Natural Product Sciences
13(1) : 11-16 (2007)

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

Naglaa M. Nazif*

Phytochemistry Department, National Research Centre, Dokki, Cairo, Egypt, 12311.

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, caffiec acid (**1**), and ferulic acid (**2**), fraction EtOAc/MeOH on further fractionation afforded 3 Flavonoids, kampferol 3-*O*-glucoside (**3**), quercetin 3-methyl ether (**4**) and 8-methoxy kampferol 3-*O*-glucoside (**5**). The isolated constituents were identified by co chromatography with authentic samples, TLC, PC., UV, MS and ^{1}H-NMR. Also the lipoidal matter of the plant was studied. The unsaponifiable matter was found to be mixture of hydrocarbons from (C_{14}-C_{28}), cholesterol, campasterol, stigmasterol, and α-amyrin. Fatty acid methyl esters were found to contain 12 fatty acids. The fatty acids containing C_{18} formed ca.65% of the total mixture.
Keywords – *Spathodea campanulata*, Bigononiaceae, Phenolic acids, Flavonoids, Antioxidants

Introduction

Spathodea campanulata P. Beauvois (Bignoniaceae), 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, diarrhea, and as poison antidote (Amusan *et al.*, 1995; Ngouela *et al.*, 1990). The moluscicidal 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 Niyozima *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; Lasure *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.

*Author for correspondence
Fax: +202-337-0931; E-mail: naglaanazif@yahoo.com

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.

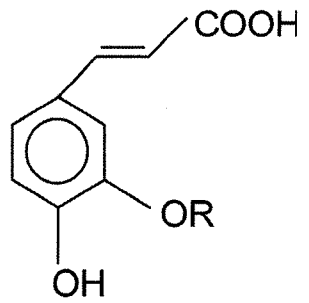
General experimental procedures – TLC was carried out on precoated silica gel F_{254} plates (Merck) (Darmstadt, Germany) developed with EtOAc-formic acid-acetic acid-H_2O (30/1.5/1.5/7) solvent (1) Paper chromatography (pc.) was carried out on Whatman 3MM, 15% acetic acid solvent (2) and butanol-acetic acid-H_2O (4-1-5), the upper layer solvent (3) for flavonoids and phenolic acids and butanol-benzene-pyridine-H_2O (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. ^{1}H-NMR was recorded on Jeol-GX at 500 MHz. using TMS as internal standard.

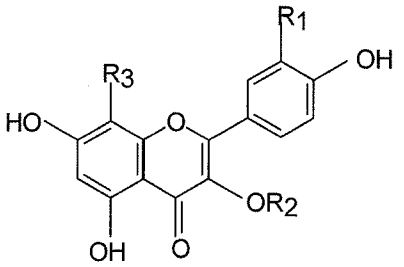
Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl chroman-2-carboxylic acid) (Aldrich chemical CO.), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma chemical CO.) and methanol HPLC grade (Riedel-de-Häen) for antioxidant activity.

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 celite (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-$CHCl_3$ (1/1), $CHCl_3$, $CHCl_3$-EtOAc (1/1) = (fraction A), EtOAc, EtOAc-MeOH (1/1) = (fraction B), and MeOH. About 1.35 gm 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 $CHCl_3$, increasing polarities with MeOH, (100 ml fractions) were collected. Fractions 9-16 coming with $CHCl_3$/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 $CHCl_3$/MeOH gradient of increasing polarity. The fractions eluted with $CHCl_3$/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) λ_{max} 326, 293, 253 nm The ^{1}H-NMR spectrum (DMSO-d_6) 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),



Compound (**1**): Caffiec acid: R = H
Compound (**2**): Ferulic acid: R = CH_3



Compound (**3**): Kampferol 3-*O*-glucoside: R_1, R_3 = H, R_2 = glycosyl
Compound (**4**): Quercetin 3-methylether: R_1 = OH, R_2 = CH_3, R_3 = H
Compound (**5**): 8-Methoxy-kamferol 3-*O*-glucoside: R_1 = H, R_2 = glucosyl, R_3 = OCH_3

Fig. 1. Structure of the isolated compounds.

