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BIOACTIVITY AND COMPOSITION OF THE FLOWERS OF *SPATHODEA CAMPANULATA* P. BEAUV

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ABSTRACT

Among plant metabolites, phenolics were found to possess a wide spectrum of biological activities. The purpose of this study was to investigate the ethanol extract of the flowers of *Spathodea campanulata* P. Beauv. (EEFSC) in terms of bioactivity and composition. Special emphasis was made on its phenolic components. The LD_{50} of EEFSC (up to 4 g/kg) indicated its safety. The growth inhibitory activity of EEFSC, and its hexane, chloroform, ethyl acetate and *n*-butanol fractions was evaluated *in-vitro* against a set of micro-organisms and 2 human tumor cell lines (breast and colon). The chloroform fraction exhibited the highest antibacterial effect and the least IC_{50} against both cell lines. The anthelmintic and acute anti-hyperglycemic potentialities of EEFSC were also explored and found noticeably significant. RP-HPLC with UV detection was employed for identification and quantification of the floral phenolics. Six compounds were isolated from the active chloroform and ethyl acetate/*n*-butanol fractions. The purified isolates included three phenolics, and were identified as phytol, *α*-methylcinnamaldehyde, *β*-sitosterol-3-acetate, naringenin, catechin-3-O-*α*-rhamnopyranoside and 5, 6, 4' trihydroxy flavonol-7-O-*α*-rhamnopyranoside, all being for the first time reported from the flowers. The observed bioactivities were correlated to the isolated constituents.

KEYWORDS: *Spathodea campanulata*, Flowers, Antitumor, Anthelmintic, Anti-hyperglycemic.

INTRODUCTION

Traditional medicine practices have been adopted in different cultures and regions without the parallel advance of international standards and methods for evaluation. Herbal treatments,

the most popular form of traditional medicine in developing countries, are now widespread in developed countries as a type of alternative or complementary medicine [1]. Among plant metabolites, phenolics are reputed to play a noticeable protective role in several health disorders [2].

A large number of ornamental plants are used as folk medicines in their native countries including *Spathodea campanulata* P. Beauv. (African Tulip, Bignoniaceae). The plant is indigenous to equatorial Africa and widely grown for its attractive red flowers in tropical and subtropical areas [3].The flowers are used as diuretic and anti-inflammatory; the leaves in curing kidney diseases, urethra inflammations and animal poisoning; and the stem bark for wound healing, treatment of skin ailments, herpes and diarrhea [4- 6]. Scientifically-based biological studies revealed that: the flowers and stem bark exerted a molluscicidal effect [4]; the stem bark exhibited hypoglycemic, anti-HIV, antibacterial and antimalarial activities [4,7-10]. Meanwhile, the aerial parts and leaves were found to act as antioxidant and antimicrobial, and to exert antimalarial effect [11-13]. In addition, flower extracts were reported to have a photoprotective activity [14]. Phytochemical investigations on *S. campanulata* were mainly performed on the stem bark, leaves and fruits; the isolated compounds included: spathodic and ursolic acids, steroids, saponins, pectic substances as well as phenolic and iridoid glycosides [15-20]. Whereas, data dealing with the chemical composition of the flowers were limited to : a GC-MS identification of 1,1-diethoxy-3-methyl-butane, *n*-hexadecanoic acid, 1,2-benzenedicarboxylic acid diisooctyl ester, and oleic acid [21]and isolation of hexadecanoic acid, tricosane, heneicosane, oleic acid and thujopsene[22] from two Indian samples. The present work was thus planned with the aim to explore the bioactivities of the flower and perform a deeper investigation of its constituents especially those phenolic in nature.

MATERIALS AND METHODS

General

Melting points were determined on Electrothermal 9100 equipment. UV absorption spectra were run on a Shimadzu 1700 spectrophotometer. Mass spectra were recorded on a Jeol Mass Spectrometer SSQ 7000, Digital DEC 300. NMR spectra were measured in DMSO-d_6; ^{1}H-NMR spectra were obtained at 500 MHz and ^{13}C-NMR spectra at 125 MHz on a JEOL GX-500 spectrometer with the chemical shifts (δ ppm) expressed relative to TMS as internal standard. TLC was performed on sheets pre-coated with silica gel 60 F_{254} (Fluka). Chromatograms were visualized under UV light (254 and 366 nm) before and after exposure

to ammonia vapor and by spraying with *p*-anisaldehyde and aluminium chloride reagents. Column chromatography was performed over silica gel 60 GF (Merck) for VLC, sephadex LH-20 (Sigma-Aldrich) and silica gel 100 C_{18}-Reversed Phase (Fluka). HPLC analysis of phenolics was carried out on an Agilent Series 1100 apparatus equipped with a Hypersil-ODS (4.6 X 250 mm, 5µm) column and a UV detector.

Chemicals and Drugs

All solvents were of analytical grade and obtained from Fisher Scientific. Gentamicin, alloxan and metformin (all purchased from Sigma-Aldrich) and mebendazole (Vermox®, from Janssen-Cilag) were used throughout the biological assessment.

Plant material

Inflorescences (buds and expanded flowers) of *Spathodea campanulata* P. Beauv. were gathered during the flowering season (June-September, 2009-2011), from trees cultivated in the National Egyptian Zoo-Garden, Giza. The plant was kindly authenticated by Mrs. Therese Labib, Herbarium Section, Orman Garden, Giza, Egypt and identity confirmed by Dr. Hassnaa Ahmed Hosny, Professor of Plant Taxonomy, Department of Botany, Faculty of Science, Cairo University, Egypt. Voucher specimens (# 013.04.30) were kept at the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University. Samples, air-dried in shade, were powdered and preserved for further study.

Experimental animals and microorganisms

All animal investigations were performed in accordance with the ethical standards for the proper care and use of laboratory animals, and upon approval of the Animal Experimentation Ethics Committee of the Faculty of Pharmacy, Cairo University. Adult male albino mice (25-30 g, procured from the animal facility at the Faculty of Pharmacy, Cairo University) were used for determination of LD_{50} and evaluation of the acute anti-hyperglycemic activity. Mice were maintained under standard hygienic conditions (temperature 22 ± 2 ºC, relative humidity 50-60 %, with 12 h day/night lighting cycle), fed with well balanced normal diet and water supplied *ad libitum*; they were left for a period of one week for accommodation before performing the experiments. Earth-worms (*Allolobophora caliginosa*; 6-8.5 cm long) were collected from Dubai Medical College Garden and used for evaluation of the anthelmintic activity.

