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Antiparasitic antioxidant phenylpropanoids and iridoid glycosides from *Tecoma mollis*

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ABSTRACT

A radical scavenging guided phytochemical study on the stem bark of *Tecoma mollis* afforded seven active phenylpropanoid glycosides (**1**–**7**), including a new one (**4**), and one iridoid (**8**). The structures of the isolated compounds were elucidated on the basis of spectroscopic evidences and correlated with known compounds. Compounds (**1**–**7**) displayed promising antioxidant activity (DPPH assay) in relation to ascorbic acid (positive control). The antimicrobial activity for compounds (**1**–**8**) was evaluated against five bacterial and five fungal strains. The isolated compounds exhibited nonselective weak to moderate antimicrobial activity. The highest antileishmanial activity against *Leishmania donovani* was observed for compound (**7**) with an IC_{50} value of 6.71 μg/ml, using pentamidine and amphotericin B as drug controls. Compound (**5**) exhibited moderate antimalarial activity (45% inhibition) against chloroquine sensitive (D6) clones of *Plasmodium falciparum*.

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1. Introduction

Tecoma mollis Humb. and Bonpl., Family Bignoniaceae is an upright shrub to a large tree attaining 7 m in height. The plant is native to South United States, Mexico to Venezuela and Argentina and is known by the following synonyms: *Tecoma velutina*, Lindl.; and *Tecoma stans var. velutina*, Hort. [1]. The plant has been introduced to Egypt in 1982 as an ornamental plant for its timber and showy bell-shaped flowers and cultivated in the experimental station of Faculty of Agriculture, Assiut Univ., Egypt. *T. mollis* is a member of genus *Tecoma* which is of economic importance both for lumber and as cultivated ornamentals [2,3]. The wood is widely used for timbering, construction and in building of ships and for export due to their hardness and resistance for environmental conditions [4–6]. To the best of our knowledge, little studies were focusing on the phytochemistry and biological activity of *T. mollis* [7], and this is the first study describing in details the chemistry of the polar constituents as well as the potential biological activities of *T. mollis*. The only reported phytochemical study of this plant exhibited the isolation of triterpenoid as well as flavonoid glycosides [7]. The reported biological studies of the leaves of *T. mollis* include analgesic, antipyretic and CNS depressant activities [7]. Also, the leaves of *T. mollis* have been used in traditional medicine as hypoglycaemic due to its alkaloidal contents [8,9]. In general, the genus *Tecoma* is rich in phytoconstituents and previous studies revealed the isolation and identification of many constituents of different chemical classes as phenylpropanoids [10,11], iridoids [11–16], flavonoids [7, 17–21], alkaloids [22–24], triterpenoids [7], phenolics [25] and indolic compounds [26]. Many biological activities were also reported for this genus such as anti-inflammatory,

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hypoglycaemic, spasmolytic, diuretic, antimicrobial and for protozoal diseases [27–35]. Other reported biological activities include antioxidant, anti-proliferative, insecticidal and as astringent for the treatment of diarrhea, dysentery and enteritis [36–40].

In the course of our ongoing research activities toward the isolation of biologically active compounds from plants growing in Egypt either wild or cultivated, in particular the species of diverse chemical constituents with various reported biological activities, and as a part of our collaboration with the National Center for Natural Products Research, School of Pharmacy, University of Mississippi, USA, for differential antimicrobial and antiparasitic screening, we had the opportunity to continue the work on the stem bark of the cultivated *T. mollis* to investigate its chemical constituents and potential biological activities.

In this study, we report the isolation of seven phenylpropanoid glycosides and one iridoid from the stem bark of *T. mollis*, one of which (**4**) is a new phenylpropanoid glycoside. All structures were established by spectroscopic analyses, as well as using accurate mass measurement.

2. Materials and methods

2.1. General experimental procedures

IR spectra were measured on an Ati Mattson Genesis Series FTIR machine. UV absorption was done on a UV–vis spectrometer (Perkin-Elmer Instruments, Lambda 25).The 1D and 2D-NMR experiments were recorded on Varian Unity INOVA 400 MHz NMR systems. Low resolution ESI-MS data were obtained using a Perseptive Biosystems Mariner LC-MS, and high-resolution ESI-MS data were obtained on a Finnigan MAT 900 XLT system. HPLC separations were carried out using a Phenomenex RP column (C18, 250×10 mm, 5 µm) and an Agilent 1200 series gradient pump monitored using a DAD G1315B variable-wavelength UV detector. Column chromatography (CC) was performed using a silica gel (Kieselgel 60 Å, 40–63 µM mesh size, Fluorochem, UK), size exclusion chromatography (Sephadex LH-20, 25–100 mm mesh size, SIGMA, Germany) and using TLC pre-coated silica-gel 60 F254 (0.25 mm, ALUGRAM® SIL G/UV_{254}, Macherey-Nagel, Germany) and RP-18 F254S plates (0.25 mm, Merck, Germany). All flash chromatography was performed on Biotage cartridge flash columns (Si 40 M and C18 HS 40 M) using a Biotage Flash system, Charlottesville, USA.

2.2. Plant materials

The stem barks were collected in June 2005 from the trees cultivated in the experimental station of Faculty of Agriculture, Assiut Univ., Egypt. The plant was authenticated by Dr. Gamal Taha, Dept. of Horticulture, Faculty of Agriculture, Assiut Univ., Egypt. A voucher specimen (no. 2011TM) was deposited at the Pharmacognosy Dept., Faculty of Pharmacy, Assiut Univ., Egypt.

2.3. Extraction and isolation

One kg of the air-dried powdered stem barks of *T. mollis* were extracted by maceration in 70% EtOH (2 L×3). The alcoholic extract was concentrated and the solvent free residue (185 g, 18.5%) was mixed with 500 mL of distilled H_2O, and defatted with $CHCl_3$. The aqueous layer was subjected to DIAION HP20 column (25×1.5 cm) and eluted with H_2O, MeOH and Me_2CO, successively. The MeOH eluant (31 g) was subjected to medium pressure liquid chromatography (MPLC) on silica gel column using $CHCl_3$–MeOH mixtures in a manner of increasing polarities. Hundred and five fractions (100 mL each) were collected and monitored on TLC (silica gel) using $CHCl_3$–MeOH–H_2O (80:20:0.5), (70:30:3) and (55:40:5), as well as on RP-C18 using H_2O–MeOH (70:30), (50:50) and (30:70) as solvent systems and Anisaldehyde/H_2SO_4 as a spraying reagent. Similar fractions were combined to yield five groups.