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, λ_{max} 322, 296, 207 nm a bathochromic shift with increase of intensity was noticed on addition of NaOH to λ_{max} 348, 310, 207 nm. The ^{1}H-NMR spectrum (DMSO-d_6) showed signals at δ 7.49 and 6.32 (2H, d, d, J = 16,16Hz, olefinic protons), δ 7.26 (1H, s, H-2), 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, s, OCH_3) (Balde, *et al.*, 1991). The EI/MS spectral data showed a molecular ion peak at m/z 194 (M^+) 193 (M^+-1), 179 (M^+-CH_3), 163 (M^+-OCH_3), 149 (M^+-CO_2H), 123 (M^+-CH = CH, COOH).

Compound (**3**) showed a UV spectrum in MeOH, λ_{max} 347.8, 290, 267.2, 255 (sh), (NaOH) λ_{max} 404.8, 330, 291, 270, ($AlCl_3$) λ_{max} 390, 301, 294, 269, ($AlCl_3$/HCl) λ_{max} 389, 290, 268, 256, (NaOAc) λ_{max} 387, 301, 281, 214, (NaOAc/H_3 BO_3) 369, 289, 267. ^{1}H-NMR spectrum (DMSO-d_6) δ 7.9 (2H, d, J = 8.7Hz, H-2', H-6'), δ 6.8 (2H, d, J = 8.7, H-3', H-5') δ 5.91 (1H, d, J = 2.1, H-8), δ

5.75 (1H, d, J=2.1, H-6), δ 5.12 anomeric proton of glucose, δ (3.07-3.65, m, 5H of glucose).

Compound (**4**) showed a UV spectrum in MeOH, λ_{max} 356, 293, 257, (NaOH) λ_{max} 402, 331, 297, 260 ($AlCl_3$) λ_{max} 446, 362, 320, 262 ($AlCl_3$/HCl) λ_{max} 406, 293, 259, (NaOAc) λ_{max} 389, 320, 297, 260, (NaOAc/H_3BO_3) 376, 301, 293, 260. EI/MS, M^+ 316, 300 (M-OH), 270 (M-OCH_3, OH), 153, 164.

Compound (**5**) showed a UV spectrum in MeOH, λ_{max} 349, 314, 274, (NaOH) λ_{max} 410, 358, 329, 282 ($AlCl_3$) λ_{max} 417, 382, 361, 323, 278, 248 ($AlCl_3$/HCl) λ_{max} 417, 380, 349, 321, 279. ^{1}H-NMR spectrum (DMSO-d_6) displayed signals at δ 8.13 (1H, d, J = 7Hz, H-2'), δ 8.11 (1H, d, J = 8.7Hz, H-6'), δ 6.92 (1H, d, J = 8.7 Hz, H-3'), δ 6.9 (1H, d, J = 8.7Hz, H-5'), δ 5.24 (1H, d, J = 10.2Hz , H-1of glucose), δ 3.89 (3H, s, OCH_3), 3.87-3.3 ppm (5H of glucose). FAB-MS +ve mode showed peaks at m/z 479 and m/z 317 and 302, 289.

Saponification of lipids – The combined petroleum ether extracts were purified by fuller's earth filtered. The filtrate was evaporated under vacuum at 40 °C and the residue (7.8 g) was dissolved in boiling acetone (350 ml), cooled and left over night. An amorphous white precipitate was formed which was separated by filtration (1.1 g) representing acetone insoluble fraction. The acetone soluble fraction was saponified (N/2 methanolic KOH) (El-Said and Amr, 1965), and unsaponifiable matter (1.9 g) was first separated by extraction with ether. The mother liquor was acidified, the liberated fatty acids mixture, was extracted with peroxide free ether, methylated (methanol, BF_3) (Harborne, 1984). Aliquots of the isolated unsaponfiable fraction and methyl esters of fatty acids were subjected to GLC analysis using the following conditions.

