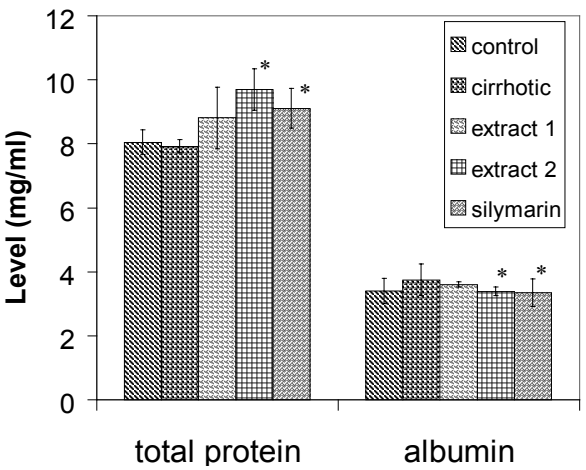

Fig. 3. Effect of oral treatment with silymarin (100 mg/kg), extract 1 (200 mg/kg) and extract 2 (200 mg/kg) for 30 days on total protein and albumin levels in adult male cirrhotic rats. Results are presented as the mean ± S.E.M. * Statistics: ANOVA and post ANOVA test. ● Significantly different from the corresponding mean value of normal control group at $P < 0.05$.

propanoids (acteoside and isoacteoside), which contain a caffeic acid moiety as a part of their structure, may contribute also to this activity (Kiso *et al.*, 1983). Thus, *B. asiatica* can be considered as a new interesting and efficient candidate for hepatoprotection, but a clinical follow-up is needed to test the safety and efficacy of the drug.

Experimental

General experimental procedures

Melting points were determined on an SMP3 apparatus (UK) and are uncorrected. IR (KBr) spectra were recorded on a Jasko FTYIR-460 plus spectrophotometer. Mass spectra were recorded at 70 eV on a Finnigan Mat 55Q-700 spectrometer. ^{1}H, ^{13}C NMR and 2D experiments (COSY and HETCOR) were recorded with a Varian Gemini 300 spectrometer at 300 and 75 MHz, respectively, or with a AM-300 spectrometer at 360 and 90 MHz, respectively, using CD_3OD or DMSO. Chemical shifts are given in ppm with TMS as internal standard. Silica gel 60 (Merck) was used for CC; precoated TLC plates (Merck) were employed to separate and isolate some of the compounds.

Plant material

Aerial flowering parts and roots of *B. asiatica* were collected in January 2005 from a private garden in Altal Alkabeer town, Ismaeleia Governorate, Egypt. The identity of the plant was confirmed by Dr. Ali Abd El-Hamid, Professor of Ornamental Plants, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. A voucher specimen has been deposited at Pharmacognosy Department, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt (accession number: Ba101).

Extraction and isolation

For the antihepatotoxic experiment the roots (0.5 kg) and for phytochemical analysis the aerial parts (2.5 kg) were extracted with ethanol (95%) by cold maceration till exhaustion. The concentrated extract was suspended in methanol and cooled at 7 °C for 72 h, then filtered to remove saturated hydrocarbons to yield 100 g residue. The obtained residue was then placed on a silica gel column (800 g) eluted with $CHCl_3$ and MeOH in a gradient elution technique to afford three main fractions: I (25% MeOH/$CHCl_3$, 20 g), II (1:1 MeOH/$CHCl_3$, 25 g) and III (100% MeOH, 52 g). Fr. I was rechromatographed over a silica gel column and eluted with $CHCl_3$/MeOH to isolate compounds **1** and **2**. Frs. II and III were chromatographed separately over silica gel columns eluted with $CHCl_3$/MeOH/H_2O (8:2:0.2, 7:3:0.3, and 6:4:0.4). Fr. II afforded compounds **3**, **4** and **5**. Compounds **6–14** were isolated from Fr. III by rechromatography over a silica gel column eluted with EtOAc/MeOH/H_2O (9:1:0.1, 8:2:0.2, and 7:3:0.3), respectively.

