Asian Journal of Pharmacy and Life Science ISSN 2231 – 4423
Vol. 2 (4), Oct-Dec,2012

GLYCOSIDES FROM *CASSIA BREWSTERI* FLOWERS GROWING IN EGYPT

Asmaa Ibrahim Owis*

Department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt.

E-mail: asmaa_owis@yahoo.com **Telephone**: 20/ 01202500017 – 20/082/2316955 , 20-01202500017

Abstract:

Five known glycoside: physcion-8-O- β-D-glucopyranoside, aloe-emodin -8-O- β-D-glucopyranoside, emodin-8-O-β-D-glucopyranoside, rhein-8-O-β-D-glucopyranoside and quercitrin were isolated for the first time from the flowers of Cassia brewsteri (F.Muell.) Benth., family Fabaceae. The structure identification of the isolated glycosides have been established on the basis of chemical and spectroscopic examination. Free and total anthraquinone content in the flowers extract were found to be (0.0343 g%) and (0.062 g%) respectively when determined spectrophotometrically as emodin-8-O- β-D-glucopyranoside. Flavonoids content in the flowers was found to be (0.195 g%) when determined spectrophotometrically as quercitrin.

Key words: *Cassia, anthraquinone, flavonoid, glycosides, determination.*

Introduction:

Cassia species are reported to be rich in anthraquinones, anthrones, flavonoids, triterpenes (1), alkaloids, chromones (2), hydroanthracenes and naphthalenic compounds (3). *Cassia brewsteri* (F.Muell.) Benth. family Fabaceae, has been identified as a potential multipurpose agroforestry species, and also as a potential source of seed galactomannans (industrial gums) (4). The main objective of this project is the continuation of the phytochemical study of *C. brewsteri* plant (5) through investigation of the flowers with emphasis on isolation and structure elucidation of different glycosidal compounds present in *C. brewsteri* flowers in addition to, spectrophotometric determination of anthraquinone and flavonoid content. The isolation was commenced by suitable chromatographic techniques.The pure fractions were crystallized from suitable solvents and physical constants of the isolated compounds were determined. Different chemical and spectroscopic methods such as MS, ^{1}H and ^{13}C-NMR were applied whenever necessary for structure elucidation. Comparison of the obtained results with the literature data about the contents of *Cassia* species was performed.

Materials and Methods:

Materials:

A. Plant material: *C. brewsteri* flowers were collected in Jan. 2011 from plants cultivated in Al-Abd Farm in Cairo-Alexandria Desert Road, Egypt. The plant was kindly identified by Prof. Dr. Abd Al Haleem Abd Al Magly Mohamed (Plant Taxonomy and Flora Research Department – Al Bsateen Research Institute – Agriculture Research Center – Ministry of Agriculture- Dokki- Giza) for whom the author is thankful.

B. Chromatographic material:

1) Adsorbents used: silica gel G for TLC; silica gel 60 for column chromatography; sephadex LH-20 (Pharmacia Fine Chemicals, Sweden); polyamide 6 S for column chromatography (Ridel De Häen AG, Seelze,Hannover, Germany).
2) Solvent systems used: S1: chloroform: MeOH (8.5:1.5v/v); S2: butanol: acetic acid: water (B.A.W.)(4:1:5).
3) Spray reagent: sulphuric acid spray reagent*; p*-anisaldehyde-sulphuric spray reagent (6); alcoholic potassium hydroxide (7); aluminium chloride (8).

Apparatus:

a) UV lamp: Hanovia lamps.
b) UV- visible spectrophotometer: Beckman DU7 and Shimadzu UV240.
c) Mass spectrometer: Jeol mass spectrophotometer, (70 eV.)
d) Varian 90 NMR (^{1}H-NMR, 300MHz, ^{13}C-NMR, 75 MHz, Japan). The NMR spectra were recorded in DMSO using TMS as an internal standard and chemical shifts values were recorded in δ ppm.
e) Buchi 520 melting microscope: for determination of melting points.

Methods:

A) Extraction: 0.5 kg of the air-dried powdered flowers was exhaustively defatted using hexane and then extracted with EtOH 70% by percolation. The residue (10.9g) was partitioned with chloroform followed by EtOAc. The dried EtOAc fraction (2.8g) was used in this study.