Group 1 (fractions 1–22, eluent: $CHCl_3$–MeOH, 8:2) (3.6 g) was re-chromatographed on RP-C18 column using eluent MeOH–H_2O (70:30) to provide seven sub-fractions. Part (40 mg) of sub-fraction 1–3 (900 mg) was purified by HPLC (Luna 5 µ C18 100 Å, 10 µm× 10 mm×250 mm) using a gradient of 0–100% CH_3CN–H_2O over 40 min to give compound (**2**) (9.7 mg, yellow powder).

Group 2 (fractions 23–60, eluent: $CHCl_3$–MeOH, 7:3) (8.3 g) was re-chromatographed on silica gel column using EtOAc–MeOH in a manner of increasing polarities to provide five sub-fractions. Part (65 mg) of sub-fraction 2–4 (3.9 g) was purified by RP HPLC using a gradient of 0–100% CH_3CN–H_2O over 40 min to afford compound (**1**) (13.6 mg, yellow powder) and compound (**6**) (19.2 mg, yellow powder).

Group 3 (fractions 61–78, eluent: $CHCl_3$–MeOH, 7:3) (6.9 g) was subjected to gel filtration chromatography using a Sephadex LH-20 column with $CHCl_3$–MeOH (3:2) to provide nine fractions. Part (80 mg) of sub-fraction 3–6 (3.4 g) was purified by RP HPLC using a gradient of 0–100% CH_3CN–H_2O over 40 min to give compound (**5**) (14.1 mg, yellow powder) and (**4**) (16.4 mg, yellow powder).

Group 4 (fractions 79–96, eluent: $CHCl_3$–MeOH, 6:4) (5.1 g) was re-chromatographed on RP-C18 column using 5-70% aq. MeOH as eluent to provide six sub-fractions. Part (60 mg) of sub-fractions 2–3 (1.8 g) were purified by RP HPLC using a gradient of 0–100% CH_3CN–H_2O over 40 min to give compound (**3**) (7.1 mg, yellow powder) and (**7**) (13.1 mg, yellow powder).

Group 5 (fractions 97–105, eluent: $CHCl_3$–MeOH, 6:4) (3.4 g) was re-chromatographed on Sephadex LH-20 column with $CHCl_3$–MeOH (1:1) to provide seven sub-fractions. Part (50 mg) of sub-fractions 4–6 (826 mg) was purified by RP HPLC using a gradient of 0–80% CH_3CN–H_2O over 40 min to give compound (**8**) (16.8 mg, yellow powder).

2.4. Acid hydrolysis of isolated glycosides [41]

Isolated glycosides (5 mg, each) were dissolved in 0.2 N H_2SO_4 (5 mL) and heated at 95 °C for 1 h. After cooling, the reaction mixture was extracted with Et_2O (2 mL×3). The aqueous layer was neutralized with $NaHCO_3$, evaporated to dryness, and extracted with pyridine. The pyridine extract

was then analyzed on silica gel TLC (EtOAc–MeOH–H_2O–AcOH 13:3:3:4). Rhamnose (R_f 0.52), Apiose (R_f 0.48) and glucose (R_f 0.32) were detected by comparison with authentic samples.

2.5. DPPH radical-scavenging assay

Firstly, radical scavenging activity of the isolated glycosides against stable DPPH$^{\bullet}$ was performed with a rapid TLC screening method using 0.2% DPPH in MeOH. 30 min after spraying, the active compounds appear as yellow spots against purple background [42,43]. In a second experiment, spectrophotometric assay was carried out according to the method of Yen and Chen [44]. Briefly, 2.0 mL of a wide range of concentrations (2.5–120 μM) of test sample (in MeOH) was added to 2.0 mL of 100 μM of methanolic solution of DPPH. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min in dark. When DPPH$^{\bullet}$ reacts with an antioxidant compound, it changes its color from deep-violet to light-yellow which is measured at 517 nm on a UV/visible light spectrophotometer. Absorption of blank sample containing the same amount of MeOH and DPPH solution was prepared and measured daily. The experiment was carried out in triplicate, using ascorbic acid as a positive control. The percentage reduction of the DPPH, Q, referred to "inhibition or quenching" was calculated by the following formula [45]:

$$Q(\%\,\text{inhibition}) = [(A_B - A_A)/A_B] \times 100$$

where: A_B—absorption of blank sample (t = 0 min);
A_A—absorption of tested extract solution (t = 30 min).

2.6. Biological assays

2.6.1. Antimicrobial assay

All organisms are obtained from the American Type Culture Collection (Manassas, VA). They include the fungi *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 90906 and the bacteria *Staphylococcus aureus* ATCC 29213, methicillin-resistant *S. aureus* ATCC 43300 (MRSA), *Pseudomonas aeruginosa* ATCC 27853, *Mycobacterium intracellulare* ATCC 23068 and *Escherichia coli* (ATCC 35218). Susceptibility testing was performed for all organisms, except *M. intracellulare*, using modified versions of the CLSI/NCCLS methods [46]. *M. intracellulare* susceptibility was tested using a modified Franzblau-method [47]. Samples dissolved in DMSO were serially diluted in DMSO/saline (20%/0.9%) and transferred in duplicate to 96-well flat bottom microplates. Microbial inocula were prepared by correcting the OD_{630} of microbe suspensions in incubation broth to afford final target inocula. Controls [fungi: amphotericin B; bacteria: ciprofloxacin (ICN Biomedicals, OH)] were included in each assay. All organisms were read at 630 or 544(ex)/590(em) nm (*M. intracellulare* and *A. fumigatus*) prior to and after incubation. Percent growth was plotted versus test concentration to afford the IC_{50}. Minimum fungicidal or bactericidal concentrations were determined by removing 5 μL from each clear well, transferring to agar, and incubating until growth was seen. The MFC/MBC is defined as the lowest test concentration that kills the organism (allows no growth on agar).