Spectroscopic data

Mixture of β-sitosterol and stigmasterol (**1**): White needles ($CHCl_3$/MeOH). – IR (KBr): λ_{max} = 3428, 2936, 2867, 1654, 1465, 1382, and 1107 cm^{-1}. – EIMS: *m/z* (%) = 414 (72), 412 (100), 399 (10.4), 397 (17.5), 396 (2.5), 394 (2.0), 381 (1.87), 329 (1.7), 327 (2.3), 314 (2.4), 303 (2.6), 301 (8.9), 300 (19.9), 299 (7.8), 275 (2.2), 273 (35.3), 271 (81.0), 255 (28.3), 253 (3.0), 246 (16.8), 231 (9.47), 299 (11.6), 213 (7.79).

Mixture of β-sitosterol and stigmasterol-O-glucoside (**2**): White crystals (MeOH). – IR (KBr): λ_{max} = 3500–3300, 2980–2930, 1640, 1465, 1375, 1170 cm^{-1}. – FAB-MS: *m/z* (%)= 577 $[M+1]^+$, 575. – EIMS: *m/z* = 414 (0.61), 412 (0.72), 399 (1.15), 397 (70), 396 (74), 394 (56), 329 (0.8), 303 (0.7), 301 (0.6), 300 (0.5), 299 (0.56), 275 (1.32), 255 (47), 213 (17), 69 (100).

β-Sitosterol-O-glucoside (**3**): White platelets (MeOH). – IR (KBr): λ_{max} = 3500–3300, 2980–2920, 1640, 1465, 1375, 1170, 1085, 1035, 810 cm^{-1}. – FAB-MS: *m/z* (%) = 577 $[M+1]^+$. – EIMS: *m/z* = 414 $[M\text{-sugar}]^+$ (9.9), 397 (73.6), 396 (100), 381 (6.9), 300 (3.6), 273 (4.3), 255 (31.8), 231 (3.6), 299 (8.3), 213 (11.5).

Isoacteoside (**4**): Orange-brown amorphous residue (MeOH/EtOAc). – IR (KBr): λ_{max} = 3470, 2929, 1694, 1602, 1446, 1155, 810, 648 cm^{-1}. – FAB-MS: *m/z* (%) = 625 $[M+1]^+$, 180, 179, 154. – ^{1}H NMR (300 MHz, CD_3OD): δ = 6.7 (1H, d, J = 2.8 Hz, H-2), 6.69 (1H, d, J = 8.1 Hz, H-5), 6.57 (1H, dd, J = 8.1, 2.1 Hz, H-6), 2.08 (2H, t, J = 7.5 Hz, H-7β), 4.11–4.04 (2H, m, H-8α), 4.38 (1H, d, J = 7.8 Hz, H-1'), 3.42 (1H, dd, J = 8.1, 10.8 Hz, H-2'), 3.84 (1H, t, J = 9 Hz, H-3'), 3.54 (1H, t, J = 9.3 Hz, H-4'), 3.61–3.57 (1H, m, H-5'), 4.92 (2H, m, H-6'), 5.19 (1H, H-1"), 3.93 (1H, d, J = 1.5 Hz, H-2"), 3.56–3.51 (1H, H-3"), 3.3 (1H, m, H-4"),

3.56–3.51 (1H, H-5"), 1.1 (3H, d, J = 6.3 Hz, H-6"), 7.06 (1H, d, J = 2.1 Hz, H-2"'), 6.79 (1H, d, J = 9 Hz, H-5"'), 6.96 (1H, dd, J = 8.4, 2.1 Hz, H-6"'), 7.61 (1H, d, J = 15.8 Hz, H-7"'β), 6.3 (1H, d, J = 15.8 Hz, H-8"'α). – ^{13}C NMR (75 MHz, CD_3OD): δ = 131.46 (C-1), 117.11 (C-2), 146.03 (C-3), 144.59 (C-4), 116.53 (C-5), 121.27 (C-6), 36.52 (C-7), 72.31 (C-8), 104.12 (C-1'), 76.15 (C-2'), 81.66 (C-3'), 70.55 (C-4'), 75.92 (C-5'), 62.33 (C-6'), 102.99 (C-1"), 72.23 (C-2"), 72.01 (C-3"), 73.76 (C-4"), 70.39 (C-5"), 18.47 (C-6"), 127.62 (C-1'"), 116.32 (C-2"'), 146.76 (C-3"'), 149.72 (C-4"'), 114.67 (C-5"'), 123.24 (C-6"'), 148.01 (C-7'"), 115.24 (C-8"'), 168.35 (C=O).