A set of five bacterial strains, kindly provided by the Dubai Specialist Medical and Research Center, were used for the antibacterial screening. These included two representatives of Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and three of the Gram-negative group (*Escherichia coli* and *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*). Microorganisms were grown on appropriate media: nutrient agar for *S. aureus, P. aeruginosa* and *S. pyogenes* and MacConkey agar for *E. coli* and *K. pneumoniae*.

Cancer cell lines

The two human tumor cell lines, used for assessment of the cytotoxic potential *viz.*, breast carcinoma (MCF7) and colon carcinoma (HCT116) were obtained from the National Cancer Institute, Cairo University, Egypt**.**

Extraction, fractionation and phytochemical screening

The air-dried powdered flowers of *S. campanulata* (1.5 Kg) were exhaustively extracted by cold maceration in 70 % ethanol (3L X 4). The solvent was evaporated under reduced pressure at 50^{o}C to provide EEFSC. A portion of this extract was suspended in water (150 ml) and successively fractionated with *n*-hexane, chloroform, ethyl acetate and *n*-butanol saturated with water; while the residual amount was saved for biological evaluation. The individual fractions were, separately, evaporated and the extractives subjected to specific chemical tests and TLC screening for qualitative identification of its components [23,24].

Determination of LD_{50}

The LD_{50} of the ethanol extract of the flowers of *S. campanulata* (EEFSC) was estimated to assess its safety [25].

Antibacterial Activity

EEFSC and its partitioned fractions, at a dose level of 100 µg, were subjected to *in-vitro* screening for evaluation of their antibacterial potentialities. The agar diffusion technique was used [26], solubilisation of the samples being assisted by sterile Tween 80. The effects were compared to those produced by the broad-spectrum antibacterial, gentamicin (30 µg). Diameters of zones of inhibition (in mm) were taken as a measure for the growth inhibitory activity against the selected strains. Results are recorded in Table 1.

Cytotoxic activity

The antitumor activity of EEFSC and its fractions was *in-vitro* assessed against the two human cell lines (MCF7 and HCT116) by the sulphorhodamine B (SRB) assay [27]. The survival curves representing the relation between the surviving cells (expressed as percent) and extractives concentrations were constructed and represented in Figures 1A and B, respectively. The doses of extractives which reduce cell viability to 50% (IC_{50}) were calculated and values displayed in Table 2.

Anthelmintic activity

The effect of EEFSC was evaluated on adult earth worms [28]. Different dilutions ranging from 25-100mg/5ml, in Tween 80, were tested and compared to Vermox® (100mg/5ml) as standard. Evaluation of the anthelmintic activity was based on the time necessary to paralyze and/or kill the worms up to the end of the test period. Results are recorded in Table 3.

Acute anti-hyperglycemic activity

Diabetes mellitus was induced in male albino mice by intraperitoneally injecting a single dose of Alloxan (150 mg/ kg b.wt). Blood samples were obtained from tail tip vein of all experimental animals and fasting blood glucose (FBG) concentration was determined using One-touch ultra glucometer (Johnson and Johnson) and compatible glucose strips [29,30]. Hyperglycemia was assessed after 72 hours of induction of diabetes and animals with FBG>140 mg/dl were selected for the study. Alloxan-treated mice were divided into three groups, each of 6 animals; Group I (diabetic control-saline treated), Group II (diabetic-EEFSC treated, 500 mg/kg b.w.) and Group III (diabetic-Metformin treated, 150 mg/kg b.w.). FBG levels were measured at zero time and after 2 and 4h following oral administration. The results are recorded in Table 4.

Statistical analysis

Experimental data were expressed as mean ± SEM, statistical analysis was performed by one way ANOVA followed by Bonferroni's multiple comparison test, *p* values <0.05 were considered as significant.

HPLC analysis of the floral phenolics

Flavonoids (expressed as their aglycones) and phenolic acids were determined by HPLC in the hydrolyzed-methanolic extract of the flowers (1g) [31]. HPLC separation was performed on a Hypersil-ODS (4.6 X 250 mm, 5µm) column. Isocratic elution was employed using

acetonitrile /15% acetic acid (40/60 v/v) as mobile phase. The flow rate of the mobile phase was 1 ml/min and the injection volumes were 40 µl for standards and samples. Standards were prepared by diluting stock standard solutions with methanol to afford a final concentration of 50 µg/ml. The UV detector was set at 330 nm. Identification of the major components was by comparing their retention times and spectral data with those of the available standards. Quantification was based on peak area measurement using the external standard method. All analyses were carried out in triplicate. Results are listed in Table 5 and the chromatogram represented in Fig. 2.

Isolation of the constituents of the chloroform extract

An accurately weighed amount of the chloroform extract (3.4 g) was subjected to VLC on a silica gel 60 GF column (25 X 5 cm). Gradient elution was carried out with hexane-chloroform, chloroform-ethyl acetate, ethyl acetate and ethyl acetate-methanol mixtures. Fractions, each of 100 ml, were collected and monitored by TLC using different mobile systems (System A, chloroform-hexane 1:1; System B, ethyl acetate-formic acid-acetic acid-water 100:11:11:10; System C, chloroform-methanol 8.5:1.5; System D, chloroform-methanol-water 8.5:1.5:0.1). Spots were located by visualization under UV_{365nm} before and after exposure to ammonia vapour and by spraying with *p*-anisaldehyde. Similar fractions were pooled to yield 5 collective fractions (**F1-F5**). Fractions **F1** and **F2** were selected for further isolation.

F1 (0.7 g; 3 spots, R_f values 0.61, 0.30 and 0.10, system A) upon rechromatography on a silica gel 100 C_{18}-RP column (20 X 1.5 cm), using methanol-water 9:1 as eluent, resulted in isolation of compounds **1** and **2**.

F2 (0.5 g; 3 spots, R_f values 0.61, 0.30 and 0.12; system C) was subjected to successive column chromatography on sephadex LH-20 (35X 3 cm; elution, methanol-water 4:1) followed by RP silica (20 X 1.5 cm; elution, methanol-water 1:1) to afford compound **3**.