2.6.2. In vitro antileishmanial assay

The *in vitro* antileishmanial activity was evaluated against a culture of *L. donovani* promastigotes grown in RPMI 1640 medium supplemented with 10% GIBCO fetal calf serum at 26 °C [48,49]. A three-day-old culture was diluted to 5×10^5 promastigotes/ml. Drug dilutions (50–3.1 μg/ml) were prepared directly in cell suspension in a 96-well plate, followed by incubation (26 °C, 48 h). Growth of leishmanial promastigotes was determined by the Alamar Blue assay (BioSource International, Camarillo, CA) [50–52].

Standard fluorescence was measured by a Fluostar Galaxy plate reader (excitation wavelength, 544 nm; emission wavelength, 590 nm). Pentamidine and amphotericin B were used as the drug controls. Percent growth was calculated and plotted against the tested concentrations in order to determine the IC_{50} and IC_{90} values.

2.6.3. In vitro antimalarial activity

The assay for antimalarial activity was based on the determination of plasmodial LDH activity. For the assay, a suspension of red blood cells infected with D6 strain of *Plasmodium falciparum* (200 μL, with 2% parasitemia and 2% hematocrit in RPMI 1640 medium supplemented with 10% human serum and 60 μg/mL amikacin) was added to the wells of a 96-well plate containing 10 μL of serially diluted test samples. The plate was flushed with a gas mixture of 90% N_2, 5% O_2, and 5% CO_2 and incubated at 37 °C, for 72 h in a modular incubation chamber (Billups-Rothenberg, CA). Parasitic LDH activity was determined according to the procedure of Makler and Hinrichs [53]. Briefly, 20 μL of the incubation mixture was mixed with 100 μL of the Malstat™ reagent (Flow Inc., Portland, OR) and incubated at room temperature for 30 min. Twenty microliters of a 1:1 mixture of NBT/PES (Sigma, St. Louis, MO) was then added and the plate is further incubated in the dark for 1 h. The reaction was then stopped by the addition of 100 μL of a 5% acetic acid solution. The plate was read at 650 nm. Artemisinin and chloroquine were included in each assay as antimalarial drug controls. IC_{50} values were computed from the dose response curves. To determine the selectivity index of antimalarial activity of compounds their *in vitro* cytotoxicity to mammalian cells was also determined. The assay was performed in 96-well tissue culture-treated plates. Vero cells (monkey kidney fibroblasts) were seeded to the wells at a density of 25,000 cells/well and incubated for 24 h. Diluted samples were added and plates were again incubated for 48 h. The number of viable cells was determined by Neutral Red assay as described earlier [54]. IC_{50} values were obtained from dose response curves. Doxorubicin was used as a positive control for cytotoxicity.

Verpectoside B (Crassoside) (**3**): yellow amorphous powder (7.1 mg). $[\alpha]^{23}_D$ +133 (*c* 0.08, MeOH); UV: λ_{max} (MeOH): 218, 301, 348 nm; IR (KBr) ν_{max} 3450, 1693, 1617, 1513 cm^{-1}; HRESIMS m/z 809.24707 ($[M+Na]^+$, calcd for $C_{35}H_{46}NaO_{20}$, 809.24801, $\Delta = -1.0$ ppm); ^1H (400 MHz, DMSO-d_6) and ^{13}C (100 MHz, DMSO-d_6) NMR data: see Table 1.

Table 1
^{1}H (400 MHz) and ^{13}C-NMR (100 MHz) data of **3** and **4** in DMSO-d_6.

No.	3	3	4	4
	δ^1H/ppm, mult, J/Hz	$\delta^{13}C$/ppm, mult.	δ^1H/ppm, mult, J/Hz	$\delta^{13}C$/ppm, mult.
Aglycone				
1	–	129.1, s	–	129.5, s
2	6.62 (1H, brs)	116.3, d	6.61 (1H, d, 1.8)	116.3, d
3	–	145.0, s	–	145.0, s
4	–	143.5, s	–	143.5, s
5	6.62 (1H, d, 8.0)	115.5, d	6.62 (1H, d, 8.0)	115.5, d
6	6.49 (1H, d, 8.0)	119.6, d	6.49 (1H, dd, 1.8, 8.0)	119.5, d
7	2.68 (2H, t, 7.8)	35.0, t	2.69 (2H, m)	35.0, t
8	a. 3.88 (1H, m) b.3.62 (1H, m)	70.3, t	a. 3.82 (1H, m) b.3.63 (1H, m)	70.3, t
Sugar 1	Glucose	Glucose	Glucose	Glucose
1′	4.57 (1H, d, 7.8)	101.3, d	4.65 (1H, d, 7.2)	101.2, d
2′	3.56 (1H, m)	81.1, d	3.62 (1H, m)	80.6, d
3′	3.86 (1H, m)	77.7, d	3.91 (1H, m)	77.2, d
4′	4.77 (1H, m)	69.2, d	4.90 (1H, m)	68.8, d
5′	3.50 (1H, m)	74.3, d	3.81 (1H, m)	70.7, d
6′	a. 3.38 (1H, m) b. 3.34 (1H, m)	60.8, t	a. 4.03 (1H, dd, 5.0, 12.0) b. 3.95 (1H, m)	62.5, t
C=O	–	–	–	170.1, s
CH_3	–	–	1.96 (3H, s)	20.6, q
Caffeoyl moiety				
1″	–	125.5, s	–	125.9, s
2″	7.00 (1H, brs)	114.7, d	7.02 (1H, brs)	114.8, d
3″	–	145.5, s	-	145.6, s
4″	-	148.5, s	-	148.5, s
5″	6.75 (1H, d, 8.0)	115.8, d	6.76 (1H, d, 8.1)	115.8, d
6″	6.98 (1H, d, 8.0)	121.4, d	6.98 (1H, d, 8.1)	121.5, d
7″	7.46 (1H, d, 16.0)	145.6, d	7.46 (1H, d, 15.9)	145.8, d
8″	6.20 (1H, d, 16.0)	113.6, d	6.19 (1H, d, 15.9)	113.2, d
C=O	–	165.7, s	–	165.6, s
Sugar2	Rhamnose	Rhamnose	Rhamnose	Rhamnose
1″′	5.03 (1H, brs)	101.3, d	5.02 (1H, brs)	101.1, d
2″′	3.81 (1H, m)	70.4, d	3.81 (1H, m)	70.4, d
3″′	3.27 (1H, m)	70.4, d	3.27 (1H, m)	70.2, d
4″′	3.11 (1H, m)	71.8, d	3.11 (1H, m)	71.7, d
5″′	3.34 (1H, m)	68.9, d	3.34 (1H, m)	69.0, d
6″′	0.96 (3H, d, 6.2)	18.2, q	0.98 (3H, d, 6.2)	18.2, q
Sugar 3	Glucose	Glucose	Glucose	Glucose
1″″	4.54 (1H, d, 7.5)	101.8, d	4.53 (1H, d, 7.5)	101.8, d
2″″	2.99 (1H, t, 7.6)	74.3, d	2.99 (1H, t, 7.6)	74.3, d
3″″	3.12 (1H, m)	77.1, d	3.12 (1H, m)	77.1, d
4″″	3.11 (1H, m)	69.7, d	3.12 (1H, m)	69.7, d
5″″	3.16 (1H, m)	77.1, d	3.16 (1H, m)	77.1, d
6″″	a. 3.68 (1H, m) b. 3.52 (1H, m)	61.0, t	a. 3.68 (1H, m) b. 3.51 (1H, m)	60.9, t