For the unsaponifiable matter – Column : Capilary column HP-5 (19091j-413) nominal length 30 m 320 μm diameter, film thickness, 0.25 μm. Temperature program: Initial temp. 80 °C, Ini. time 2 min. program rate 8 °C/min., final temp. 275 °C, final time 50 min., Injection temp. 260 °C, detector (FID), T = 300 °C, flow rate of carrier gases N_2 : 30 ml/min., H_2 : 35 ml/min., air 230 ml/min.

For fatty acid methyl esters – Methyl esters of fatty acids were analyzed on Capillary column (Ag-Bp-70), Polysilphenylene-siloxane, 60 m. length, 320 μm. internal diameter, 0.25 μm. film thickness, oven initial temp. 70 °C, FID, detector, temp., 280 °C, injector temp. 250 °C flow rate of carrier gases N2: 40 ml/min, H2: 40 ml/min., air: 45 ml/min., injected volume 2 ul, sensitivity 32×10^{-9} with (FID), column program temp. 70 °C (2 °C/min.) up to 240 °C.

Table 1. GLC analysis of fatty acid methyl esters of *Spathodea campanulata*

Peak No.	RRT	Relative %	Constituents
1	0.73	0.09	$C_{12(0)}$ lauric
2	0.86	0.34	$C_{14(1)}$ tetracosaenoic
3	0.87	1.43	$C_{14(0)}$ Myristic
4	0.91	0.731	$C_{15(0)}$ Pentadecanoic
5	1	19.119	$C_{16(0)}$ Palmitic
6	1.05	1.38	$C_{16(1)}$ palmitoliec
7	1.09	17.20	$C_{18(0)}$ stearic
8	1.10	29.60	$C_{18(2)}$ Linoliec
9	1.13	18.71	$C_{18(3)}$ linolenic
10	1.22	3.57	$C_{19(0)}$ Nonadecanoic
11	1.37	6.38	$C_{20(0)}$ behenic
12	1.40	1.45	$C_{22(1)}$ Erucic

RRT = Relative to retention time of palmitic acid $C_{16(0)}$ (20.71 min.)

Anti-oxidant activity – Determination of the scavenging activities was carried out according to the method of Chen & Ho, 1997.

An aliquot of 0.1 mM methanol solution of DPPH was mixed with the methanolic solution of the sample, so that the relative concentration of plant material versus the stable radical in the cuvette was 0.13. Then the solution with samples to be tested were shaken vigorously. The absorbance was monitored at the start and after 10 & 30 minutes after being kept in the dark against a blank of methanol without DPPH. All tests were run in duplicates and averaged. The antioxidant activities of these samples were compared with trolox and expressed as percent radical scavenging activity (% RSA).

Results

Fatty acid methyl esters – The fatty acid methyl esters Table 1 were found to be a mixture of 12 fatty acids in which linoleic acid represents the main component (29.6%), followed by palmitic acid (19.2%), linolenic (18.71) and stearic acid (17.2 %).

The unsaponifiable matter – GLC analysis for the unsaponifiable fraction detected the presence of mixture of hydrocarbons from (C_{14}-C_{28}), cholesterol, campasterol, stigmasterol, β-sitosterol and α-amyrin. The Identification of the components was carried out by comparing their retention times with the available references and the results were tabulated in Table 2.