Isolation of the constituents of the combined ethyl acetate / *n*-butanol extracts

The combined ethyl acetate/ *n*-butanol residue (14 g) was fractionated by VLC on a silica gel 60 GF column (25 X 5 cm; gradient elution, chloroform-ethyl acetate mixtures, ethyl acetate and ethyl acetate-ethanol mixtures). Fractions, 100 ml each, were collected, monitored by TLC (Systems B, C or D). Similar fractions were pooled to yield 8 collective fractions (P1-P8). Fractions **P1**, **P**2 and **P8** were selected for further isolation.

Rechromatography of **P1** (0.9 g; TLC, 3 spots; R_f: 0.73, 0.46 and 0.23 System C) and **P2** (1.2 g; TLC, 3 spots; R_f: 0.77, 0.5 and 0.26 System B) on sephadex LH-20 (35X 3 cm; elution, methanol-water 4:1) yielded compounds **4** and **5** from **P1** and **P2**, respectively. Meanwhile, CC of P 8 (2.3 g; TLC, 5 spots; R_f: 0.74, 0.69, 0.60, 0.33 and 0.21System D) on sephadex LH-20 (35 X 3 cm; elution, methanol-water 9:1) followed by repeated purification on two successive RP silica columns (20 X 1.5 cm; elution, methanol-water 7:3 and 1:1, respectively) yielded compound **6**.

The structural formulae of compounds **1-6** are represented in Fig.3.

RESULTS AND DISCUSSION

Yield of extractives

Extraction of the powdered flowers with ethanol 70% afforded EEFSC as a dark brown residue in a yield of 90.0 g. Partitioning 60.0 g of this extract with *n*-hexane, chloroform, ethyl acetate and *n*-butanol yielded 15.3, 3.4, 3.1 and 10.9 g of dried extractives, respectively.

Determination of median lethal dose (LD_{50})

Oral administration of EEFSC in doses up to about 4 g/kg body weight produced neither mortality nor signs of morbidity or behavioral changes in any of the treated animal groups under examination. The wide safety margin of EEFSC stimulated further exploration of its biological activities before and/or after fractionation, as well as isolation of the phytoconstituents responsible of these effects.

Phytochemical and TLC screening of the ethanolic extract and fractions

Preliminary phytochemical screening of EEFSC and its partitioned fractions revealed the presence of flavonoids in the parent extract and its chloroform, ethyl acetate and *n*-butanol fractions; and of steroids in the hexane and chloroform fractions. TLC monitoring for phenolics revealed a close similarity in chromatographic profiles for the ethyl acetate and *n*-butanol extractives hence these were combined before CC fractionation.

Evaluation of the antibacterial activity

Results, displayed in Table 1, revealed that at a concentration level of 100µg/ml, all the tested samples, excluding the hexane fraction, exhibited variable antibacterial activities with inhibition zones ranging from 13 to 25 mm in diameter. The chloroform fraction showed the highest growth inhibitory activity against *Streptococcus pyogenes*, *Escherichia coli* and

Klebsiella pneumoniae, while EEFSC exhibited highest activity against *Staphylococcus aureus* and the *n*-butanol fraction appeared the most active against *Pseudomonas aeruginosa* as compared to gentamicin. In addition, the ethyl acetate and *n*-butanol fractions exhibited similar effects against *Streptococcus pyogenes*. EEFSC and its chloroform, ethyl acetate and *n*-butanol fractions exhibited significant antibacterial activities as compared to gentamicin. This effect may be referred to any of the identified or isolated components *viz.*, phenolics [32], β-sitosterol [33] and phytol (noticeably active against *Staphylococcus aureus*) [34] either through individual action or in a synergistic way. In fact, a number of phenolics have been detected in all the extracts; β-sitosterol and phytol have been isolated and identified from the chloroform fraction and three phenolics from the combined ethyl acetate/ *n*-butanol fraction. In addition, the observed results are in agreement with those formerly reported for the stem bark of the plant [7].

Table (1): Antibacterial effect of the ethanol extract of the flowers of *S. campanulata* [EEFSC] and its partitioned fractions as compared to Gentamicin

Microorganism	Diameter of zone of inhibition (mm)					
	EEFSC	Hex. fr.	Chl. fr.	Et. Ac. fr.	But. fr.	Gentamicin
Staphylococcus aureus	25	-	15	17	15	20
Streptococcus pyogenes	18	19	22	20	20	15
Pseudomonas aeruginosa	15	-	14	15	18	20
Escherichia coli	15	-	17	13	15	19
Klebsiella pneumoniae	16	-	20	15	10	22

Hex., hexane; Chl., chloroform; Et. Ac., ethyl acetate; But., butanol; fr., fraction.

Evaluation of the cytotoxic potential

Under the experimental conditions adopted and from data displayed in Table 2 and represented in Fig. 1 A and B, it could be concluded that the tested extracts exhibited variable antitumor activities against the two tested cell lines *viz.*, Mammary Carcinoma (MCF7) and Human Colon Carcinoma (HCT116) cell lines. In this respect, EEFSC and its chloroform fraction demonstrated the lowest IC_{50} (17.6 and 17.8 µg/ml) against mammary carcinoma (MCF7 cells), meanwhile, both the hexane and *n*-butanol fractions showed identical and relatively higher IC_{50} against the same cell line (21.0 µg/ml). Whereas, the effect exerted by the tested samples on human colon carcinoma (HCT116) was milder or negligible (IC s_{50},

21.4-30.4 µg/ml), the lowest IC_{50} being recorded for the chloroform extract. Data obtained on evaluation of the cytotoxic potential of EEFSC and its partitioned fractions are in accordance with those reported for both phenolics and steroids [35, 36], which appear to constitute the major makeup of the most effective chloroform fraction. While, the cytotoxic activity of certain Bignoniaceous extracts and metabolites has been previously reported [37] yet, the present study appears the first concerning *Spathodea campanulata*.