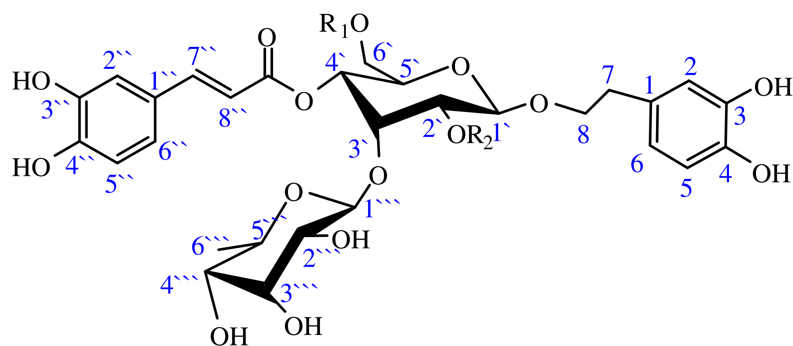
Tocomoloside (6′-*O*-acetyl verpectoside B) (**4**): yellow amorphous powder (16.4 mg). $[\alpha]^{23}_{D}$ +116.3 (*c* 0.2, MeOH); UV:λ_{max}^{MeOH}: 220, 291, 345 nm ; IR (KBr) v_{max} 3433, 1730, 1688, 1624, 1523 cm^{-1}; HRESIMS m/z 851.25623 ($[M+Na]^+$, calcd for $C_{37}H_{48}NaO_{21}$, 851.25858, $\Delta=-3.0$ ppm); ^{1}H (400 MHz, DMSO-d_6) ^{13}C (100 MHz, DMSO-d_6) NMR data: see Table 1.

3. Results and discussion

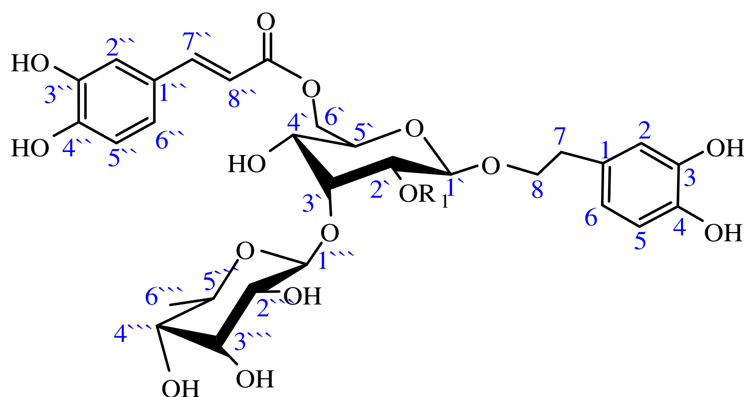
A combination of flash or medium-pressure liquid chromatography (MPLC), gel filtration and HPLC of the MeOH fraction obtained from the stem bark of *T. mollis* afforded seven phenylpropanoids (**1**–**7**) and one iridoid (**8**) (Fig. 1). Their structures were elucidated by extensive 1D, 2D NMR analysis, accurate mass measurements and by comparing with the reported data.

Six known phenylpropanoids (**1**–**3**, **5**–**7**) were identified as acteoside (Verbascoside) (**1**) [55,56], 6′-*O*-acetylverbascoside (**2**) [57], verpectoside B (Crassoside) (**3**) [58], luteoside A (**5**) [41,59], isoacteoside (isoverbascoside) (**6**) [57,60], luteoside B (**7**) [41,61] and one iridoid, ixoside (**8**) [61–63]. All physical and spectral data of these compounds were in agreement with the respective published data.

Compound **4** was obtained as a yellow amorphous powder ($[\alpha]^{23}_{D}$ +116.3 (*c* 0.2, MeOH)). HRESIMS showed pseudomolecular ion peaks at m/z 829.27435 $[M+H]^+$ and 851.25623 $[M+Na]^+$ consistent with a molecular weight of 828 amu.



1	R_1= H	R_2= H
2	R_1= $COCH_3$	R_2= H
3	R_1= H	R_2= Glc
4	R_1= $COCH_3$	R_2= Glc
5	R_1= $COCH_3$	R_2= Api



6	R_1= H
7	R_1= Api

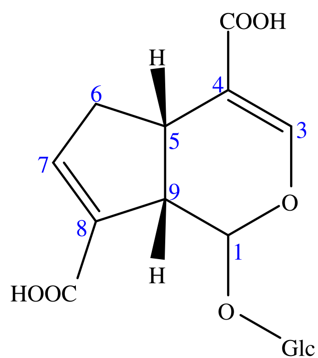


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Fig. 1. Chemical structures of isolated compounds **1–8** (Glc = Glucose, Api= Apiose).