Phenolic components – Compound (**1**) was isolated from fraction A, followed by PPC on 3MM, and solvent (3). Band 1, having R_f. of 0.79, appeared as intense blue

Table 2. GLC analysis of unsaponifiable matter of *Spathodea campanulata*

Peak No.	RRT	Relative %	Constituents
1	0.098	1.77	Tetradecance C_{14}
2	0.135	3.55	pentadecane C_{15}
3	0.21	5.85	Hexadecane C_{16}
4	0.306	3.52	Heptadecane C_{17}
5	0.38	2.01	OctadecaneC_{18}
6	0.41	2.844	Nonadecane C_{19}
7	0.556	2.751	Octadecane C_{18}
8	0.57	6.37	Nonadecane C_{19}
9	0.59	2.21	Eicosane C_{20}
10	0.65	2.215	Dodacosane C_{22}
11	0.81	5.25	Tricosane C_{23}
12	0.86	2.193	Tetracosane C_{24}
13	0.92	8.99	Heptacosane C_{27}
14	0.95	4.087	Cholesterol
15	1	4.79	Campasterol
16	1.05	2.65	Stigmasterol
17	1.08	28.46	β-Sitosterol
18	1.12	10.5	α-amyrine

RRT = Relative to retention time of β-sitosterol (36.51 min.)

fluorescent spot. It showed UV (MeOH) λ_{max} 326, 293, 253 nm. On addition of NaOH shift reagent the compound showed a bathochromic shift of λ_{max} to 349, 305, 256 nm followed by decomposition. This behavior is similar to a hydroxylated cinnamic acids. The EI/MS spectral data showed a fragmentation pattern was found to be in accordance to that of caffiec acid (Subramanian *et al.*, 1973).

Compound (**2**) was isolated from fraction A, followed by PPC on 3MM, and solvent (3). Band 2, having R_f. of 0.87, appeared as intense blue fluorescent spot in UV light (366 nm). The EI/MS spectral data showed a molecular ion peak at m/z 194 (M^+) 193 (M^+-1), 179 (M^+-CH_3), 163 (M^+-OCH_3), 149 (M^+-CO_2H), 123 (M^+-CH=CH, COOH) This fragmentation pattern was found to be in agreement with that reported for ferulic acid (Niyonizima, *et al.,* 1991).

Compound (**3**) appeared as dull spot in UV at 366 nm on silica gel TLC, changed to intense yellow on spraying with N.A. reagent having R_f. of 0.31 solvent system (1). The UV spectrum in methanol showed absorption maxima at λ_{max} 347.8 nm for (band I, cinnamyl system) and λ_{max} 267.2 nm (band II, benzoyl system), which suggesting it to be a flavonol-3-O-glycosyl. There is a bathochromic shift of 57 nm in band I with increase in intensity, on addition of NaOH, indicating the presence of free 4'-OH group. Also UV spectrum with NaOH showed a shoulder peak at 330 nm indicating the presence of free -7-OH group. UV spectrum with $AlCl_3$ diagnostic reagent showed a bathochromic shift of 43 nm in band I, indicating the presence of free 5-OH group. On addition of HCl the shift remains the same 43 nm which confirms the presence of free -5-OH group and the absence of O-dihydroxy system in ring B. $AlCl_3$/HCl showed double peaks at 256 & 268 nm which is typical for 3-substituted flavone with a phloroglucinol type A ring (Voirin, 1983). There is 14 nm bathochromic shift of band II in NaOAc diagnostic reagent confirms the presence of free 7-hydroxyl group. Acid hydrolysis of the compound afforded kampferol in the organic layer and glucose in the aqueous layer (TLC, solvent system 1 & PC. solvent system 4 with authentics). EI/MS of the aglycone showed a molecular ion peak at m/z 286, M^+-1 at 285 which constitutes to a molecular formula of $C_{15}H_9O_6$ and the fragmentation pattern was found to be in accordance with that found in literature for kampferol. From the aforementioned data the compound was identified as kampferol -3-*O*-glucoside (Mabry *et al.*, 1970; Makhram, 1982).