Table (2): IC_{50} of the ethanol extract of the flowers of *S. campanulata* [EEFSC] and its partitioned fractions on MCF7 and HCT116 cell lines

Cell line	IC_{50}(µg/ml)				
	EEFSC	Hex. fr	Chl. fr	Et. Ac. fr.	But. fr
MCF7	17.6±0.73	21±0.92	17.8±0.67	20±1.01	21±1.98
HCT116	24.6±1.21	22.6±1.27	21.4±1.43	27.8±1.79	30.4±2.31

Mean ±S.E.M; Hex., hexane; Chl., chloroform; Et. Ac., ethyl acetate; But., butanol; fr., fraction; MCF7, breast carcinoma cell line; HCT116, colon carcinoma cell line.

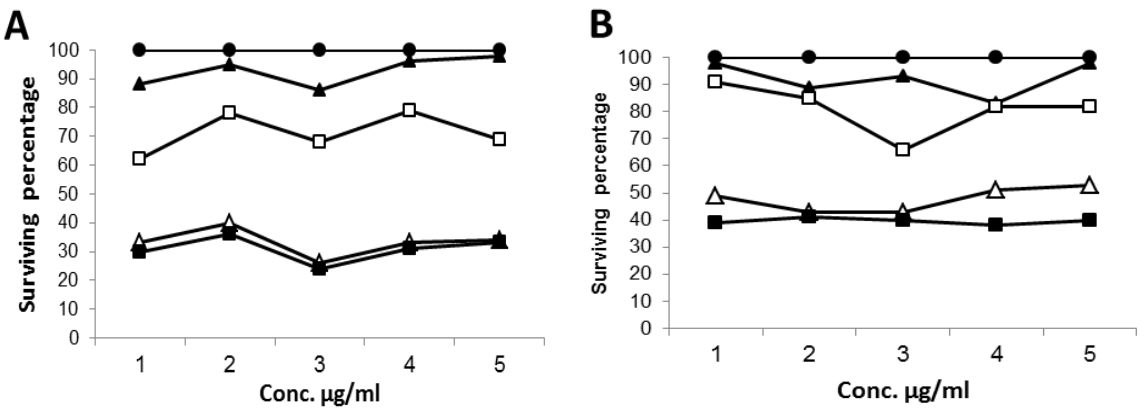

Figure (1): Cytotoxic effect of the ethanol extract of the flowers of *S. campanulata* P. Beauv. and its fractions on (A) MCF7 (breast) cell line and (B) HCT116 (colon) cell line.

(1): Ethanol extract; (2): Hexane fr.; (3): Chloroform fr.; (4) Ethyl acetate fr.; (5): n-Butanol fr.;(●): 0 µg/ml; (▲): 5 µg/ml; (□): 12.5 µg /ml; (Δ): 25 µg/ml; (■): 50 µg/ml

Evaluation of the anthelmintic activity

The anthelmintic effect of EEFSC (100 mg/ml) was found significant, dose-dependent and comparable to the well known anthelmintic agent Vermox® (Table 3). The non-parasitic earth worm, *Allolobophora caliginosa*, was selected as test organism for evaluating the anthelmentic activity due to its close relationship with intestinal parasitic nematodes [38]. Among numerous bioactivities attributed to phenolics, they are reported to hinder energy

generation in parasitic helminths through uncoupling oxidative phosphorylation [39]. The occurence of these compounds in EEFSC may account for its obvious activity against earth worms [40]. The present findings closely correlate with the antiparasitic (antimalarial and schizontocidal) effects reported for the stem bark and leaf of the plant [11].

Table (3): Anthelmintic effect of the ethanol extract of the flowers of *S. campanulata* [EEFSC], as compared to Mebendazole

Extract / Standard drug	Dose (mg/5ml)	Death time (min)
EEFSC	25	74.33±2.96
	50	53.67±2.33
	100	23.00±1.53*
Vermox ® (Mebendazole)	100	45.67±2.96*

Mean ±S.E.M.; *p<0.05.Each experiment was carried out in triplicate.

Evaluation of the acute anti-hyperglycemic activity

EEFSC exerted a significant time-dependent anti-hyperglycemic effect in alloxan-induced diabetic mice, comparable to that of metformin, at a dose level of 500 mg/kg (Table 4). The antihyperglycemic activity of EEFSC may, similarly, be correlated to its phenolic components, namely flavonoids. In fact, the latter have been reported to act as aldose-reductase inhibitors blocking the sorbitol pathway that is linked to many problems associated with diabetes [2]. Moreover, results of this investigation are in accordance with an earlier report on the hypoglycemic activity of the stem bark of the plant [8].

Table (4): Effect of oral administration of the ethanol extract of the flowers of *S. campanulata* [EEFSC] on blood glucose level, in Alloxan-treated diabetic mice, as compared to Metformin

Animal group	Blood glucose level (mg/dl)			% Decrease	
	At zero time	After 2 h	After 4 h	After 2 h	After 4 h
Diabetic + saline	270.25±44.11	267.00±44.07	264.75±43.56	-	-
Diabetic +EEFSC (500 mg/kg)	279.50±22.64	224.75±24.13	136.75±22.11*	15.8	48.3
Diabetic + Metformin (150 mg/ kg)	258.25±24.92	167.00±24.14*	130.75±31.06*	37.5	50.6

Mean ± S.E.M., *p <0.05. Each experiment was carried out in triplicate

HPLC analysis of the floral phenolics

HPLC analysis of the hydrolyzed-methanol extract allowed the identification and quantitation of 12 phenolics (Table 5 and Fig. 2). The overall composition was dominated by ellagic acid (relative %, 29.69) followed by naringenin and caffeic acid (14.28 and 10.23%). Among phenolic acids formerly reported in the leaves [18]chlorogenic, caffeic and ferulic acids were also detected, *via* HPLC, in the hydrolysed methanolic extract of the flowers; while gallic acid appeared absent. On the other hand, ellagic acid was identified in the flowers only, and in a relatively high amount.