Table 2
DPPH radical scavenging activity of fractions and isolated compounds.

Sample	IC_{50} in μg/ml (μM)
Total ext.	99.3
Chloroform fraction	>200
Hydro methanolic fraction	52.8
Group 1	39.6
Group 2	18.7
Group 3	28.8
Group 4	14.1
Group 5	128.6
Verbascoside (**1**)	6.1 (9.8)
6′-*O*-acetylverbascoside (**2**)	7.5 (11.2)
Verpectoside B (**3**)	8.6 (10.9)
Tocomoloside (**4**)	12.8 (15.5)
Luteoside A (**5**)	11.7 (14.6)
Isoverbascoside (**6**)	8.5 (13.7)
Luteoside B (**7**)	12.7 (16.8)
Ixoside (**8**)	42.4 (109.2)
Ascorbic acid	2.1 (11.9)

The molecular formula was established as $C_{37}H_{48}O_{21}$, thus implying 14 degrees of unsaturation. The UV:λ_{max}^{MeOH}: 220, 291, 345 nm, while IR spectrum showed strong absorption bands attributed to the hydroxyl and ester groups at 3433 and 1730, 1688 cm^{-1}, respectively. The 1D and 2D NMR spectra in DMSO-d_6 (Table 1) of **4** revealed the phenylpropanoid glycosides pattern and were very similar to those of **3**[58]. The ^1H NMR spectral data showed the presence of two sets of ABX system for β-(3,4-dihydroxyphenyl) ethoxy moiety and caffeoyl moiety, as well as two trans-olefinic protons, three anomeric protons for two β-glucopyranosyl and one α-rhamnopyranosyl units, and an acetyl signal. The ^1H and ^{13}C NMR spectra were very similar to those of **3** except for the additional signal of acetyl group (δ_H 1.96 for $COCH_3$), (δ_C 20.6 for CH_3) and (δ_C 170.1 for CO). Comparison of the ^{13}C NMR spectral data of **4** with those of **3** (Table 1) revealed downfield shift in the position of C-6′ (+1.7 ppm) and upfield shift of C-5′ (3.6 ppm) of the glucosyl moiety indicating that the acetyl group is at C-6′ of the glucosyl moiety. Furthermore, the HMBC spectrum showed the three bond correlation between H-6′ (δ_H 3.95 and 4.03) of the glucosyl moiety and carbonyl carbon (δ_C 170.1) of the acetyl group. Acid hydrolysis of **4** yielded sugars rhamnose and glucose. Therefore, the structure of compound **4** was elucidated as 6′-*O*-acetyl-verpectoside B. Compound **4** was identified as a new natural product for which we propose the name tocomoloside.

Phenylpropanoid glycosides are water soluble natural products widely distributed in the plant kingdom. Their significant biological activities such as enzyme inhibition, immunomodulatory, antibacterial and cytotoxic activities have been reported [64]. Recently, phenylpropanoid glycosides were demonstrated *in vitro* assays to have antioxidative and free radical scavenging activities [65–70]. The antioxidant activity of the isolated phenylpropanoids (**1**–**7**) was determined by using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging system. The IC_{50s} ranged from 9.8 to 16.8 μM (Table 2). The antioxidative effect of these compounds was related to the number of free phenolic hydroxyl groups in the form of 3,4-dihydroxy (catechol) moiety in their structures which explain the close similarity of their antioxidant activity. Absence or blocking of the hydroxyl groups by methyl group leads to dramatic decrease of the antioxidant activity [71]. The sequence of the strength of the antioxidant activity was shown to be **1**>**3**>**2**>**6**>**5**>**4**>**7**. The iridoid glycoside (ixoside) (**8**) exhibited very weak antioxidant activity with $IC_{50}=109.2$ μM. The free radical scavenging effects of the phenylpropanoids proven here may play an important role in the actions of the *Tecoma* species, and partly explain the mechanisms of the activities of phenylpropanoids against neoplasma [66,72] and inflammation [72,73] in which free radical are seriously involved.

The antifungal and antibacterial activity for the isolated compounds was evaluated against Gram positive, Gram negative bacteria and fungi. The percentage of inhibition (% inh.) of each strain was determined and compared with those for commercially available antibiotics (Table 3). In general, weak activity was observed for isolated compounds against fungal strains particularly *A. fumigatus* ATCC 90906. The highest activity was observed for compounds (**6**) and (**7**) with inhibition of 30% and 40% respectively. Similar was observed against Gram +ve and −ve bacteria with highest inhibition occurred for *Mycobacterium intracellulare* by compound (**7**).

Compounds (**1–8**) were tested against the protozoan parasite *L. donovani*, using pentamidine and amphotericin B as drug controls (Table 4). The highest *in vitro* antileishmanial activity was observed for compounds **1**, **5**, and **7** with percentage of inhibition [83% (IC_{50} 30.08), 92% (IC_{50} 15.07) and 92% (IC_{50} 6.71), respectively] (Table 4). Compounds **2** and **6** showed relatively moderate activity with percentage of inhibition [62% (IC_{50}>40), and 78% (IC_{50}>40), respectively], while the least active compounds are **3**, **4** and **8** with percentage of inhibition (19%, 21% and 13%, respectively). It is obvious from Table 4

Table 3
Antimicrobial activity of isolated compounds (**1–8**) against different bacteria and fungi.

Compound number	Compound conc. (μg/ml)	*S. aureus* % inh.	MRS % inh.	*E. coli* % inh.	*P. aeruginosa* % inh.	*M. intracellulare* % inh.	*C. Albicans* % inh.	*C. glabrata* % inh.	*C. krusei* % inh.	*A. fumigatus* % inh.	*C. neoformans* % inh.
1	13	7	5	6	2	8	16	11	12	1	14
2	13	11	0	14	6	6	9	2	5	0	3
3	13	9	5	10	0	0	21	14	8	0	6
4	13	8	8	2	6	4	7	6	5	1	12
5	13	11	0	5	3	16	8	9	1	4	4
6	13	10	8	3	4	6	11	30	9	0	11
7	13	9	10	7	0	33	14	41	0	8	6
8	13	8	1	1	4	6	17	0	11	0	10

Table 4
In vitro antileishmanial activity of isolated compounds (**1**–**8**).

