Phytochemical and Antioxidant Activity of *Spathodea campanulata* P. Beauvois. Growing in Egypt

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**Abstract** – Alcoholic extract of *Spathodea campanulata* P. aerial parts, and two of the isolated fractions from celite column showed strong antioxidant activity (92, 94 & 89% RSA, Radical Scavenging Activity). Phytochemical investigation of chloroform/EtOAc fraction of this column led to the isolation of phenolic acids, caffeic acid (1), and ferulic acid (2), fraction EtOAc/McOH on further fractionation afforded 3 Flavonoids, kaempferol 3-O-glucoside (3), quercetin 3-methyl ether (4) and 8-methoxy kaempferol 3-O-glucoside (5). The isolated constituents were identified by co chromatography with authentic samples, TLC, PC., UV, MS and 1H-NMR. Also the lipidal matter of the plant was studied. The unsaponifiable matter was found to be mixture of hydrocarbons from (C14-C28), cholesterol, campasterol, stigmasteryl, and α-amyrin. Fatty acid methyl esters were found to contain 12 fatty acids. The fatty acids containing C18 formed ca.65% of the total mixture.

**Keywords** – *Spathodea campanulata*, Bignoniacae, Phenolic acids, Flavonoids, Antioxidants

**Introduction**

*Spathodea campanulata* P. Beauvois (Bignoniacae), also called the African tulip, is widely distributed through Africa and is cultivated as an ornamental tree elsewhere in the tropics (Irvine, 1961). The seeds were received to be cultivated in Egypt in 1910 (Bircher, 1960). It is used in folkloric medicine by the Africans to treat many diseases, such as edema, dysentery, ulcers, filarial, gonorrhea, diarrhoea, and as poison antidote (Amusan et al., 1995; Ngouela et al., 1990). The molluscicidal activity of leaves and stem bark extracts has been reported by several authors (Makinde et al., 1987; Makinde et al., 1988; Makinde et al., 1990; Makinde et al., 1996).

Also the plant was used to treat diabetes mellitus in the traditional medicine of central Africa, hypoglycemic activity of stem bark decoction was reported by Niyonzima et al., 1990; 1993.

Moreover, in recent years, many medicinal plants including *Spathodea campanulata* have been reported for their complement modulating & anti HIV activity (Chang and Yeung, 1988; Lashe et al., 1995; Locker et al., 1996; Vlietinch et al., 1997; Vlietinch et al., 1998; Niyonizima et al., 2000) Spathodic acid a triterpenoid acid was isolated from the stem bark of the plant (Ngouela et al., 1990). Some anthocyanins were isolated from the flowers (Scogin, 1980). No phytochemical or biological investigation of *Spathodea campanulata* growing in Egypt, so the present work deals with the phytochemical investigation of the aerial parts (leaves & terminal branches) of the plant and their antioxidant activities as a guide for further biological activities.

**Experimental**

**Plant material** – Aerial parts (leaves and terminal branches) of *Spathodea campanulata* P. Beauvois were collected from Giza Zoo in May 2005, and kindly identified by professor Dr. K. H. El Batanony, professor of Botany, Faculty of Science, Cairo University, Cairo, Egypt. A voucher specimen is kept in the Herbarium of, National Research Centre, Cairo, Egypt. The plant was shade dried and minced.

**General experimental procedures** – TLC was carried out on precoated silica gel F254 plates (Merck) (Darmstadt, Germany) developed with EtOAc-formic acid-acetic acid-H2O (30/1.5/1.5/7) solvent (1) Paper chromatography (pc.) was carried out on Whatman 3 MM, 15% acetic acid solvent (2) and butanol-acetic acid-H2O (4-1-5), the upper layer solvent (3) for flavonoids and phenolic acids and butanol-benzene-pyridine-H2O (5/1/3/3) (for sugars) solvent (4). Spots were detected by examining the chromatoplates and or the chromatograms in the UV light at 366 nm.
before and after exposure to ammonia vapour and also by using N.A. reagent (Naturstoff reagent, Diphenyl-boric acid-ethanolamine complex, 1% solution in methanol) and aniline phthalate for sugars. Column chromatography was performed on silica gel 70-230 mesh (Merck) and Sephadex LH-20 (Pharmacia). Mass spectra were recorded on a JEOL-JMS-AX 500 Mass spectrometer (EI-MS) and (FAB-MS) using glycerol as liquid matrix. 1H-NMR was recorded on Jeol-GX at 500 MHz using TMS as internal standard.