Table (5): Phenolics identified by HPLC analysis of the hydrolysed methanolic extract of the flowers of *S. campanulata*

Identified peak No	Retention time	Identified Constituent	Relative area %
1	6.489	Catechin	1.00
2	6.794	Chlorogenic acid	1.93
3	7.459	Caffeic acid	10.23
4	7.691	Syringic acid	1.33
5	8.582	Ellagic acid	29.69
6	8.807	Ferulic acid	2.69
7	9.452	Quercetin	2.17
8	10.762	Coumarin	0.66
9	11.577	Naringenin	14.28
10	12.194	Apigenin	0.42
11	12.545	Kaempferol	0.51
12	14.624	Chrysin	0.16
Total identified constituents			**65.07**

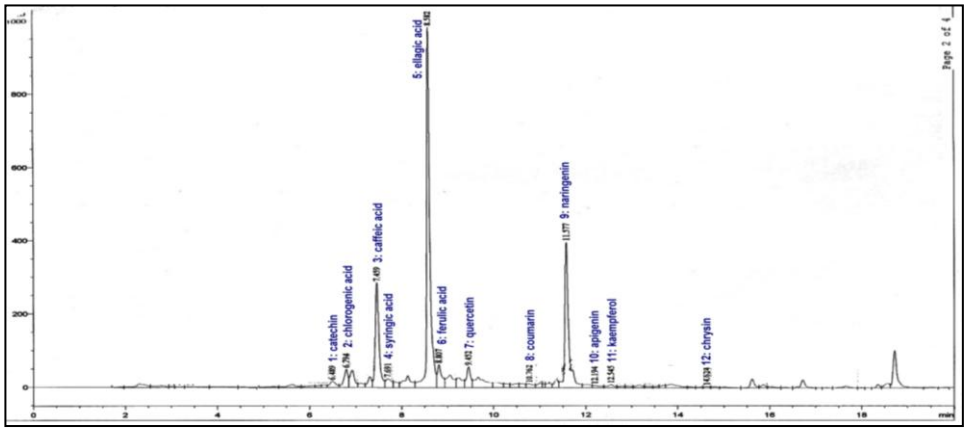

Figure (2): HPLC chromatogram of the hydrolysed methanolic extract of the flowers of *S. campanulata*.

Characterization of the isolated constituents

Phytol (1): (3, 7, 11, 15 tetramethyl-2-hexadecen-1-ol): $C_{20}H_{40}O$, colorless semisolid; 15 mg;soluble in chloroform and methanol; R_f: 0.61 (System A); EI-MS m/z (% rel. intensity), 123 (20), 111 (7), 95(11), 83 (12), 71 (50), 55 (18); ^{1}H-NMR, δ ppm (500 MHz, DMSO), 0.81 (6 H, d, *J*=7 Hz, H-16, 17), 1.4 (2 H, m, H-5), 1.05-1.46 (m, other aliphatic protons), 1.76 (3 H, s, H-20), 2.23 (2 H, t, *J*=6.8 , 14.5 Hz, H-4), 4.12 (2 H, d, *J*=8 Hz, H-1), 5.40 (1H, t, *J*=6.7,1.2 Hz, H-2).

β-Sitosterol-3-acetate (2): $C_{31}H_{52}O_2$, white needle crystals;12mg; m.p. 132-134^{o}C (methanol), R_f: 0.30 (System A); soluble in chloroform-methanol mixture. ^{1}H-NMR, δ ppm (500 MHz, DMSO): 0.71 (3H , s , Me-18) , 0.78 (3H , d , *J*=7.2 Hz, H-26) , 0.80 (3H, d , *J*=7.2 Hz, H-27), 0.90 (3H, t , *J*=8.4 Hz , H-29), 0.91 (3H, d , *J*=6 Hz, H-21), 1.10 (3H, s , H-19), 2.04 (3H, s, $\underline{CH_3}OC=O$), 4.62 (1H, m, H-3), 5.36 (1H, s , H-6).

α-Methyl cinnamaldehyde (3) (2-methyl-3-phenylprop-2-enal): $C_{10}H_{10}O$, yellow oily liquid; 7 mg;miscible with chloroform and ethanol; faint yellow under UV, yellowish brown in visible light; R_f: 0.61 (System C); UV λmax (methanol) nm, 246,276; ^{1}H-NMR, δ ppm (500 MHz, DMSO): 2.0 (3H, s, CH_3), 7.1 (2 H, m, H-3,5), 7.33 (2H, d, *J*=7.6 Hz, H-2, 6), 7.49 (1H, m, H-4), 7.68 (1 H, d, *J*=12 Hz, H-β), 9.59 (1H, d, *J*=6 Hz CHO).

Naringenin (4) (5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one): $C_{15}H_{12}O_5$, yellow crystalline powder; 10 mg; m.p. 248-251^{o}C, soluble in methanol, dull brown under UV_{365nm}, greenish brown in visible light; R_f: 0.73 (system C); UV λ_{max} nm: CH_3OH, 280, 316 sh; CH_3ONa, 260, 330; $AlCl_3$, 315, 380, $AlCl_3$/HCl, 315, 380; $NaOCOCH_3$, 384 sh, 323; NaOAc/H_3BO_3, 280, 321; ^{1}H-NMR, δppm (500 MHz, DMSO), 2.7 (1H, dd, *J*=5, 17 Hz, H-3 eq); 2.8 (1H, dd, *J*=11, 17 Hz, H -3 ax); 5.1 (1H, dd, *J*=5, 11 Hz, H-2 ax), 6.39 (1H, d, *J*=1.2,H-6), 6.64 (1H, d, *J*=1.2, H-8), 6.9 (2 H, d, *J* =8.5, H -3', H-5'), 7.4 (2 H, d , *J*=8.5, H-2', H-6').

Catechin-3-O-α-rhamnoside (5) (2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-5,7-diol-3-*α*-*L*-rhamnopyranoside): $C_{21}H_{24}O_{10}$, yellow amorphous powder; 8 mg;soluble in methanol, dull brown under UV_{365nm} and in visible light; R_f:0.26 (system D);UV λ_{max} nm, CH_3OH, 270,331; CH_3ONa, 295, 384; $AlCl_3$ 280, 400 ; $AlCl_3$/HCl 280,393; ^{1}H-NMR, δppm (500MHz, DMSO), 1.2 (3 H, d, *J* = 6 Hz, H-6"), 2.46 (1H, dd, *J*=3.5, 16.3 Hz, H-4a), 2.75 (1H, dd, *J*=4.6, 16.3 Hz, H-4b), 3.22- 3.82 (H-2" -H-5"), 3.75 (1 H, m, H-3), 4.2 (1H, s, H-

1"), 4.9 (1 H,dd , *J*=5,11 Hz, H-2), 6.3 (1H, d, *J*=1.2 Hz, H-6), 6.57 (1H, d, *J*=1.8 Hz, H-2'), 6.63 (1H, d, *J*=1.2 Hz, H-8), 6.9 (1H, d, *J*=8 Hz, H-5'), 7.37 (1H, dd, *J*=1.2, 8 Hz, H-6'), 9.2 (1H, d, 5-OH).