Compound Number	Compound conc. (μg/ml)	*L. donovani* % inh.	IC_{50} (μg/ml)	IC_{90} (μg/ml)
1	80	83	30.08	>40
2	80	62	>40	>40
3	80	19	ND	ND
4	80	21	ND	ND
5	80	92	15.07	>40
6	80	78	>40	>40
7	80	92	6.71	22.28
8	80	13	ND	ND
Amphotericin B[a]	80	100	0.34	0.43
Pentamidine[a]	80	100	1.37	1.62

IC_{50}: concentration causing 50% growth inhibition. IC_{90}: concentration causing 90% growth inhibition.
ND: not determined. *a*: control.

that introduction of five membered ring sugar moiety, such as apiose, in the position 2′ lead to improve the antileishmanial activity, while introduction of six membered sugar moiety such as glucose lead to dramatic decrease in the activity. Acetylation at position 6′ has neglectable effect. Not many phenylpropanoids have been reported for antileishmanial activity and little records considered the antileishmanial activity of phenylpropanoids [74]. The present study tries to highlight on the potential antileishmanial activity for this class of compounds and further investigation is required to establish and optimize their activity.

The *in vitro* antimalarial activity of (**1–8**) against chloroquine sensitive (D6) clones of *P. falciparum* was evaluated based on the determination of plasmodial LDH activity (Table 5). Compound (**5**) showed moderate antimalarial activity with (45%) inhibition against chloroquine sensitive (D6) clones of *P. falciparum*. The rest of the compounds exhibited weak antimalarial activity.

In conclusion, we report here the isolation of 7 phenylpropanoid glycosides and 1 iridoid from the stem bark of *T. mollis* cultivated in Egypt, one of which (**4**) is a new phenylpropanoid glycoside.

The phenylpropanoids with ortho phenolic dihydroxy group showed significant antioxidant activity comparable with ascorbic acid as natural antioxidant while the iridoid exhibited very weak antioxidant activity. None of the isolated compounds exhibited significant antimicrobial and antimalarial activity. Compounds **5** and **7** showed the highest antileishmanial activity, and further investigation is required for this class of compounds to use as antileishmanial drugs. Moderate antimalarial activity was observed with compound **5** against chloroquine sensitive (D6) clones of *P. falciparum*.

Table 5
Antimalarial activity of isolated compounds (**1**–**8**).

Compound number	*P. falciparum* (D6 clone) % inh.
1	22
2	0
3	2
4	5
5	45
6	10
7	0
8	9

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.fitote.2011.12.025.

References

[1] Bailey LH. The standard cyclopedia of horticulture. Twentieth Printing, Vol. III. New York: The MacMillan Co.; 1963. p. 3316–7.
[2] Lawrence GHM. Taxonomy of vascular plants. New York: The MacMillan Company; 1968. p. 698–700.
[3] Wood JRI. A revision of *Tecoma* Juss. (Bignoniaceae) in Bolivia. Bot J Linn Soc 2008;156:143–72.
[4] Bianco A, Passacantilli P, Nicoletti M, Alves de Lima R. Iridoids in equatorial and tropical flora. Planta Med 1982;46:33–7.
[5] Manners GD, Jurd L. New natural products from marine borer resistant woods. A review. J Agric Food Chem 1977;25:726–30.
[6] Rizzini CT, Morsin WP. Botânica Econômica Brasileira, Edn. São Paulo: EPU and EDUSP; 1976. p. 166.
[7] El-Emary NA, Khalifa AA, Bekheet AY, Abdel-Mageed WM. Phytochemical and biological studies on the leaf of *Tecoma mollis* Humb. and Bonpl. cultivated in Egypt. Bull Pharm Sci Assiut Univ 2002;25:207–28.
[8] Colin GG. Further observations on the anti-diabetic properties of *Tecoma mollis*. J Pharm Sci 1927;16:199–203.
[9] Colin GG. Study on the anti-diabetic properties of *Tecoma mollis*. Preliminary report. J Pharm Sci 1926;15:556–60.
[10] Pletsch M, Piacente S, Pizza C, Charlwood BV. The accumulation of phenylpropanoid glycosides in tissue cultures of *Tecoma sambucifolium*. Phytochemistry 1993;34:161–5.
[11] Guiso M, Marra C, Piccioni F, Nicoletti M. Iridoid and phenylpropanoid glucosides from *Tecoma capensis*. Phytochemistry 1997;45:193–4.
[12] Bianco V, Passacantilli P, Polidori G, Nicoletti M, de Lima RA. New iridoids from *Tecoma heptaphylla*. Planta Med 1982;45:153-153.
[13] Bianco A, Passacantilli G, Polidori M, Nicoletti M, Alves De Lima R. Isolation of 6-epimonomelittoside from *Tecoma heptaphylla* and its conversion into monomelittoside. Phytochemistry 1983;22:1189–91.
[14] Bianco A, Massa M, Oguakwa JU, Passacantilli P. 5-deoxystansioside, an iridoid glucoside from *Tecoma stans*. Phytochemistry 1981;20:1871–2.
[15] Bianco A, Passacantilli P. Iridoids in equatorial and tropical flora-III: isolation and partial synthesis of 6-epiaucubin a new glucosidic iridoid. Tetrahedron 1982;38:359–62.
[16] von Poser GL, Schripsema J, Henriques AT, Jensen SR. The distribution of iridoids in Bignoniaceae. Biochem Syst Ecol 2000;28:351–66.
[17] Prakash EO, Rao JT. A new flavone glycoside from the seeds of *Tecoma undulate*. Fitoterapia 1999;70:287–9.
[18] Srivastava BK, Reddy M. Flavonoids from the flower extract of *Tecoma stans*. Asian J Chem 1995;7:679–80.
[19] Ahmad M, Jain N, Kamil M, Ilyas M. Isolation and characterization of 2 new flavanone disaccharides from the leaves of *Tecoma-Grandiflora* Bignoniaceae. J Chem Res-S 1991;5:109-109.
[20] Taha MM. The carotenoids of the petals of two species of *Tecoma*. Biochem J 1954;58:413–5.
[21] Lopez-Laredo AR, Ramirez-Flores FD, Sepulveda-Jimenez G, Trejo-Tapia G. Comparison of metabolite levels in callus of *Tecoma stans* (L.) Juss. ex Kunth. cultured in photoperiod and darkness. In Vitro Cell Dev Biol-Plant 2009;45:550–8.
[22] Nagata K, Hirai KI, Koyama J, Wada Y, Tamura T. Antimicrobial activity of novel furanonaphthoquinone analogs. Antimicrob Agents Chemother 1998;42:700–2.
[23] Lins AP, Felicio JD. Monoterpene alkaloids from *Tecoma stans*. Phytochemistry 1993;34:876–8.