Compound (**4**) appeared as dark purple color in UV (366 nm, changed yellow with ammonia and N.A. reagent of R_f (0.56, 0.29, 3MM solvents 2 & 3 respectively). The UV spectrum in methanol showed band I at λ_{max} 356 nm, band II at λ_{max} 257 nm indicates the presence of 3-substituted flavonol nucleus. 4'-free OH group was proved by the bathochromic shift of (46 nm) of band I, on addition of NaOH. Bathochromic shift of (90 nm) was observed on addition of $AlCl_3$, which followed by a hypsochromic shift of (40 nm) on further addition of HCl proved the presence of ortho-dihydroxy system with 5-free OH group. NaOAc/H_3BO_3 confirmed the presence of O-di hydroxyl system by (20 nm) shift of band I. EI/MS, showed a molecular ion peak at m/z 316, and at m/z 300 (M-OH), and at m/z 270 (M-OCH_3 , OH), 153 (ring A^+), and 164 (ring B^+). From all the previous data the compound was identified as querecetin -3O-methyl ether (Mabry *et al.*, 1970; Makhram, 1982).

Compound (**5**) appeared as dark purple spot on silica gel TLC, of R_f. (0.35) solvent (1), changed to yellowish green spot on spraying with N.A. reagent, this chromatographic behaviour indicates that the compound may be monoglycoside. It showed band I at λ_{max} 349 nm, and band II at λ_{max} 274 nm which corresponds to a flavonol with 3-substituted nucleus. Bathochromic shift of band I of 61 nm with NaOH diagnostic reagent with increase of intensity and formation of small peak at 329 nm indicates the presence of free 4' OH and free 7-OH groups respectively. There is a 68 nm bathochromic shift of band

Table 3. Antioxidant activities of isolated compounds

Tested sample	Absorbance at λ_{max} 516 mm/ reaction period (min.)		% RSA
	10 min	30 min.	
Total alcohol extract	0.062	0.062	92.01
Fraction (A)	0.047	0.049	94
Fraction (B)	0.086	0.086	89
Compound (**1**)	0.053	0.052	93.23
Compound (**2**)	0.060	0.059	92.34
Compound (**3**)	0.145	0.144	81
Compound (**4**)	0.196	0.206	75
Compound (**5**)	0.18	0.181	77
Trolox	0.057	0.05	92.7
Blank	0.784	0.784	–

The absorbance readings at each reaction period are means of 2 measurements.

I on addition of $AlCl_3$ diagnostic reagent confirms the presence of free 5-OH group, no hypsochromic shift on addition of HCl confirms the presence of free 5-OH and absence of O-dihydroxy system in ring B (Voirin, 1983).

Positive FAB/MS showed molecular ion peak at m/z 479, and 317 (M^+-hexose), an ion peak at m/z 302 (aglycone-CH_3), and other fragmentation pattern was found to be in accordance with that reported for 8-methoxy kampferol 3-*O*-glucoside. Acid hydrolysis of compound (**5**) afforded, glucose and 8-methoxy kampferol which was identified by PC. For sugar with solvent 4, and by EI/MS and ^{1}H-NMR for the aglycone. So the isolated compound was found to be 8-methoxy-kampferol-3-*O*-glucoside (Drewes, 1974).

Discussion

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical is a dyed free radical, due to its odd electron , DPPH radical gives a strong absorption band at 516 nm (deep violet color). When this electron becomes paired off in the presence of free radical scavenger the absorbance vanishes, and the change of absorbance produced in this reaction is assessed to evaluate the radical scavenging potential of test samples.