6-O-(3",4"-Dimethoxycinnamoyl) catalpol (**5**): Brown amorphous residue (EtOAc/MeOH); m.p. 100–102 ºC. – IR (nujol): λ_{max} = 3415, 1716, 1614 cm^{-1}. – FAB-MS: m/z = 553 $[M+1]^+$, 523, 361, 331. – ^{1}H NMR and ^{13}C NMR (CD_3OD, 300 and 75 MHz, respectively): see Table I.

Methylcatalpol (**6**): Yellowish-white crystals (MeOH); m.p. 235–237 ºC. – FAB-MS: m/z = 377 $[M+1]^+$, 197. – EIMS: m/z (%) = 197 (43.2), 182 (5.3). – ^{1}H NMR and ^{13}C NMR (DMSO, 300 and 75 MHz, respectively): see Table I.

Catalpol (**7**): Yellowish-brown residue (MeOH/EtOAc); m.p. 203–205 ºC. – FAB-MS: m/z = 363 $[M+1]^+$. – EIMS: m/z (%) = 182 (23.8). – ^{1}H NMR and ^{13}C NMR (DMSO, 300 and 75 MHz, respectively): see Table I.

Acteoside (**8**): Reddish-brown amorphous residue (MeOH/EtOAc). – IR (KBr): λ_{max} = 3470, 2929, 1694, 1620, 1520, 1446, 1155, 810, 648 cm^{-1}. – FAB-MS: m/z = 624 $[M]^+$, 180, 179, 154. – ^{1}H NMR (360 MHz, CD_3OD): δ = 6.69 (1H, d, J = 2.5 Hz, H-2), 6.68 (1H, d, J = 7.9 Hz, H-5), 6.56 (1H, dd, J = 7.9, 2.1 Hz, H-6), 2.77 (2H, t, J = 7.5 Hz, H-7β), 3.99–4.07 (2H, m, H-8α), 4.37 (1H, d, J = 7.9 Hz, H-1'), 3.41 (1H, dd, J = 7.9, 10 Hz, H-2'), 3.80 (1H, t, J = 9 Hz, H-3'), 3.94 (1H, t, J = 9.4 Hz, H-4'), 3.62–3.57 (1H, m, H-5'), 3.62–3.57 (2H, m, H-6'), 5.18 (1H, H-1"), 3.93 (1H, d, J = 1.4 Hz, H-2"), 3.56–3.50 (1H, H-3"), 3.29 (1H, m, H-4"), 3.56–3.50 (1H, H-5"), 1.09 (3H, d, J = 6.5 Hz, H-6"), 7.06 (1H, d, J = 2.2 Hz, H-2"'), 6.78 (1H, d, J = 8 Hz, H-5"'), 6.95 (1H, dd, J = 8.2, 2.0 Hz, H-6"'), 7.6 (1H, d, J = 15.8 Hz, H-7"'β), 6.29 (1H, d, J = 15.8 Hz, H-8"'α). – ^{13}C NMR (90 MHz, CD_3OD): δ = 131.48 (C-1), 117.14 (C-2), 146.03 (C-3), 144.58 (C-4), 116.55 (C-5), 121.31 (C-6), 36.49 (C-7), 72.29 (C-8), 104.10 (C-1'), 76.12 (C-2'), 81.69 (C-3'), 70.55 (C-4'), 75.90 (C-5'), 62.31 (C-6'), 102.97 (C-1"), 72.02 (C-2"), 72.21 (C-3"), 73,75 (C-4"), 70.39 (C-5"), 18.45 (C-6"), 127.62 (C-1'"), 116.36 (C-2"'), 146.74 (C-3"'), 149.71 (C-4"'), 114.66 (C-5"'), 123.26 (C-6"'), 148.05 (C-7'"), 115.29 (C-8"'), 168.35 (C=O).