[24] Costantino L, Lins AP, Barlocco D, Celotti F, El-Abady SA, Brunetti T, et al. Characterization and pharmacological actions of tecostanine, an alkaloid of *Tecoma stans*. Pharmazie 2003;58:140–2.

[25] Okarter TU, Schiff PL, Knapp JE, Slatkin DJ. Lipid and phenolic constituents of *Tecoma radicans*. Phytochemistry 1976;15:436-436.

[26] Kunapuli SP, Vaidyanathan CS. Indolic compounds in the leaves of *Tecoma stans*. Phytochemistry 1984;23:1826–7.

[27] Alguacil LF, Galán de Mera A, Gómez J, Llinares F, Morales L, Muñoz-Mingarro MD, et al. *Tecoma sambucifolia*: anti-inflammatory and antinociceptive activities, and '*in vitro*' toxicity of extracts of the 'huarumo' of Peruvian Incas. J Ethnopharmacol 2000;70:227–33.

[28] Aguilar-Santamaría L, Ramírez G, Nicasio P, Alegría-Reyes C, Herrera-Arellano A. Antidiabetic activities of *Tecoma stans* (L.) Juss. ex Kunth. J Ethnopharmacol 2009;124:284–8.

[29] Alonso-Castro AJ, Zapata-Bustos R, Romo-Yañez J, Camarillo-Ledesma P, Gómez-Sánchez M, Salazar-Olivo LA. The antidiabetic plants *Tecoma stans* (L.) Juss. ex Kunth (Bignoniaceae) and *Teucrium cubense* Jacq (Lamiaceae) induce the incorporation of glucose in insulin-sensitive and insulin-resistant murine and human adipocytes. J Ethnopharmacol 2010;127:1–6.

[30] Costantino L, Raimondi L, Pirisino R, Brunetti T, Pessotto P, Giannessi F, et al. Isolation and pharmacological activities of the *Tecoma stans* alkaloids. Il Farmaco 2003;58:781–5.

[31] Karen S, William GC. Natural products used for diabetes. J Am Pharm Assoc 2002;42:217–26.

[32] Naseri MKG, Moghaddam MA, Bahadoram S. Antispasmodic effect of *Tecoma stans* (L.) Juss leaf extract on rat ileum. DARU-J Fac Pharm 2007;15:123–8.

[33] Porez H, Diaz F, Medina JD. Chemical investigation and *in vitro* antimalarial activity of *Tabebuia ochracea* ssp. neochrysantha. Int J Pharmacogn 1997;35:227–31.

[34] Ganapaty S, Nyamathulla S, Srilakshmi GVK, Kumar PVR. Iridoid compounds and antimicrobial activity of the roots of *Tecoma stans* (L) Juss. Asian J Chem 2008;20:4493–7.

[35] Binutu OA, Lajubutu BA. Antimicrobial potentials of some plant species of the Bignoniaceae family. Afr J Med Sci 1994;23:269–73.

[36] Marzouk MSA, Gamal-Eldeen AM, Mohamed MA, El-Sayed MM. Antioxidant and anti-proliferative active constituents of *Tecoma stans* against tumor cell lines. Nat Prod Commun 2006;1:735–43.

[37] Marzouk M, Gamal-Eldeen A, Mohamed M, El-Sayed MM. Anti-proliferative and antioxidant constituents from *Tecoma stans*. Z Naturforsch C -J Biosci 2006;61:783–91.

[38] Sechagui DR. The insecticidal property of petals of several common plants of India Econ. Botany 1957;11:274–6.

[39] Hashimoto G. Brasil shokubutu ki—Knowledge of popular, useful plants. Japan: Teikoku Shoin; 1962. p. 193.

[40] Piocorrea I. Dicionario das Plantas Uteis do Brasil e das Cultivadas Exoticas, IV. Edn. Rio do Janeiro: Ministerio da Agricultara; 1952. p. 318.

[41] Kanchanapoom T, Kasai R, Yamasaki K. Phenolic glycosides from *Markhamia stipulata*. Phytochemistry 2002;59:557–63.

[42] Braca A, Sortino C, Politi M, Morelli I, Mendez J. Antioxidant activity of flavonoids from *Licania licaniaeflora*. J Ethnopharmacol 2002;79:379–81.

[43] Yrjönen T, Peiwu L, Summanen J, Hopia A, Vuorela H. Free radical-scavenging activity of phenolics by reversed-phase TLC. J Am Oil Chem Soc 2003;80:9–14.

[44] Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric Food Chem 1995;43:27–32.

[45] Abdel-Mageed WM, Milne BF, Wagner M, Schumacher M, Sandor P, Pathom-aree W, et al. Dermacozines, a new phenazine family from deep-sea dermacocci isolated from a Mariana Trench sediment. Org Biomol Chem 2010;8:2352–62.

[46] (a) National Committee for Clinical Laboratory Standards (NCCLS) (Wayne, Pa.): Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved Standard — Second Edition. Document M27-A2, 2002, 22. (b) NCCLS: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard — Seventh Edition. Document M7-A7, 2006, 26. (c) NCCLS: Susceptibility testing of mycobacteria, nocardia, and other aerobic actinomycetes; Tentative Standard — Approved Standard. Document M24-A, 2003, 23. (d) NCCLS: Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; Approved Standard. Document M38-A, 2002, 22.