5,6,4' trihydroxy flavonol 7-O-*α*-rhamnoside (6) (3,4',5,6-tetrahydroxy-2-phenylchromen-4-one-7-*α*-*L*-rhamnopyranosyl): $C_{21}H_{20}O_{11}$, yellow amorphous powder; 13 mg; soluble in methanol; reddish brown under UV_{365nm}, yellow in visible light; R_f : 0.74 (system B); UV λ_{max}nm, CH_3OH, 265, 354; CH_3ONa, 265, 300 sh, 397; $AlCl_3$/HCl 265, 296sh, 359, 390; $NaOCOCH_3$, 265, 288sh, 363; ^{1}H-NMR δ ppm, (500MHz, DMSO), 1.2 (3 H, d, *J* =6 Hz, H-6"), 3.82-3.22 (H-2"- H-5"), 5.3 (1H, s, H-1"), 6.58 (1 H, d, *J*=2.5 Hz, H-8), 7.1(2 H, d, *J*=8.5 Hz, H-3' , H-5'), 8.1 (2 H, d , *J* =8.5 Hz, H-2' , H-6'). ^{13}C-NMR δ ppm (125 MHz, DMSO)147.5 (C-2), 137.8 (C-3), 177.2 (C-4), 151.1 (C-5), 130.9 (C-6),162.8 (C-7), 93.2 (C-8), 151.4 (C-9), 103.3 (C-10), 122.1 (C-1'), 131.6 (C-2'), 118.1(C-3'), 159.2 (C-4'),113.3 (C-5'), 129.3(C-6'), 102.9 (C-1"), 74.2 (C-2"), 76.4 (C-3"), 70.4 (C-4"), 76.1 (C-5"), 18.5 (C-6").

Structure elucidation of the isolated compounds **1-6** (Fig. 3) was based on determination of physical and spectral data (m.p., EI-MS, UV, ^{1}H-NMR and ^{13}C NMR), and comparison with published literature.The molecular formula of compound **1** was established *via* its EI-MS spectrum as $C_{20}H_{40}O$. Through interpretation of its ^{1}H-NMR spectral data and by comparison with those reported [20], it was identified as the diterpene alcohol Phytol. The ^{1}H-NMR spectrum of compound **2** demonstrated the characteristic features of a steroidal nucleus with 6 methyl substituent groups and a C-24 ethyl group [41]; in addition, the presence of an acetate unit was confirmed by the appearance of a singlet at δ 2.04; thus it was identified as *β*-sitosterol-3-acetate [41].The ^{1}H-NMR spectrum of compound **3** revealed the presence of a doublet at δ 9.59 with *J*=6 Hz corresponding to an aldehydic group, one doublet and two multiplets appear at 7.1-7.49 indicating the presence of protons at positions 2, 6; 4;3 and 5 respectively and one doublet at δ 7.68 with *J*=12 Hz indicative for H-*β* together with a methyl group at δ 2.0; compound **3** was therefore identified as *α*-methyl cinnamaldehyde, a common ingredient in balsamic cosmetics and perfumes[42]. The UV λ_{max} of compound **4** suggested that it possesses a flavanone skeleton; this was supported by its ^{1}H-NMR spectrum and through comparison with previously reported data [43], and allowed its identification as the flavanone aglycone, naringenin. The UV λ_{max} of compound **5** pointed to a free 5-OH in ring A of its skeleton, and an *ortho*-dihydroxy group in ring B (bathochromic shift in the sodium methoxide spectrum persistent in $AlCl_3$ and decreased in $AlCl_3$/HCl);in the ^{1}H-NMR

spectrum, 5 doublets ranging from δ 6.30-7.37, were assigned for H-6, H-8, H-2', H-5' and H-6';while the doublet of doublet appearing at 4.9 and the multiplet at 3.75 were representative for protons at C-2 and C-3 (dihydro) positions; whereas, the doublet of doublet at δ 2.46 and 2.75 with *J*=3.5, 16.3 Hz and 4.6, 16.3 was characteristic for H-4a, b; the sugar moiety was identified as *α*-rhamnose attached at position 3 [44] (TLC of acid hydrolysate and ^{1}H-NMR spectral data); compound **5** was thus identified as catechin 3-O-α-rhamnoside.The UV spectral data of compound **6** indicated a flavonol substituted skeleton with a 4' hydroxyl group and lack of free 7-hydroxyl group; its ^{1}H-NMR spectrum revealed the occurrence of a doublet signal at δ 6.58 (*J*=2.5 Hz), indicating the presence of a C-8 and absence of C-6 protons in ring A. The appearance of 2 doublets at δ 7.1 and 8.1 with *J* value = 8.5 Hz assigned for H-3', H-5' and H-2' and H-6', respectively and indicated a 1',4' di-substituted system in ring B[45] ; the sugar moiety was identified as *α*-Rhamnose attached at 7 position (TLC, ^{1}H-NMR, ^{13}C-NMR) [45, 46]; from the aforementioned data, compound **6** could be identified as 5,6,4' trihydroxyflavonol 7-O-*α*-rhamnopyranoside (6-hydroxy kaempferol-7-*α*-rhamnospyranoside).