Aqueous alcoholic extracts of *Spathodea campanulata* aerial parts (leaves & terminal branches) showed strong antioxidant activity (92% RSA, Radical Scavenging Activity) as shown in Table 3. Further fractionation of the total extract using celite (545) column led to the isolation of 7 subfractions. Two of them showed promising antioxidant activities, (94%, 89% RSA). When fraction A subjected for further chromatographic fractionation led to the isolation of phenolic acids (caffiec acid and ferulic acid). This result was potentiated by the reported data of Chen and Ho, 1997 who studied the antioxidant activity of phenolic acids. Ferulic acid was isolated from *Spathodea campanulata* stem bark by Niyonzima *et al.*, 1991. Caffiec acid and quercetin were previously isolated from *Spathodea campanulata* stem bark by Subramanian *et al.*, 1973. Further fractionation of fraction B, led to the isolation of 3 flavonoidal constituents, kampferol 3-*O*-glucoside, quercetin 3-methyl ether, and 8-methoxy-kampferol 3-*O*-glucoside, which showed an antioxidant activities of, (81, 75, 77% RSA) respectively. This is the first time to isolate and studying the antioxidants of the flavonoids from *Spathodea campanulata* (leaves and terminal branches) grown in Egypt. This antioxidant property of the plant extract may indicate the presence of other pharmacological activity like hepato-protection. The Study of lipoidal matter revealed the presence of terpenoidal constituents, campasterol, β-sitosterol, and stigmasterol in the unsaponifiable fraction, also triterpene as a-amyrene was detected, spathodic acid which is a triterpene acid and spathodol which is polyhydroxysterol were previously isolated by Ngouela *et al.*, 1988 & 1991 from the stem bark of the plant.

Acknowledgments

The author is deeply indebted to Prof. Dr. Faiza, M. Hammouda, Prof. Dr. M. Sief El-Nasr, Prof. Dr. S.I. Ismail, Prof. Dr. M. M. El-Missiry, and N. M. Hassan, for their kind help. The author thanks the National Research centre and the Academy of Science and technology for financial support which made this work possible.

References

Amusan, O.O.G., Musonthi, J.D., and Makhubu, L.P., Molluscicidal Activity of *Spathodea campanulata*, *Andrachne ovalis*, *Phytolacca dodecandera*, and *Hypoxis rooperi*, *Fitoterapia* **LXVI 2**, 113-116 (1995).

Balde, A.M., Claeys, M., Pieters, L.A., Wray, V., and Vlietinck, A.J., Ferulic acid esters from stem bark of *Pavetta owariensis*, *Phytochem.* **30**(3), 1024-26 (1991).

Bircher, W.H., Gardens of Hesperides. The Anglo Egyptian Bookshop, Cairo, 1960.

Chang, R.S. and Yeung, H.W., Inhibition of growth of human immunodeficiency Virus in vitro by crude extracts of Chinese medicinal herbs, *Antiviral Res.*, **9**, 163-176 (1988).

Chen, J.H. and Ho, C.T., Antioxidant activity of Caffiec acid and its related hydroxycinnamic acid compounds, *J. Agric. Food Chem.* **45**, 2374-2378 (1997).

Drewes, S. E., Chroman and related compounds, Vol. 2, Verlag Chemie, Printed in Germany 1974.

El-Said, M.E. and Amr, M.M., Oils, Fats, Waxes, and surfactants, Anglo

Egyptian Book Shop; Cairo, Egypt 1965, p. 55.

Harborne, J.B., Phytochemical Methods, (2nd) Ed. Chapmann and Hall, London, New York 1984, p. 156-157.

Irvine, F.R., Woody Plants of Ghana. Oxford University Press, London 1961, p. 739-740.

Lasure, A., Van Poel, B., Declerk, L.S., Bridts, C.H., Stevens, W.J., Rwangabo, P.C., Pieters, and Vlietinch, A.J. Screening of Rwandese plant extracts for their influence on lymphocytes proliferation, *Phytomed.*, **4**, 303-307 (1995).

Locker, C.P., Witvrouw, M., De Bethune, M.P., Burch, M.T. Mower, H.F., Davis, H., Lasure, A., Pauwels, R., DeClercq, E., and Vieltinch, A.J. Antiviral activity of Hawaiian plants against human immunodeficiency virus type-1-(HIV-1), *Phytomed.*, **2**(3), 259-264 (1996).

Mabry, T.J., Markham, K.R., and Thomas, M.B., The systematic identification of flavonoids, Springer-Verlag, Berlin-Heidelberg New York. 1970.

Makinde, J.M., Adesogan, E.K., and Amusan, O.O.G, Antimalarial active principles of *spathodea campanulata* stem bark, *Phytother. Res.*, **10**, 692-693 (1996).