B) Fractionation: The obtained residue (2.8g) was chromatographed on silica gel column chromatography. Elution was started with chloroform (100%) then the polarity was increased till MeOH (100%) to afford ten pooled fractions. Fraction III [eluted with chloroform-MeOH (92:8) (112mg)] was further purified on silica gel column chromatography, to yield subfraction [eluted with chloroform-MeOH (90:10) (47mg)] after evaporation. Then subjected to further purification on Polyamide 6 column, starting with water then 10% gradual decrease in polarity with MeOH as an eluant to yield on concentration compound **1** (9mg).

Fraction IV [eluted with chloroform-MeOH (90:10) (400mg)] was further purified on silica gel column chromatography, to yield subfraction A [eluted with chloroform- MeOH (90:10) (58mg)] and subfraction B [eluted with chloroform- MeOH (86:14) (64mg)] after evaporation. Both were separately subjected to further purification on Polyamide 6 column, starting with water then 10% gradual decrease in polarity with MeOH as an eluant to yield on concentration compound **2** (6mg) and compound **3** (8mg). Fraction VI [eluted with chloroform- MeOH (85:15) (148mg)] was further purified on silica gel column chromatography, to yield subfraction [eluted with chloroform-MeOH (86:14) (57mg)] after evaporation. Then subjected to further purification on Polyamide 6 column, using MeOH as an eluant to yield on concentration compound **4** (5mg). Fraction VIII [eluted with chloroform- MeOH (70:30) (148mg)] was further purified on silica gel column chromatography, to yield subfraction [eluted with chloroform- MeOH (70:30) (57mg)] after evaporation. Then subjected to further purification on Polyamide 6 column, using MeOH as an eluant to yield on concentration compound **5** (3mg).

C) Characterization and identification of isolated compounds: The isolated compounds were identified by MS, ^{1}H-NMR and ^{13}C-NMR spectral data, as well as, by determining their melting points.

D) Acid hydrolysis of isolated glycosides: 3mg of each isolated compound was separately treated with 1.5N HCl in aqueous MeOH for 2 hours at 100^{o}C. Each hydrolysate was, then separately, extracted with EtOAc and the extract was subjected to TLC investigation alongside authentic aglycones. The mother liquor was neutralized with sodium bicarbonate and used for the study of the sugar moieties (9).

E) Quantitative estimation of anthraquinone content of flowers of *C. brewsteri* by spectrophotometric method:

i) Calibration curve: Different aliquots amounts (0.5-2.5 ml) of (0.01%) solution of emodin-8-O-β-D-glucopyranoside in MeOH (equivalent to 50-250 µg) were separately transferred to test tubes and evaporated to dryness. The residues obtained were treated with 10ml 10% sodium hydroxide. The absorbance of the developed rose-red colour was measured at λ_{max} 520 nm immediately against blank, prepared in the same way but replacing emodin-8-O- β-D-glucopyranoside solution by MeOH, using UV spectrophotometer. For each concentration, three determinations were carried out and the average of the obtained absorbances was plotted versus the concentration.

ii) Estimation of anthraquinone content:

a) Estimation of free anthraquinone content: 5 gm of the air-dried powdered material was exhaustively extracted with MeOH (70%) by sonication for 30 minutes and repeated till negative Borntrager test. The MeOH extract was filtered and transferred to a volumetric flask 250 ml capacity and completed to volume with MeOH (70%) [ext. A]. From ext.A, 10 ml was evaporated to dryness.The residue was extracted with chloroform untill negative Borntrager test. The chloroformic extract was washed with distilled water, reject washing and adjust the volume to 50 ml in 50-ml volumetric flask [ext. B]. 10 ml of ext. B was transferred to a clean test tube and evaporated to dryness. The residue was dissolved in 10% sodium hydroxide and the procedure continued as mentioned before.

b) Estimation of total anthraquinone content: To 20 ml of ext. B, 10 ml hydrochloric acid was added, heated on boiling water bath under reflux for 30 minutes, cooled, and extracted with successive portions of chloroform till negative Borntrager test. The combined chloroformic extract was washed with distilled water and the washings were rejected. The chloroform solution (free original and free librated after hydrolysis) was completed to 100ml in 100-ml volumetric flask with chloroform. 10ml of the chlororformic solution was transferred to a clean test tube and evaporated to dryness. The residue was dissolved in 10% sodium hydroxide and the procedure continued as mentioned before.

