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ORIGINAL ARTICLE

# Flavonoid constituents, cytotoxic and antioxidant activities of *Gleditsia triacanthos* L. leaves

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**Abstract** *Gleditsia triacanthos* L. is a deciduous tree belonging to the family Fabaceae. It possesses important biological activities as anti-mutagenic, anticancer, cytotoxic and treating rheumatoid arthritis. The total ethanol extract (EtOHE) and successive extracts (petroleum ether, chloroform, ethyl acetate, and aqueous ethanol) were prepared from the leaves. Eight flavone glycosides and two flavone aglycones named vicenin-I (**1**), vitexin (**2**), isovitexin (**3**), orientin (**4**), isoorientin (**5**), luteolin-7-*O*-ß-glucopyranoside (**6**), luteolin-7-*O*-ß-galactopyranoside (**7**), apigenin-7-*O*-ß-glucopyranoside (**8**), luteolin (**9**) and apigenin (**10**) were isolated from the aqueous ethanol extract of *G. triacanthos* L. leaves. Potent cytotoxic activity of the EtOHE extract was observed against the liver ($IC_{50}$ = 1.68 µg), breast ($IC_{50}$ = 0.74 µg), cervix ($IC_{50}$ = 1.28 µg), larynx ($IC_{50}$ = 0.67 µg) and colon ($IC_{50}$ = 2.50 µg) cancer cell lines. Cytotoxic activity of compounds **2**, **4**, **6** and **8** against, the liver, breast and colon cancer cell lines was also proved. Evaluation of the *in-vivo* antioxidant activity of the EtOHE and successive extracts revealed that the highest activity was exhibited by 100 mg of EtOHE (97.89% potency) as compared with vitamin E (100% potency). Compound **6** showed 91.8% free radical scavenging activity.



## 1. Introduction

Genus *Gleditsia* comprises 14 species of deciduous trees which can reach a height of 20–30 m (Huxley et al., 1992). *Gleditsia* species have been widely used in traditional Chinese medicine. The fruits and thorns are used for treating apoplexy, headache, productive cough, asthma and suppurative skin diseases (Zhong Yao Da Ci Dian, 1979). It was reported that *Gleditsia triacanthos* seed extracts can be used as a natural source of phenolic compounds and as antioxidants, also extracts of

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*Gleditsia* plant possess important pharmacological activities in treating rheumatoid arthritis, as anti-mutagenic, anticancer and they have significant cytotoxic activity against different cell lines (Miguel et al., 2010). Phytochemical studies were carried out on members of *Gleditsia* fruits that indicated the presence of triterpenoidal saponins which possess anti-inflammatory activity. (Yamahara et al., 1975; Shin and Kim, 2000; Ha et al., 2008, Dai and Hou, 2006; Hou et al., 2006). Triacanthosides saponin A1, G and C together with Gleditschosides A, B, C, D and E were reported to be isolated from *Gleditsia* fruits species (Badalbaeva et al., 1972a,b, 1973a,b). Triacanthoside C, oleanolic and echinocystic acids were reported to be isolated from the pericarp of *G. triacanthos* in addition to Gleditschosides A, B, C, D, and E (Badalbaeva et al., 1973a). Triacanthine alkaloid was isolated from the leaves of *G. triacanthos* (Panova et al.,1971; Masterova' et al., 1977). Vitexin, luteolin, isovitexin, quercetin were reported to be isolated from *G. triacanthos* (Panova and Georgieva, 1972; Leibovici et al., 1986). Aim of the study is to prove the cytotoxic and antioxidant activities of the plant which were reported in folk medicine as well as isolation and identification of the flavonoid compounds of the leaves (see Fig. 1).

## 2. Materials

### 2.1. Plant material

Fresh leaves of *G. triacanthos* L. were obtained from the Zoo in May 2007. It was identified by Mrs. Terase Labib, plant taxonomist of Orman Garden, Giza, Egypt. A voucher specimen (M 96) was deposited by Dr. Mona Marzouk and kept in the herbarium of NRC (National Research Center).

### 2.2. Material for in-vitro cytotoxic activity

HepG2 (liver), MCF7 (breast), HeLa (cervix), HEp-2 (larynx) and HCT116 (colon) cancer cell lines were obtained from the American Type Culture Collection, University Boulevard, Manassas, USA.

### 2.3. Material for in-vitro antioxidant activity

Vitamin E (α-tocopheryl acetate) (Pharco Pharmaceutical Co.) is available in the form of gelatinous capsules each contains 400 g it was used as a reference antioxidant drug. Alloxane (Sigma Co.): was used for induction of diabetes in rats. DPPH (1,1-Diphenyl-2-picryl-hydrazil) is a relatively stable free radical.