Makinde, J.M., Adesogan, E.K., and Amusan, O.O.G, The Antimalarial activity of chromatographic fractions of *Spathodea campanulata* stem bark extract against plasmodium berghei berghei in mice, *Phytother. Res.* **4**(2), 53-56 (1990).

Makinde, J.M., Adesogan, E.K., and Amusan, O.O.G, The Antimalarial activity of *Spathodea campanulata* stem bark extract on Plasmodium berghei in mice, *Planta med.* **54**, 122-125 (1988).

Makinde, J.M., Adesogan, E.K., and Amusan, O.O.G, The Schizontocidal activity of *Spathodea campanulata* leaf extract on Plasmodium berghei in mice, *Phytother. Res.* **1**(2), 65-68 (1987).

Markham, K.R., Techniques of flavonoid identification. Academic press, A subsidiary of Harcourt Brace, Jouvanovich publishers 1982.

Ngouela, S.B., Nyasse, E., Tsamo, B.L., and Sondengam Connolly, J.D., Spathodic acid: a triterpene acid from the stem bark of *Spathodea campanulata*, *Phytochem.* **29**(12), 3959-3961 (1990).

Ngouela, S., Tsamo, E., and Sondengam, B.L., Spathodol, a new polyhydroxysterol from the leaves of *Spathodea campanulata*; *J. Nat. Prod.* **54**(3), 873- 876 (1991).

Ngouela, S., Tsamo, E., and Sondengam, B.L., Extractives from bignoniaceae: constituents of stem bark of *Spathodea campanulata*, *Planta med.*, **54**, 476-477 (1988).

Niyonizima, G, Laekman, G.M., Scharpe, S., Mets, T., and Vlietinck. A.J., Hypoglycemic activity of *Spathodea campanulata* stem bark decoction on streptozotocin-diabetic mice, *Planta med.*, **56**, 682 (1990).

Niyonizima, G, Laekman, G.M., Scharpe, S., Mets, T., and Vlietinck. A.J., Hypoglycemic activity of *Spathodea campanulata* stem bark decoction on streptozotocin-diabetic mice, *Phytother. Res.* **7**, 64-67 (1993).

Niyonizima, G, Laekman, G.M., Witvrouw, M., Van Poel, B., Pieters, L., Paper, D., DeClercq, E., Franz, G, and Vlietinck, A.J., Hypoglycemic, Anticomplement, and Anti-HIV activities of *Spathodea capanulata* stem bark, *Phytomed.*, **6**(1), 45-49 (2000).

Niyonzima, G, Pieters, L., Balde, A.M., Claeys, M., Laekeman, G.M., and Vlietinck, A.J., Isolation of 6-O-caffeolcatalpol and some other compounds from *Spathodea campanulata*. *Planta medica*, **57**, suppl. **2**, A85-A86 (1991).

Scogin, R., Anthocyanins of the Bignoniaceae, *Biochem. Syst.Ecol.* **8**, 273-276 (1980).

Subramanian, S.S., Nagarian, S., and Sulochanc, N., Caffiec acid from the leaves of *Spathodea campanulata*, *Curr. Sc.*, **42**(11), 403 (1973).

Vlietinch, A.J., De Bruyne, T., and Vanden Berghe, D., Plant substances as antiviral agents, *Current Organic Chem.* **1**, 307-344 (1997).

Vlietinch, A.J., De Bruyne, T., Apers, S., and Pieters, L., Plant derived compounds for chemotherapy of human immunodefieciency virus (HIV) infection, *Planta Med.* **64**, 97-107 (1998).

Voirin, B., UV Spectral Differentiation Of 5-hydroxy-and 5-hydroxy-3-methoxyflavones with mono-(4';), DI-(3',4') or Tri-(3',4',5')-substituted B rings, *Phytochem*, **22**(10), 2107-45 (1983).

(Accepted November 6, 2006)