Aucubin (**9**): Reddish-brown crystals (MeOH/EtOAc); m.p. 179–182 ºC. – FAB-MS: m/z = 347 $[M+1]^+$. – EIMS: m/z (%) = 180 (5.38), 166 [M-sugar]$^+$, (38.0). – ^{1}H NMR (360 MHz, CD_3OD): δ = 5.11 (1H, d, J = 6.3 Hz, H-1), 6.16 (1H, d, J = 6.4 Hz, H-3), 5.06 (1H, dd, J = 1.4, 4.3 Hz, H-4), 2.66 (1H, m, H-5), 4.48 (1H, d, J = 7.5 Hz, H-6), 5.76 (1H, brs, H-7), 2.90 (1H, t, J = 6.8 Hz, H-9), 4.18 (2H, d, J = 13.6 Hz, H-10), 4.68 (1H, d, J = 7.5 Hz, H-1'), 3.62 (1H, d, J = 9 Hz H-2'), 3.48 (1H, m, H-3'), 3.18 (1H, d, J = 10 Hz, H-4'), 3.29 (1H, ddd, J = 5.2, 5.5, 7 Hz, H-5'), 3,67 (1H, dd, J = 5.5, 11.1 Hz, H-6'a), 3.96 (1H, m, H-6'b). – ^{13}C NMR (90 MHz, CD_3OD): δ = 95.29 (C-1), 141.79 (C-3), 105.75 (C-4), 46.22 (C-5), 83.62 (C-6), 130.29 (C-7), 148.0 (C-8), 49.88 (C-9), 61.58 (C-10), 99.94 (C-1'), 77.86 (C-2'), 79.55 (C-3'), 74.89 (C-4'), 78.22 (C-5'), 62.33 (C-6').

Mimengoside A (**10**): White powder (MeOH/EtOAc); m.p. 264–268 ºC. – IR (KBr): λ_{max} = 3428, 2928, 1066 cm^{-1}. – FAB-MS: m/z = 1095 $[M+Na]^+$, 1073 $[M+1]^+$, 926, 764, 602, 456, 438. – ^{1}H NMR (360 MHz, CD_3OD): δ = 1.0/1.84* (2H, d, J = 13.4 Hz, H-1), 2.07 (2H, d, J = 8.1 Hz, H-2), 3.93 (1H, m, H-3), 1.51 (1H, H-5), 1.76/1.5* (2H, H-6), 1.46 (2H, H-7), 1.92 (1H, brs, H-9), 5.93 (1H, d, J = 10.7 Hz, H-11), 5.36* (1H, H-12), 2.08*/1.8 (2H, d, J = 13.1 Hz, H-15), 4.55* (2H, H-16), 1.61 (1H, H-18), 1.84*/1.27 (2H, H-19), 1.65/1.18 (2H, H-21), 1.5* (2H, d, J = 13.6 Hz, H-22), 4.55*/3.7 (2H, d, J = 10.9 Hz, H-23), 1.07 (3H, s, H-24), 0.96 (3H, s, H-25), 1.32 (3H, s, H-26), 0.98 (3H, s, H-27), 3.39/4.4 (2H, d, J = 7.9 Hz, H-28), 0.93 (3H, s, H-29), 0.88 (3H, s, H-30), 4.91 (1H, d, J = 7.7 Hz, H-1'), 5.33 (1H, d, J = 7.3 Hz, H-1"), 5.31 (1H, d, J = 7.3 Hz, H-1"'), 5.36* (1H, H-1""), 1.62* (3H, d, J = 6.0 Hz, H-6""), the rest of sugar proton signals at δ = 3.6–4.0; *overlapped. – ^{13}C NMR (90 MHz, CD_3OD): δ = 38.3 (C-1), 26.9 (C-2), 84.9 (C-3), 44.44 (C-4), 48.34 (C-5), 17.9 (C-6), 32.0 (C-7), 42.71 (C-8), 54.63 (C-9), 37.08 (C-10), 134.04 (C-11), 131.4 (C-12), 86.9 (C-13), 45.08 (C-14), 32.0 (C-15), 26.5 (C-16), 42.84 (C-17), 52.4 (C-18), 37.55 (C-19), 36.5 (C-20), 32.55 (C-21), 26.5