The mean percentage of free and total anthraquinone content, calculated as emodin-8-O-β-D-glucopyranoside in *C. brewsteri* flowers, is deduced from the pre-established calibration curve (10), (11).

F) Quantitative estimation of flavonoid content of flowers of *C. brewsteri* by spectrophotometric method:

(1) Calibration curve: Different aliquots amounts (0.5 - 3 ml) of (0.004%) solution of quercitrin in 95% ethanol (equivalent to 20-120 µg) were separately transferred to test tubes and evaporated on a water bath. The dried residues obtained were, each treated with 5 ml 0.1 M Aluminium chloride. The absorbance of the developed yellow colour was measured at λ_{max} 420 nm immediately against blank, prepared in the same way but replacing quercitrin solution by ethanol (95%), using UV-visible spectrophotometer. For each concentration, three determination were carried out and the average of the obtained absorbances was plotted versus the concentration.

(2) Estimation of flavonoid content:1 gm of the air-dried powdered material was defatted with petroleum ether then exhaustively extracted with ethanol (95%). The ethanolic extract was transferred to a measuring flask 100 ml capacity and completed to volume with ethanol (95%). From the ethanolic extract, 0.5 ml was transferred to a test tube and evaporated. To the residue, 5 ml of 0.1M Aluminium chloride was added and the procedure continued as mentioned before. The mean percentage of flavonoid content, calculated as quercitrin in *C. brewsteri* flowers, is deduced from the pre-established calibration curve (12)

Results and discussion:

Compound 1 (Table 1, 3; Fig.1): obtained as yellow needles, soluble in MeOH ; gives positive Borntrager's test which indicates its anthraquinone nature**; m.p**.: 230 -231 ^{0}C (from MeOH); **TLC:** showed a single spot, at R_f = 0.52 in S1; **Acid hydrolysis** afforded physcion as aglycone of the compound. The sugar moiety was identified as glucose. **EI-MS** showed a molecular ion peak at m/z = 284 ascribed to [aglycone]$^+$ ($C_{16}H_{12}O_5$); **^{1}H-NMR (TMS) δppm**: showed two signal sets of *meta*-coupled aromatic protons; one at δ 7.79 (br s, 1H, **H-4**) and 7.13 (br s, 1H, **H-2**) and the other at δ 7.40 (d, J=2.0 Hz, 1H, **H-5**) and 6.91 (d, J=2.0 Hz, 1H, **H-7**) and two singlet signals for a methoxy and a methyl group at δ 3.90 (s, 3H, OCH_3), 2.42 (s, 3H, CH_3). These signals are characteristic for **physcion** aglycone with additional signals of sugar moiety at 5.01 (d, J = 7.6 Hz, 1H, **H-1'**) and (3.38-3.89, m) indicating β-D-glucoside moiety.**^{13}C-NMR (TMS) δppm**: showed typical 16 carbon signals of physcion and 6 carbon signals of glucose. Accordingly, compound **1** is designated to be **physcion-8-O-β-D-glucopyranoside** (13).