## 3. Methods

### 3.1. Experimental

#### 3.1.1. General procedure

The structure of the compounds was identified by spectroscopic methods including: UV/VIS (Ultraviolet and Visible Absorption Spectrometer, Labomed Inc.) for measuring UV spectral data of the isolated compounds, in the range of 200–500 nm in methanol and with different diagnostic shift reagents. NMR (Nuclear Magnetic Resonance Spectrophotometer, JEOL EX, 500 MHz for determination of $^{1}$H NMR and 125 MHz for determination of $^{13}$C NMR), ESI/MS (Electrospray Ionization Mass Spectrometer, Thermo Finnigan (ion trap)) were carried out for determination of molecular weight of compounds, CC was carried out on Polyamide 6S (Riedel-De-Haen AG, Seelze Haen AG, D-30926 Seelze Hanver, Germany) and Sephadex LH-20 (Pharmazia). PC (descending) Whatman No. 1 and 3 MM papers, using solvent systems 15% HOAc ($H_2O$–HOAc 85:15), BAW (*n*-BuOH:HOAc:$H_2O$ 4:1:5, upper layer). Complete acid hydrolysis for *O*-glycosides (6, 7, 8) was carried out & followed by CO-chromatograph with authentic samples to identify the aglycone and sugar moieties, the sugar unit of (1, 2, 3, 4, 5) was determined using ferric chloride degradation (Mabry et al., 1970). Source of solvents used for plant extraction: SDFCL (Industrial Estate, 248 Worli Road, Mumbai-30, India).

### 3.2. Extraction and purification

Dried powdered leaves of *G. triacanthos* L (1 kg) were exhaustively extracted with solvents of increasing polarities, petroleum ether (Pet. ether 49 g), chloroform (Chlo. 21 g), ethyl acetate (EtOAc: 14 g) and 70% aqueous ethanol (AEtOH: 135 g) at room temperature, the previous extracts were dried under reduced pressure and subjected to *in-vivo* antioxidant assay. 80 g of AEtOH was dissolved in water then applied on the top of polyamide S6 column (120 × 5 cm) eluted by solvent systems of decreasing polarities starting from 100% water to 100% methanol. A total of 100 fractions were collected (250 ml each). Similar fractions were combined according to PC analysis (1MM) sheets using the following eluents: *n*-butanol:acetic acid:water (4:1:5), acetic acid:water (15:85). Components were detected under UV and by spraying with $AlCl_3$ to give six main pooled fractions (**A**–**F**). Fraction **A** was chromatographed on cellulose column eluted by 10% MeOH-$H_2O$ to give one main subfraction, purification on Sephadex using MeOH-$H_2O$ (10%) yielded compounds **1** (10 mg). **B** was subjected to cellulose column eluted by saturated butanol to give one main subfraction which was purified on silica gel column eluted by $CHCl_3$: MeOH 9:1 to give compounds **2** and **3** (20, and 18 mg respectively). **C** was chromatographed on PPC (3 MM) using acetic acid: water (15:85) followed by Sephadex and eluted by saturated butanol to give compounds **4** and **5** (20, and 10 mg). **D** was chromatographed on PPC (3 MM) using acetic acid:water (15:85) and purified on Sephadex with saturated butanol to give compound **6** and **7** (15, and 15 mg). **E** was purified on Sephadex using MeOH-$H_2O$(10%) to give compound **8**. **F** was subjected to Sephadex using MeOH-$H_2O$ (1:1) to yield compounds **9** (12 mg) and **10** (15 mg). Final purification of all compounds was achieved by Sephadex LH20 column using MeOH as eluent.

#### 3.2.1. Compound *1* (vicenin-I)

Negative ESI-MS Molecular ion peak $(M–H)^-$ at *m*/*z* 563, the ESI-MS fragmentation pattern: 503, 473, 443, 383. UV: λ max (nm): MeOH: 272, 327; NaOMe: 282, 333 (sh), 390; $AlCl_3$: 305, 345, 389; $AlCl_3$/HCl: 305, 345, 489; NaOAc: 281, 307, 392; NaOAc/$H_3BO_3$: 279, 344. $^{1}$H NMR: (500 MHz, DMSO-$d_6$, δ ppm): 6.30(1H, s, H-3), 7.83 (2H, d, $J$ = 8.4 Hz, H-2′/H-6′), 6.78(2H, d, $J$ = 8.4 Hz, H-3′/H-5′), 8-*C*-β-Glc: 4.35 (1H, d, $J$ = 9.8 Hz, H-1‴), 6-*C*-β-xylose: 3.8 (1H, d, $J$ = 9.6 Hz H-1″, Xylose).