(C-22), 63.56 (C-23), 12.61 (C-24), 18.50 (C-25), 20.1 (C-26), 19.5 (C-27), 77.8 (C-28), 34.03 (C-29), 23.9 (C-30), 103.49 (C-1'), 78.32 (C-2'), 85.68 (C-3'), 72.45 (C-4'), 70.71 (C-5'), 16.9 (C-6'), 105.13 (C-1"), 75.45 (C-2"), 78.63 (C-3"), 71.32 (C-4"), 77.72 (C-5"), 61.75 (C-6"), 104.73 (C-1"'), 76.06 (C-2"'), 77.88 (C-3"'), 78.01 (C-4"'), 76.78 (C-5"'), 63.0 (C-6"'), 102.87 (C-1""), 72.73 (C-2""), 72.44 (C-3""), 73.77 (C-4""), 70.70 (C-5""), 17.88 (C-6"").

Diosmin (**11**): Off-white platelets (MeOH); m.p. 277–280 °C. – FAB-MS: *m/z* = 609 $[M+1]^+$, 301 $[M+1\text{-sugar}]^+$. – EIMS: *m/z* (%) = 300 $[M]^+$ (100), 272 (2.3), 257 (19.2), 153 (15.3), 152 (3.6), 151 (2.07), 148 (6.8), 136 (9.3), 124 (5.5), 123 (4.8). – ^{1}H NMR (360 MHz, CD_3OD): δ = 6.80 (1H, ss, H-3), 6.45 (1H, d, *J* = 2.1 Hz, H-6), 6.75 (1H, d, *J* = 2.0 Hz, H-8), 7.43 (1H, d, *J* = 2.1 Hz, H-2'), 7.12 (1H, d, *J* = 8.5 Hz, H-5'), 7.56 (1H, dd, *J* = 2.1, 8.3 Hz, H-6'), 5.07 (1H, d, *J* = 7.5 Hz, H-1"), 4.53 (1H, brs, H-1"'), 1.07 (3H, d, *J* = 5.9 Hz, H-6"'), 3.76–3.14 (other sugar protons), 3.82 (3H, s, O–CH_3). – ^{13}C NMR (90 MHz, CD_3OD): δ = 162.89 (C-2), 103.76 (C-3), 181.89 (C-4), 161.15 (C-5), 99.53 (C-6), 164.14 (C-7), 94.73 (C-8), 156.9 (C-9), 105.39 (C-10), 122.82 (C-1'), 113.07 (C-2'), 146.71 (C-3'), 151.25 (C-4'), 112.19 (C-5'), 118.88 (C-6'), 99.85 (C-1"), 73.00 (C-2"), 76.20 (C-3"), 69.51 (C-4"), 75.54 (C-5"), 65.97 (C-6"), 100.46 (C-1"'), 70.26 (C-2"'), 70.66 (C-3"'), 71.98 (C-4"'), 68.26 (C-5"'), 17.74 (C-6"'), 55.73 (O–CH_3).