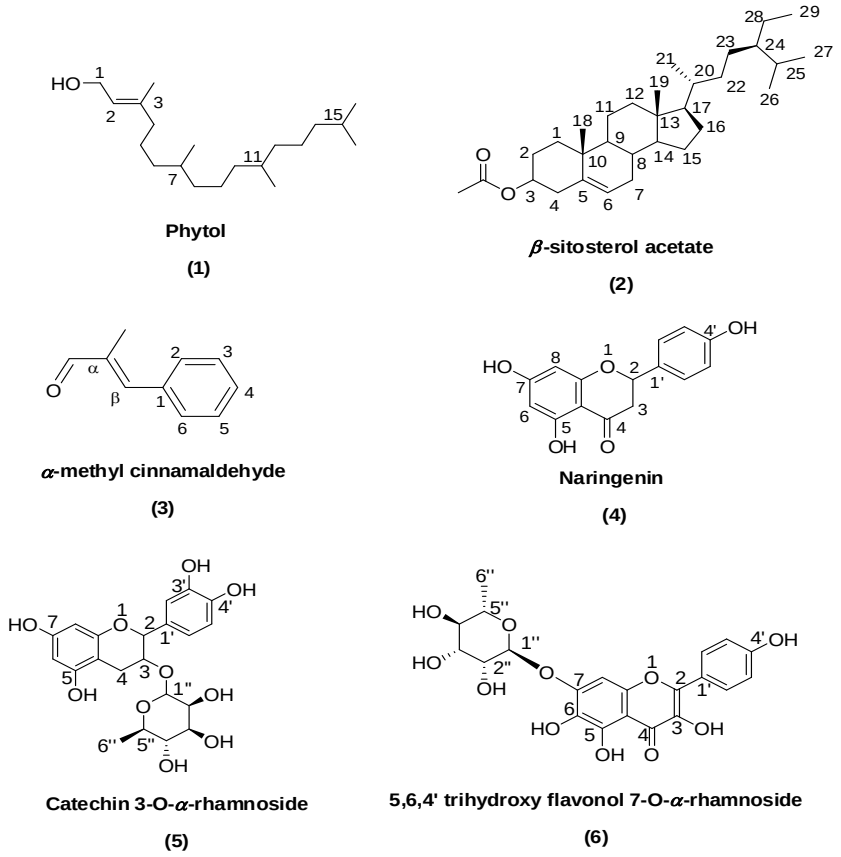

Figure 3: Compounds isolated from the flowers of *S. campanulata*

CONCLUSION

To the best of our knowledge, this is the first report on the bioactivity of the flowers of *Spathodea campanulata* P. Beauv. cultivated in Egypt. The ethanol extract (and/or its fractions) can be recommended, after further laboratory and clinical trials, to be used as antibacterial, antitumor, anthelmintic and antihyperglycemic; especially that it showed a high safety margin. The bioactive extractives, on fractionation, afforded six compounds among which three phenolics, all for the first time recorded from the flowers.

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REFERENCES

1. WHO. Traditional medicine: 2008.
2. Hung TM, Na MK, Thoung PT, Su ND, Sok DE, Song KS, Seong YH, Bae K. Antioxidant activity of caffeoylquinic acid derivatives from the roots of *Dipsacus asper* Wall. J Ethnopharmacol, 2006;108: 188-192.
3. Joly AB. Introdução à taxonomia vegetal, 7th ed., SãoPaulo: Ed. Nacional: 1985, p.503
4. Mendes NM, de Souza CP, Araujo N, Pereira JP, Katz N. Molluscicidal activity of some natural products on *Biomphalaria glabrata*. Mem Inst Oswaldo Cruz, 1986; 81(1): 87–91.
5. Ilodigwe EE, Akah A. *Spathodea campanulata*: an experimental evaluation of the analgesic and anti-inflammatory properties of a traditional remedy. Asian J Med Sci, 2009; 1(2): 35-36.
6. Ofori-Kwakye K, Kwapong AA, Bayor MT. Wound Healing Potential of Methanol Extract of *Spathodea campanulata* Stem Bark Formulated Into A Topical Preparation. Afr J Tradit Complemen Altern Med, 2011; 8(3):218-223.
7. Mbosso EJT, Ngouela S, Nguedia JC A, Beng VP, Rohmer M, Tsamo E. Spathoside, a cerebroside and other antibacterial constituents of the stem bark of *Spathodea campanulata*. Nat Prod Res, 2008; 22(4): 296-304

8. Niyonzima G, Laekeman G, Witvrouw M, Van poel B, Pieters L, Paper D, De Clercq E, Franz G, Vlietinck AJ. Hypoglycemic, anticomplement and anti-HIV activities of *Spathodea campanulata* stem bark. Phytomedicine**,** 1999; 6 (1): 45-49.
9. Aarthi N, Murugan K. Larvicidal and smoke repellent activities of *Spathodea campanulata* P. Beauv. against the malarial vector *Anopheles stephensi* Lis. Diptera: Culicidae. J Phytology, 2010; 2(8):61-69.
10. Kowti R, Harsha R, Ahmed MG, Hareesh AR, Gowda Th, Dinesha R, Kumar S, Ali I. Antimicrobial activity of ethanol extract of leaf and flower of *Spathodea campanulata P*. Beauv. Res J Pharm, Biol& Chem Sci, 2010; 1(3): 691-698.
11. Makinde JM, Amusan OOG, Adesogan EK.The antimalarial activity of chromatographic fractions of *Spathodea campanulata* stem bark extracts against *Plasmodium berghei berghei* in mice. Phytother Res, 1990; 4(2): 53-56.
12. Nazif NM. Phytochemical and antioxidant activity of *Spathodea campanulata* P. Beauvois. Growing in Egypt. Nat Prod Sciences, 2007; 13 (1): 11-16.
13. Dhanabalan R, Doss A, Balachandar S, Kezia E, Jagadeeswari M, Karthik H. *In vitro* phytochemical screening and antibacterial activity of organic leaf extracts of *Spathodea campanulata* P. Beauv. against hospital isolated bacteria strains. Ethnobotanical Leaflets, 2008; 12: 1022-28.
14. Patil VV, Patil SB , Kondawar MS , Naikwade NS, Magdum CS. Study of methanolic extract of flower of *Spathodea campanulata* L. as an anti-solar. I J G P, 2009; 3(3): 248-249.
15. Ngouela S, Nyasse B, Tsamo E, Sondengam B L, Connolly JD. Spathodic acid: a triterpene acid and sitosterol from the stem bark of *Spathodea campanulata*. Phytochemistry, 1990; 29(12):3959-3961.
16. Ngouela S, Tsamo E, Sondengam BL. Extractives from Bignoniaceae: Constituents of the Stem Bark of *Spathodea campanulata*. Planta Med, 1988; 54(5):476.
17. El-Hela AA. A new iridoid glucoside from *Spathodea campanulata* P. Beauvois leaves. Az J Pharm Sci, 2001a; 27: 115-120.
18. El-Hela AA. Phenolics from *Spathodea campanulata* P. Beauvois leaves. Az J Pharm Sci, 2001b; 27: 152-162.
19. Pianaro A, Pinto JP, Ferreira DT, Ishikawa NK, Filho RB. Iridoid glucoside and antifungal phenolic glycosides from *Spathodea campanulata* roots. Semina: Ciencias Agrarias, Londrina**,** 2007; 28(2):251-256.