Compound 2 (Table 1, 3; Fig.1): obtained as yellow crystals, soluble in MeOH ; gives positive Borntrager's test which indicates its anthraquinone nature **; m.p**.: 224 - 226 ^{0}C (from MeOH); **TLC:** showed a single spot, at R_f =0.48 in S1; **Acid hydrolysis** afforded aloe-emodin as aglycone of the compound. The sugar moiety was identified as glucose. **EI-MS** showed a molecular ion peak at m/z = 270 ascribed to [aglycone]$^+$ ($C_{15}H_{10}O_5$); **^{1}H-NMR (TMS) δppm**: showed two *meta*-coupled aromatic proton signals at δ 7.65 (d, J=1.7 Hz, 1H, **H-4**) and 7.30 (d, J=1.7 Hz, 1H, **H-2**), three aromatic proton signals at δ 7.89 (dd, J=7.6, 8.3 Hz, 1H, **H-6**) [due to *ortho* coupling between H-6 and H-7, H-5, respectively], 7.85 (dd, J=1.2, 7.6 Hz, 1H, **H-7**) [due to *meta, ortho* coupling between H-7 and H-5, H-6, respectively], 7.70 (dd, J=1.2, 8.3 Hz, 1H, **H-5**) [due to *meta, ortho* coupling between H-5 and H-7, H-6, respectively]. In addition to, one proton signal at δ 5.52 (s, 1H, **OH-3**) and one signal at δ 4.63 (s, 2H, **CH_2-3**) assigned for a hydroxymethylene group. These signals are characteristic for **aloe-emodin** aglycone with additional signals of sugar moiety at 5.17 (d, J = 7.6 Hz, 1H, **H-1')** for an anomeric proton in a β-configuration and (3.21-3.64, m) indicating β-D-glucoside moiety. **^{13}C-NMR (TMS) δppm**: showed typical 15 carbon signals of aloe-emodin and 6 carbon signals of glucose. Accordingly, compound **2** is designated to be **aloe-emodin -8-O-β-D-glucopyranoside** (14), (15), (16), (17), (18).

Compound 3 (Table 1, 3; Fig.1): obtained as orange needles, soluble in MeOH; gives positive Borntrager's test which indicates its anthraquinone nature **; m.p**.: 188-189^{0}C (from MeOH); **TLC:** showed a single spot at R_f =0.38 in S1; **Acid hydrolysis** afforded emodin as aglycone of the compound. The sugar moiety was identified as glucose. **EI-MS** showed a molecular ion peak at m/z = 270 ascribed to [aglycone]$^+$ ($C_{15}H_{10}O_5$). **^{1}H-NMR (TMS) δppm**: exhibited two signal sets of *meta*-coupled aromatic protons; one at δ 7.65 (s, 1H, **H-4**) and 6.85 (s, 1H, **H-2**) and the other at δ 7.32 (d, J=2.0 Hz, 1H, **H-5**) and 7.18 (d, J=2.0 Hz, 1H, **H-7**) and one singlet signal for a methyl group at δ 2.40 ppm. These signals are characteristic for **emodin** aglycone with additional signals of sugar moiety at 4.90 (d, J = 7.6 Hz, 1H, **H-1')** and (3.25-3.87, m) indicating β-D-glucoside moiety.**^{13}C-NMR (TMS) δppm**: showed typical 15 carbon signals of emodin and 6 carbon signals of glucose (19). Accordingly, the structure for compound **3**, was designated as **emodin-8-O-β-D-glucoside.**

Compound 4 (Table 1, 3; Fig.1): obtained as yellow powder, soluble in MeOH; gives positive Borntrager's test which indicates its anthraquinone nature **; m.p**.: 197-199^{0}C (from MeOH); **TLC:** showed a single spot at R_f =0.27 in S1; **Acid hydrolysis** afforded rhein as aglycone of the compound. The sugar moiety was identified as glucose. **EI-MS** showed a molecular ion peak at m/z = 284 ascribed to [aglycone]$^+$ (C_{15} H_8 O_6). **^{1}H-NMR (TMS) δppm**: showed two *meta*-coupled aromatic proton signals at δ 8.25 (s, 1H, **H-4**) and 7.86 (s, 1H, **H-2**), three aromatic proton signals at δ 7.71 (dd, J=7.4, 7.3 Hz, 1H, **H-6**) [due to *ortho* coupling between H-6 and H-7, H-5, respectively], 7.84 (dd, J=1.5, 7.4 Hz, 1H, **H-7**) [due to *meta, ortho* coupling between H-7 and H-5, H-6, respectively], 7.91 (dd, J=1.5, 7.3 Hz, 1H, **H-5**) [due to *meta, ortho* coupling between H-5 and H-7, H-6, respectively]. These signals are characteristic for **rhein** aglycone with additional signals of sugar moiety at 5.08 (d, J = 7.5 Hz, 1H, **H-1')** for an anomeric proton in a β-configuration and (3.10-3.72, m) indicating β-D-glucoside moiety. **^{13}C-NMR (TMS) δppm**: showed typical 15 carbon signals of rhein and 6 carbon signals of glucose (18), (20), (21). Accordingly, the structure for compound **4**, was designated as **rhein-8-O-β-D-glucoside.**