Linarin (**12**): Yellowish residue (MeOH); m.p. 265–267 °C. – FAB-MS: *m/z* = 593 $[M+1]^+$. – EIMS *m/z* (%) = $[M\text{-sugar}]^+$ (100), 283 (9.5), 256 (6.0), 153 (2.5), 152 (11.5), 135 (3.0), 132 (28.1), 124 (9.0), 123 (4.0), 69 (12.5). – ^{1}H NMR (300 MHz, CD_3OD): δ = 6.95 (1H, s, H-3), 6.45 (1H, d, *J* = 3 Hz, H-6), 6.79 (1H, d, *J* = 3 Hz, H-8), 8.06 (1H, d, *J* = 9 Hz, H-2'), 7.15 (1H, d, *J* = 9 Hz, H-3'), 7.15 (1H, d, *J* = 9 Hz, H-5'), 8.06 (1H, d, *J* = 9 Hz, H-6'), 5.07 (1H, d, *J* = 6 Hz, H-1"), 4.49 (1H, brs, H-1"'), 1.08 (3H, d, *J* = 6 Hz, H-6"'), 3.66–3.11 (other sugar protons), 3.85 (3H, s, O–CH_3). – ^{13}C NMR (75 MHz, CD_3OD): δ = 162.99 (C-2), 103.84 (C-3), 182.09 (C-4), 161.18 (C-5), 99.68 (C-6), 163.98 (C-7), 94.81 (C-8), 159.01 (C-9), 105.49 (C-10), 122.70 (C-1'), 128.51 (C-2'), 114.75 (C-3'), 162.46 (C-4'), 114.75 (C-5'), 128.51 (C-6'), 99.93 (C-1"), 73.10 (C-2"), 76.27 (C-3"), 70.76 (C-4"), 75.68 (C-5"), 66.12 (C-6"), 100.57 (C-1"'), 70.39 (C-2"'), 69.61 (C-3"'), 72.08 (C-4"'), 68.38 (C-5"'), 17.92 (C-6"'), 55.61 (O–CH_3).

Mannitol (**13**): Colourless needles (MeOH); m.p. 167–170 °C. – ^{1}H NMR (360 MHz, CD_3OD): δ = 3.3–3.6. – ^{13}C NMR (90 MHz, DMSO): δ = 64.5 (C-1, C-6), 70.34 (C-3, C-4), 71.9 (C-2, C-5).

Sucrose (**14**): White crystals (MeOH); m.p. 188–190 °C. – FAB-MS: *m/z* (%) = 343 $[M+1]^+$ (19.9), 163 (6.3). – ^{1}H NMR (360 MHz, DMSO): δ = 5.14 (d, *J* = 6.3 Hz, H-1'), 3.18 (dd, *J* = 9.4, 3.6 Hz, H-2'), 3.38* (H-3'), 3.14 (dd, *J* = 9.9, 9.1 Hz, H-4'), 3.62 (brd, *J* = 10 Hz, H-5'), 3.530* (H-6'), 3.75 (d, *J* = 12.2 Hz, H-1), 3.85 (d, *J* = 8.2 Hz, H-3), 3.75* (d, *J* = 7.6 Hz, H-4), 3.77* (d, *J* = 7.2 Hz, H-5), 3.53* (H-6), 4.34–5.18 (OH- protons); *overlapped. – ^{13}C NMR (DMSO, 90 MHz): δ = 91.79 (C-1'), 71.83 (C-2'), 73.14 (C-3'), 70.08 (C-4'), 73.02 (C-5'), 60.76 (C-6'), 62.33 (C-1), 104.21 (C-2), 77.34 (C-3), 74.52 (C-4), 82.67 (C-5), 62.33 (C-6).

Antihepatotoxic activity

Adult male albino rats (180–200 g) were used. The animals were purchased from the animal house of the National Research Center (NRC), Dokki, Cairo, Egypt and divided into 5 groups (8–12 in each group) according to the following design: Group (1) received liquid paraffin (0.3 ml/kg, i.p.) for 4 weeks and served as an untreated control group. Group (2) received CCl_4 three times a week for 4 weeks in a dose of 25 μl/100 g body weight, i.p., diluted 1:6 with liquid paraffin and served as cirrhotic control group (Hernandez-Munzo *et al.*, 1997). Group (3): Cirrhotic animals received silymarin (100 mg/kg) orally daily for 30 d. and acted as standard group. Group (4): Cirrhotic animals received extract 1 (polar fraction of the aerial parts) orally (200 mg/kg) for 30 d. Group (5): Cirrhotic animals received extract 2 (polar fraction of the roots) orally (200 mg/kg) for 30 d. Venous blood samples (3–5 ml) were collected in clean dry test tubes from the retro-orbital sinus of rats using heparinized microcapillary tubes according to the method of Riley (1960) and Sorg and Buckner (1964). The tubes were centrifuged for 15 min using a Heraeus sepatech centrifuge (Labofuge 200). The serum was collected and used immediately for the determination of AST, ALT, total protein and albumin levels.

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