20. Elusiyan CA, Ani NC, Adewunmi CO, Olugbade TA. Distribution of iridoid glucosides and antioxidant compounds in *Spathodea campanulata* parts. Afr J Tradit Complement Altern Med, 2011; 8 (1):27-33.
21. Kumaresan M, Palanisamy PN and Kumar PE. Chemical investigation of the flower of *Spathodea campanulata* by GC-MS. J Nat Prod Plant Resour, 2011; 1 (2): 14-17.
22. Zaheer Z, Paithankar A P, Deshpande SD, Khan S. Optimization of extraction process and phytochemical investigations of *Spathodea campanulata* flowers. Afr J Pharm Pharmacol, 2011; 5(20):2226-2231.
23. Farnsworth NR. Biological and phytochemical screening of plants. J Pharm Sci, 1994; 55: 225-276.
24. Wagner H, Bladt S. Plant drug analysis*:* A thin layer chromatography atlas, 2nd ed., Springer Verlag, New York: 1996.
25. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol, 1983; 54: 275-387.
26. Lorian V. Antibiotics in Laboratory Medicine, 5 th ed., Philadelphia, PA, Lippincott Williams and Wilkins: 2005.
27. Skehan P, Storeng R, Scudiero A, McMahon J., Vistica D, Warren JT, Bokesch H Kenney S, Boyd RM.New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. J Natl Cancer Inst, 1990; 82 (13): 1107-1112.
28. Dash GK, Mishra B, Panda A, Patro CP, Ganapathy S. Anthelmintic activity of *Evolvulus nummularius*. Indian J Nat Prod, 2003; 19 (3):24-25.
29. Henry JD. Clinical diagnosis and Management by Laboratory Methods, 7th ed., Philadelphia PA, WB Saunders: 1984.
30. Nidhi Sh , Veena G. Antidiabetic and antioxidant potential of ethanolic extract of *Butea monosperma* leaves in alloxan-induced diabetic mice. Indian J Biochem Biophys, (IJBB), 2009; 46: 99-105.
31. Mattila P, Astola J, Kurnpulainen J. Determination of Flavonoids in Plant Material by HPLC with Diode-Array and Electro-Array Detections. J Agric Food Chem, 2000; 48 (12): 5834-5841.
32. Rajaei A, Barzegar M, Mobarez AM, Sahari MA, Esfahani ZH. Antioxidant, anti-microbial and antimutagenicity activities of pistachio (*Pistachia vera*) green hull extract. Food Chem Toxicol, 2010; 48: 107–112.

33. Kiprono PC, Kaberia F, Keriko J M, Karanja J N. The in-vitro anti-fungal and anti-bacterial activities of beta-sitosterol from *Senecioly ratus* (Asteraceae). Z Natur forsch C, 2000; 55 (5-6): 485-488.
34. Yoshihiro I, Toshiko H, Akiko S, Kazuma H, Hajime H, Shigeki K. Biphasic effects of geranyl geraniol, terpenone and phytol on the growth of *Staphylococcus aureus.* Antimicrob Agents Chemother, 2005; 49(5):1770-1774.
35. Awad AB, Chan K, Downie A, Fink CS. Peanuts as a source of B-sitosterol, a sterol with anticancer properties. Nutr Cancer, 2000; 36(2): 238-241.
36. Nandi S, Vracko M, Bagchi MC. Anticancer activity of selected phenolic compounds: QSAR studies using ridge regression and neural networks. Chem Biol Drug Des, 2007;70 (5):424-36.
37. Hashem FA, Sengab AE, Shabana MH, Khaled S. Antioxidant activity of *Mayodendron igneum* Kurz and the cytotoxicity of the isolated terpenoids. J M A P , 2012; 1(3):88-97
38. Thorn GW, Adams RD, Braunwald E, Esselbacher KJ , Petersdorf RG *Harrisons's* Principles of Internal medicine. New York, McGraw Hill Co: 1977.
39. Martin RJ. Mode of action of anthelmintic drugs. Vet J, 1997;154 :11-34.
40. Deore SL, Khadabadi SS, Kamdi KS, Ingle VP. *In vitro* Anthelmintic activity of *Cassia tora*. Int J Chem Tech Res, 2009;1(2):177-179.
41. Goad LJ, Akihisa T. Analysis of Sterols, 1st ed., Blackie Academic and Professional, London, Weinheikm, New York, Tokyo, Melbourne and Madras: 1997.
42. John E Mc M. Organic Chemistry, 8 th ed., Chapter 11, Brooks/Cole Pub Co.:2001.
43. Hammami S, Jannet HB, Bergaoui A, Ciavatta L, Cimino G, Mighri Z. Isolation and Structure Elucidation of a Flavanone, a Flavanone Glycoside and Vomifoliol from *Echiochilon fruticosum* Growing in Tunisia. Molecules, 2004; 9: 602-608.
44. Ogechukwu OE, Ogoamaka OP, Kawamura A, Hassan A, Debbab Ab, Okechukwu ECh, Sylvester N Ch , Ngozi N, Peter P. Three -(-) Catechin-O-Rhamnosides from the Eastern Nigeria Mistletoe with Potent Immunostimulatory and Antioxidant Activities. J B M R T, 2012; 1: 102.
45. Wang H , Nair MG , Strasburg GM , Booren AlM , Gray JI. Antioxidant Polyphenols from Tart Cherries (*Prunus cerasus*). J Agric Food Chem, 1999; 47: 840-844.
46. Agrawal PK. Carbon-^{13} NMR of flavonoids. Elsevier, Amsterdam, Oxford, New York, Tokyo: 1989.