Compound 5 (Table 2; Fig.2): obtained as yellow powder, soluble in MeOH**; m.p**.: 177-179^{0}C (from MeOH); **TLC:** showed a single spot at R_f =0.7 in S2; **Acid hydrolysis** afforded quercetin as aglycone of the compound. The sugar moiety was identified as glucose. **EI-MS** showed a molecular ion peak at m/z = 302 ascribed to [aglycone]$^+$ (C_{15} H_{10} O_7). **^{1}H-NMR (TMS) δppm**: showed a sharp singlet signal at δ 11.98 for protons of four hydroxyl groups, a multiplet signal at δ 7.65 for H-2' and 6' due to coupling with each other and with H-5', a doublet signal at δ 6.84

with (J=8.1Hz) for H-5' due to ortho-coupling with H-6' of ring B. In addition to two doublet signals at δ 6.41 with (J=2.1Hz) for H-8 and at δ 6.21 with (J=2.1Hz) for H-6 of ring A. These signals are characteristic for **quercetin** aglycone [3, 5, 7, 3', 4' hydroxyl derivative (absence of 3, 5, 7, 3', and 4' protons)] with additional signals of sugar moiety at 4.61 ppm (H-1" with J1"*2" equatorial, s) and 1.10 ppm (3 protons of methyl, doublet with J=5.7Hz) indicated α-rhamnose protons (22), (23). Accordingly, the structure for compound **5**, was designated as **quercitrin.**
In addition to the presence of astragalin, rutin which were previously isolated from the leaves (5).

Quantitative estimation of anthraquinone content of flowers of C. brewsteri by spectrophotometric method: revealed that free and total anthraquinones content in the flower extract were found to be (0.0343 g%) and (0.062 g%) respectively calculated as emodin-8-O-β-D-glucopyranoside.

Table (1): ^{1}H-NMR spectral data of compounds [1-4] (300.0 MHz, DMSO-d_6):

δ ppm proton No.	Compound			
	1	**2**	**3**	**4**
1	-	-	-	-
2	7.13 (br s, 1H)	7.30 (d, J=1.7 Hz, 1H)	6.85 (s, 1H)	7.86 (s)
3	-	-	-	
4	7.79 (br s, 1H)	7.65 (d, J=1.7 Hz, 1H)	7.65 (s, 1H)	8.25 (s)
5	7.40 (d, J=2.0 Hz, 1H)	7.70 (dd, J=1.2, 8.3 Hz, 1H)	7.32 (d, J=2.0 Hz, 1H)	7.91 (dd, J=1.5, 7.3 Hz, 1H)
6	-	7.89 (dd, J=7.6, 8.3 Hz, 1H)	-	7.71 (dd, J=7.4, 7.3 Hz, 1H)
7	6.91 (d, J=2.0 Hz, 1H)	7.85 (dd, J=1.2, 7.6 Hz, 1H)	7.18 (d, J=2.0 Hz, 1H)	7.84 (dd, J=1.5, 7.4 Hz, 1H)
CH_3-3	2.42 (s, 3H, methyl)	-	2.40 (s, 3H, methyl)	-
CH_2-3	-	4.63 (s, 2H)	-	-
OCH_3-6	3.90 (s, 3H, methoxy)			-
1'	5.01 (d, J = 7.6 Hz, 1H)	5.17 (d, J = 7.6 Hz, 1H)	4.90 (d, J = 7.6 Hz, 1H)	5.08(d, J = 7.5 Hz, 1H)
Sugar protons	3.38-3.89 (m)	3.21-3.64 (m)	3.25-3.87 (m)	3.10-3.72 (m)
OH-1	12.32(br s, 1H)	12.87(br s, 1H)	12.95 (br s, 1H)	12.62(br s, 1H)
OH-3	-		12.07 (br s, 1H)	-
CH_2OH-3	-	5.52 (s, 1H)	-	-

Quantitative estimation of flavonoid content of flowers of C. brewsteri by spectrophotometric method: revealed that the percentage of flavonoid calculated as quercitrin was found to (0.195 g%) .