[47] Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A, et al. Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue assay. J Clin Microbiol 1998;36:362–6.

[48] Jain M, Khan SI, Tekwani BL, Jacob MR, Singh S, Singh PP, et al. Synthesis, antimalarial, antileishmanial and antimicrobial activities of some 8-quinolinamine analogues. Bioorg Med Chem 2005;13:4458–66.

[49] Bharate SB, Khan SI, Tekwani BL, Jacob M, Khan IA, Singh IP. S-Euglobals: biomimetic synthesis, antileishmanial, antimalarial, and antimicrobial activities. Bioorg Med Chem 2008;16:1328–36.

[50] Ma G, Khan SI, Jacob MR, Tekwani BL, Li Z, Pasco DS, et al. Antimicrobial and antileishmanial activities of hypocrellins A and B. Antimicrob Agents Chemother 2004;48:4450–2.

[51] Mikus J, Steverding D. A simple colorimetric method to screen drug cytotoxicity against Leishmania using the dye Alamar Blue. Parasitol Int 2000;48:265–9.

[52] Hamid R, Rotshteyn Y, Rabadi L, Parikh R, Bullock P. Comparison of alamar blue and MTT assays for high through-put screening. Toxicol in Vitro 2004;18:703–10.

[53] Makler MT, Hinrichs DJ. Measurement of the lactate dehydrogenase activity of *Plasmodium falciparum* as an assessment of parasitemia. Am J Trop Med Hyg 1993;48:205–10.

[54] Mustafa J, Khan SI, Ma G, Walker LA, Khan I. Synthesis and anticancer activities of fatty acid analogs of podophyllotoxin. Lipids 2004;39:162–72.

[55] Budzianowski J, Skrzypczak L. Phenylpropanoid esters from *Lamium album* flowers. Phytochemistry 1995;38:997–1002.

[56] Xiong Q, Kadota SH, Tani T, Namba T. Antioxidative effects of phenylethanoids from *Cistanche deserticola*. Chem Pharm Bull 1996;19:1580–5.

[57] Kanchanapoom T, Kasai R, Yamasaki K. Phenolic glycosides from *Barnettia Kerrii*. Phytochemistry 2002;59:565–70.

[58] Saracoglu I, Harput US, Inoue M, Ogihara Y. New phenylethanoid glycosides from *Veronica pectinata* var. *glandulosa* and their free radical scavenging activities. Chem Pharm Bull 2002;50:665–8.

[59] Kernan MR, Amnarquaye A, Chen JL, Chan J, Sesin DF, Parkinson N, et al. Anti-viral phenylpropanoid glycosides from medicinal plant *Markhamia lutea*. J Nat Prod 1998;61:564–70.

[60] Kobayashi H, Oguchi H, Takizawa N, Miyase T, Ueno A, Usmanghani K, et al. New phenylethanoid glycosides from *Cistanche tubulosa* (schrenk) Hook. f.1. Chem. Pharm Bull 1987;35:3309–14.

[61] El-Naggar LJ, Beal JL. Iridoids. A review. J Nat Prod 1980;43:649–707.

[62] Takeda Y, Nishimura H, Inouye H. Studies on monoterpene glucosides and related natural products. XXXII. Iridoid glucosides of *Tarenna kotoensis* var. *gyokushinka*. Chem Pharm Bull 1976;24:1216–8.

[63] Yang XW, Ma YL, He HP, Wang YH, Di YT, Zhou H, et al. Iridoid constituents of *Tarenna attenuate*. J Nat Prod 2006;69:971–4.

[64] Jimenez C, Riguera R. Phenylethanoid glycosides in plants: structure and biological activity. Nat Prod Rep 1994;11:591–606.

[65] Thuan ND, Ha do T, Thuong PT, Na MK, Lee JP, Lee JH, et al. A phenylpropanoid glycoside with antioxidant activity from *Picria tel-ferae*. Arch Pharm Res 2007;30:1062–6.

[66] Chen RC, Su JH, Yang SM, Li J, Wang TJ, Zhou H. Effect of isoverbascoside, a phenylpropanoid glycoside antioxidant, on proliferation and differentiation of human gastric cancer cell. Acta Pharmacol Sin 2002;23:997–1001.

[67] Korkina LG. Phenylpropanoids as naturally occurring antioxidants: from plant defense to human health. Cell Mol Biol 2007;53:15–25.

[68] Yousuf S, Choudhary MI, Ur Rahman A. Separation of phenylpropanoids and evaluation of their antioxidant activity. Methods Mol Biol 2009;594:357–77.

[69] Daels-Rakotoarison DA, Seidel V, Gressier B, Brunet C, Tillequin F, Bailleul F, et al. Neurosedative and antioxidant activities of phenylpropanoids from *ballota nigra*. Arzneimittelforschung 2000;50:16–23.

[70] Gálvez M, Martín-Cordero C, Ayuso MJ. Pharmacological activities of phenylpropanoids glycosides. Stud Nat Prod Chem 2006;33:675–718.

[71] Hamerski L, Bomm MD, Silva DHS, Young MCM, Furlan M, Eberlin MN, et al. Phenylpropanoid glucosides from leaves of *Coussarea hydrangeifolia* (Rubiaceae). Phytochemistry 2005;66:1927–32.

[72] Murai M, Tamayama Y, Sansei N. Phenylethanoids in the herb of *Plantago lanceolatae* and inhibitory effects on arachidonic acid-induced mouse ear edema. Planta Med 1995;61:479–80.

[73] Chang C, Zhang L, Chen RY, Kuo LY, Huang J, Huang H, et al. Antioxidant and anti-inflammatory phenylpropanoid derivatives from *Calamus quiquesetinervius*. J Nat Prod 2010;73:1482–8.

[74] Kaur A, Singh R, Dey CS, Sharma SS, Bhutani KK, Singh IP. Antileishmanial phenylpropanoids from *Alpinia galangal* (linn.) willd. Indian J Exp Biol 2010;48:314–7.