**Extraction and isolation** – About 1.5 kg of minced air dried aerial parts of *Spathodea campanulata* (leaves and terminal branches) was exhaustively extracted with petroleum ether in a soxhlet apparatus then filtered and the dried marc was re-extracted with 80% MeOH in a percolator. The collected MeOH extracts was concentrated under vacuum at 40 °C, left overnight in the refrigerator, and then filtered. About 50 g of the MeOH extract was mixed with 250 g cellie (545) and dried under vacuo at 40 °C. The mixture was then applied on a column (60 cm × 5 cm i.d.). Elution was affected using different solvents: n-hexane, n-hexane-CHCl₃ (1/1), CHCl₃, CHCl₃-CH₃OH (1/1) – (fraction A), EtOAc, EtOAc-MeOH (1/1) – (fraction B), and MeOH. About 1.35 g of fraction A was fractionated by ppc using solvent (3) to afford compounds 1 & 2 they further purified by passing through sephadex LH-20 column eluted with MeOH. About 5 g of fraction B was subjected to column chromatography on silica gel (column 80 × 4 cm, 250 g). Elution of the column was carried out by means of CHCl₃, increasing polarities with MeOH, (100 ml fractions) were collected. Fractions 9-16 coming with CHCl₃/MeOH (9:1) were collected and subjected to further column chromatography on Sephadex LH-20 (solvent MeOH) to give compound 3. Fractions 20-35 of the same eluent were collected and subjected to column chromatography on silica gel using a CHCl₃/MeOH gradient of increasing polarity. The fractions eluted with CHCl₃/MeOH (80/20) were collected and subjected to ppc on 3MM using solvent system (2), then purified on Sephadex LH-20 using 90% MeOH, yielding compounds 4 & 5.

**Compound (1)** showed UV (MeOH) λmax 326, 293, 253 nm.

The 1H-NMR spectrum (DMSO-d₆) showed signals at δ 7.41 and δ 6.17 (2H, d, d, J = 16, 16Hz, olefinic protons), δ 7.01 (1H, s, H-2), δ 6.75 (1H, d, J = 8Hz, H-6), δ 6.94 (1H, d, J = 6.7Hz, H-5), δ 9.13 (2H, s, phenolic OH), 12.01 (1H, s, acidic OH). The EI/MS spectral data showed a molecular ion peak at m/z 180, 179 (M⁻1), and fragments at m/z 163 (M-OH), 145, 136, 134.

**Compound (2)** showed a UV spectrum in MeOH, λmax 322, 296, 207 nm a bathochromic shift with increase of intensity was noticed on addition of NaOH to λmax 348, 310, 207 nm. The 1H-NMR spectrum (DMSO-d₆) showed signals at δ 7.49 and 6.32 (2H, d, d, J = 16,16Hz, olefinic protons), δ 7.26 (1H, s, H-1), δ 6.78 (1H, d, J = 8Hz, H-6), δ 7.06 (1H, d, J = 2.3Hz, H-5), δ 9.49 (1H, s, phenolic OH), δ 12.1 (1H, s, acidic OH) and at δ 3.8 (3H, acidic CH₃) (Balde, et al., 1991). The EI/MS spectral data showed a molecular ion peak at m/z 194 (M⁺1) 193 (M⁺-1), 179 (M⁺-CH₃), 163 (M⁺-OCH₃), 149 (M⁺-CO₂H), 123 (M⁺-CH₂-CH₂), 107 (M⁺-CH₂), 29 (CH₂-CH₂-CH₂). The 1H-NMR spectrum (DMSO-d₆) showed signals at δ 7.86 (2H, d, J = 8Hz, H-4), δ 6.85 (2H, d, J = 8Hz, H-5). 1H-NMR spectrum (DMSO-d₆) δ 7.9 (2H, d, J = 8.7Hz, H-2', H-6').

Fig. 1. Structure of the isolated compounds.