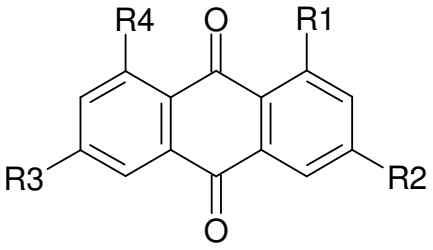
It is interesting to note that all the previous compounds were isolated and identified for the first time from C. brewsteri although it has been isolated from another Cassia species.

Table (2): ^{1}H-NMR spectral data of compounds [5] (300.0 MHz, DMSO-d_6):

δ ppm proton No.	Compound 5
6	6.21(d,J=2.1Hz, 1H)
8	6.41 (d, J=2.1Hz, 1H)
5'	6.84 (d J=8.1Hz, 1H)
2',6'	7.65 (m, 2H)
1"	4.61(s, 1H)
rhamnosyl	3.63-4.02 (m)
6"	1.10 (d,H=5.7Hz, 3H)

Table (3): ^{13}C-NMR spectral data of compounds [1- 4] (75.0 MHz, DMSO-d_6):

δ ppm carbon No.	Compound			
	1	2	3	4
1	163.5	162.98	162.44	162.76
2	121.8	116.11	122.12	123.55
3	149.8	151.66	145.39	142.77
4	124.7	120.56	120.38	123.10
5	106.9	120.94	108.23	120.98
6	162.4	136.21	164.51	136.27
7	106.6	115.82	108.43	118.35
8	161.06	159.43	161.78	160.20
9	186.9	187.44	185.67	188.57
10	181.2	183.72	180.54	181.23
11	136,8	133.45	110.97	135.58
12	107.6	122.85	134.67	117.04
13	114.22	115.88	113.10	121.59
14	131.20	134.52	131.63	132.43
CH_3	21.70	-	21.37	-
CH_2-OH	-	60.38	-	-
OCH_3	55.8			-
COOH	-	-	-	167.91
1'	101.12	101.50	101.40	101.22
2'	73.80	72.94	73.29	73.58
3'	77.45	76.01	76.34	77.81
4'	70.11	69.98	69.59	69.42
5'	76.99	77.81	77.70	77.80
6'	63.19	62.54	60.41	61.07



Compound	R1	R2	R3	R4
physcion-8-O- β-D-glucopyranoside	**OH**	**CH_3**	**OCH_3**	**O-gl**
aloe-emodin -8-O- β-D-glucopyranoside	**OH**	**CH_2OH**	**H**	**O-gl**
emodin-8-O-β-D-glucoside	**OH**	**OH**	**CH_3**	**O-gl**
rhein-8-O-β-D-glucoside	**OH**	**COOH**	**H**	**O-gl**

Figure (1) Anthraquinone glycoside

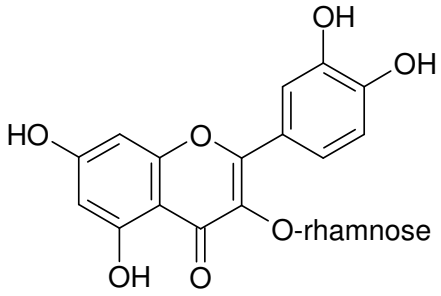


Figure (2) Quercitrin

Conclusion:

Five known glycoside: physcion-8-O-β-D-glucopyranoside, aloe-emodin -8-O-β-D-glucopyranoside, emodin-8-O-β-D-glucopyranoside, rhein-8-O-β-D-glucopyranoside and quercitrin were isolated for the first time from the flowers of *Cassia brewsteri* (F.Muell.) Benth. Free and total anthraquinone content in the flowers extract were found to be (0.0343 g%) and (0.062 g%) respectively when determined spectrophotometrically as emodin-8-O-β-D-glucopyranoside. Flavonoids content in the flowers was found to be (0.195 g%) when determined spectrophotometrically as quercitrin.

Acknowledgments:

I would like to acknowledge and extend my heartfelt gratitude to Mr. Mostafa El Abd who donates the plant.

References:

(1) Anu S.J., and Rao J.M. Oxanthrone esters from the aerial parts of *Cassia kleinii*. Phytochemistry. 2001; 57: 583-585.

(2) Ingkaninan K., Ijzerman A.P., Verpoorte R. Luteolin, a compound with Adenosine A1, receptor-binding activity, and chromone and dihydronaphthalenone constituents from *Cassia siamea*. J.Nat.Prod. 2000; 63: 315-317.

(3) Kitanaka S., and Takido M. (S)-5,7'-Biphyscion 8-glucoside from *Cassia torosa*. Phyochemistry.1995; 39: 717-718.
(4) Cunningham D.C., Anderson E.R., Walsh K.B.. Ecology and biogeography of *Cassia brewsteri*: assessment of potential sites for cultivation. Austr. J. Exp. Agric. 2002; 42: 1071-1080.
(5) Selim A.M., El Shabrawy O.A., Abou Zeid F.S., Owis I.A..Tannin and flavonoid compounds from *Cassia brewsteri* growing in Egypt. Egypt.J.Biomed.Sci. 2010; 33: 196-208.
(6) Stahl E.. Thin Layer Chromatography. 2nd Ed. Springer-Verlag: Berlin, 1969.
(7) Thomson R.H.. Naturally Occurring Quinones. 2nd Ed.. Academic Press: London and New York, 1971.
(8) Mabry T.J., Markham K.R., Thomas M.B.;.The Systematic Identification of Flavonoids. Springer-Verlag: Berlin, 1970.
(9) Harborne J.B., Mabry T.J., Mabry H.. The Flavonoids. Acad. Press, Inc.: New York, San Francisco, Chapman and Hall, London, 1975.
(10) Fluck A.A.J., Mitchell W., Wood S.A.. The chemical assay of *Cascara.* J.Pharm.Pharmacol. 1956; 18: 100.
(11) Kussmaul W., and Becker A.. Die kolorimetrische Bestimmung der Sennaglucoside. Chem.Acta. 1947; 30: 59.
(12) Karawya M.S., and Aboutabl E.A.. Phytoconstituents of *Tabernacemontana cornaria* Jac Q. Willd and *Dichotoma roxb.* growing in Egypt. Part IV: The flavonoids. Bull.Fac.Pharm. Cairo Univ. 1982; 21: 41-49 .
(13) Zhang C., Wang R., Liu B., Tu G. Structure elucidation of a sodium salified anthraquinone from the seeds of *Cassia obtusifolia* by NMR technique assisted with acid-alkali titration. Magn.Reson.Chem. 2011; 49: 529–532.
(14) Fairbairn J.W., and Shrestha A.B. Aloe-emodin glycosides of *Senna* leaf. J.Pharm.Pharmacol. 1966; 18: 467-470.
(15) Kinjo J., Ikeda T., Watanabe K., Nohara T.. An anthraquinone glycoside from *Cassia angustifolia* leaves. Phytochemistry. 1994; 37: 1685-1687.
(16) Nakajima K., Yamauchi K., Kuwano S.. Isolation of a new aloe-emodin dianthrone diglucoside from *Senna* and its potentiating effect on the purgative activity of sennoside A in mice. J.Pharm.Pharmacol. 1985; 37: 703-706 .
(17) Romanova A.S., Ban'kovstii A.I., Semakina N.D., Meshcheryakov A.A.. Anthracene derivatives of *Cassia acutifolia*. Lek.Rast. 1969; 15: 524-528.
(18) Romanova A.S., Semakina N.D., Ban'kovstii A.I.. Anthracene derivatives isolated from the pod valves of *Cassia acutifolia*. Rast.Rsur. 1968; 4: 342-343.
(19) Kalashnikova G.K., Romanova A.S., S-chavlinskii A.N.. Anthracene derivatives from the roots of *Cassia acutifolia*. Khim.Farm.Zh. 1985; 19: 569-573.
(20) Crellin J.K., Fairbairn J.W., Friedmann C.A., Ryan H.A. New glycosides from *Senna*. J. Pharm. Pharmacol. 1961; 13 : 639-640.
(21) Duquenois P., and Anton R.. Chemical study of *Cassia sieberiana* leaves. Planta med. 1968; 16: 184-190.
(22) Ilyas M., Parveen M., Khan M.S.. Flavonoids of *Cassia nodosa.* Fitoterapia. 1995; 66: 227-228 .
(23) Mishra A., and Ansari M.H.. Phytochemical investigation and anthelmintic activity of the leaf extract of *Cassia obtusifolia.* Plant archives 2007; 7: 241-243.