Chemical Constituents and Cytotoxic activity of Cassia *glauca* Lan. Leaves

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Abstract: The defatted 85 % methanolic extract from the leaves of *Cassia gluaca* Lan. (Family Leguminaceae) showed high cytotoxic effect against liver carcinoma cell line (HepG2) (IC_{50} = 17 µg /ml). Therefore this extract was fractionated using different organic solvents; chloroform, ethyl acetate and *n*-butanol. Each fraction was submitted to chromatographic separation and the structure of the isolated compounds were elucidated using physical properties and certain spectroscopic analysis. From the chloroform fraction, three compounds; Di-(2-ethylhexyl) phthalate (DEHP) **(1),** apigenin **(2)** and luteolin **(3)** were isolated. Quercetin **(4)**, quercetin-3-*O*-β-D-glucopyranoside **(5)** and kaempferol-3-*O*-rutinoside **(6)** were isolated and identified from ethyl acetate fraction whereas from the *n*-butanol fraction three compounds were identified as D (+)-pinitol **(7),** quercetin-3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (Rutin) **(8)** and quercetin-3-*O*-[α-L-rhamnopyronosyl-(1→2)-α-L-rhamnopyronosyl-(1→6)-]-β-D-glucopyronoside **(9).** The cytotoxicity of the isolated compounds **5, 6** and **9** was evaluated against HepG2 cell lines. The results showed that the three compounds **5, 6** and **9** have promising cytotoxicity with IC_{50} = 16.1, 14.3 and 15.2 µg/ml respectively. From the above results and our previous reports, the methanol extract of the leaves of *Cassia glauca* could be used as promising antioxidant and anticancer agent after more *in vitro* and *in vivo* studies.
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1. Introduction

Cancer is one of the most dangerous diseases in humans and presently there is a considerable scientific discovery of new anticancer agents. One possible solution is to explore the potential antioxidant and anticancer properties of plant extracts or isolated products of plant origin **(Namiki**, **1990; Qiu *et al.*, 2009**; **Woraratphoka *et al.*, 2012).** It is well known that many polyphenolic compounds, such as phenolic acids, flavonoids, anthocyanidins, and tannins, which possess remarkable antioxidant and anticancer activities, are rich in plant materials **(Mohsen & Ammar, 2009; Khan *et al.*, 2012**; **Saeed *et al.*, 2012**). Many studies showed that there is a positive correlation between the total phenolic compounds in the plant material and their antioxidant and anticancer properties **(Noyeem & Karnekar 2010; El-Sayed *et al.*, 2011; Saeed *et al.*, 2012; Eman *et al.*, 2013)**. In our previous studies **(El-Hashash *et al.*, 2010**; **El-Sayed *et al.*, 2011**) it has been found the methanolic extract of *Cassia glauca* Lan. (Family Leguminaceae) and certain fractions derived from it have high total phenolic and flavonoid contents as well as they showed high antioxidant activity. To our best knowledge, no previous investigation has been done on the isolation of chemical constituents and cytotoxic activity of the compounds isolated from this plant. Therefore, the aim of this study was to chromatographic separation and investigation the cytotoxicity effect of the crude extract and some isolated flavonoids from *C. glauca.*

2. Materials and Methods

2.1. General experimental procedures

Melting points were determined on an electrothermal apparatus (Electrothermal 9200). The nuclear magnetic resonance as ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Brucker Avance-500 and JEOL GX-spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C). The chemical shifts were expressed in δ (ppm) with TMS as reference and coupling constant (*J*) in Hertz. UV spectra (λ_{max}) of the isolated compounds were determined in methanol before and after addition of different reagents on Perkin Elmer UV/Vis spectrophotometer. Mass spectra were measured using Micromass Q-TOF Micro instrument. Infra red analysis was performed using IR spectrometer JASCO, FT/IR-6100 LE, USA. Silica gel 60 (70-230 mesh, Merck) and Sephadex LH-20 (25-100 µm, Sigma) were used for column chromatography. Analytical and preparative thin layer chromatographies (TLC) were performed on silica gel GF_{254} pre-coated plates (Merck). Paper

chromatography (PC) was carried out on Whatmann No. 1 or No. 3 paper sheets. Spots were visualized by absorption of UV radiation and spraying with ethanolic $AlCl_3$ (2%), $FeCl_3$ (1%) and 10% H_2SO_4 followed by heating for flavonoids phenolic compounds and triterpenoids.

2.2. Plant Materials

The leaves of *Cassia glauca* (Syn. *Senna surattensis*) were collected from Garden of Faculty of Agriculture, Cairo University, Giza, Egypt. They were authenticated by Mrs. Treaza Labib, high specialist of plant taxonomy, Department of flora and taxonomy, Orman Botanical Garden Giza, Egypt. Voucher specimen of the plant was deposited at laboratory of medicinal chemistry, Theodor Bilharz Research Institute, Giza, Egypt. The collected plant leaves were dried in shad and finally powdered by electric mill to give 1.6 Kg.

2.3. Extraction and Fractionation

A total of 1.6 kg of air-dried powder of the leaves of *C. glauca* was extracted by 85% aqueous methanol on cold till exhaustion. The solvent was distilled using rotatory evaporator under vacuum till dryness. The dried extract (450 g) was defatted with petroleum ether (60-80 °C) and the aqueous defatted methanolic extract (400 g) was successively partitioned with chloroform, ethyl acetate and *n*-butanol. The obtained extracts were evaporated under reduced pressure to dryness. The chloroform, ethyl acetate and butanolic extracts (23, 32 and 38 g) were kept for chromatographic separation process.

2.3.1. Chromatographic Separation of Chloroform Extract

The chloroform extract (20 g) was submitted to column chromatography (80 × 4 cm) packed with silica gel 60 (70-230 mesh, Merck) as stationary phase. Elution started with pure chloroform followed by chloroform/methanol gradient. Fractions 150 ml were collected, concentrated and examined by TLC (Silica gel, solvent system $CHCl_3$:MeOH:H_2O; 7:3:0.5) and PC using solvent system (*n*-BuOH:AcOH: H_2O; 4:1:5). Fractions eluted with $CHCl_3$:MeOH (90:10) were evaporated and purified on preparative TLC with solvent system (Toluene:$CHCl_3$; 7:3) to gave compound **1** as a colorless oily liquid. Fractions eluted with $CHCl_3$:MeOH (85:15) were concentrated and subjected to re-chromatography on a Sephadex LH-20 column with methanol as the eluting solvent to obtain compounds **2** and **3**.

Compound 1 was obtained as a colorless oily liquid. It is soluble in chloroform, ethyl acetate and ethanol but insoluble in water. It appeared as a single spot on TLC with R_f value = 0.6 (solvent system: Toluene:$CHCl_3$; 7:3). IR υ_{max} (KBr): 3430.42, 2930.31, 2866.67, 2734.57, 1944.88, 1729.83, 1481.78, 1381.76 1279.64, 1126.22, 1072.23, 956.62 and 743.42 cm^{-1}. ^{1}H-NMR δ: 7.67 (2H, dd, J = 7.5 Hz, 1.55 Hz), 7.48 (2H, dd, J = 7.5 Hz), 7.67 (2H, dd, J =7.50 Hz, 1.55 Hz), 4.21 (4H, d, J = 6.1 Hz), 1.66 (2H, m), 1.32 (2H, m), 1.29 (2H, m), 0.89 (3H, t) and 0.89 (3H, t). ^{13}C-NMR δ: 132.53 (C-1, C-2), 128.85 (C-3), 130.94 (C-4), 128.85 (C-6), 167.78 (C-7, C-8), 68.17 (C-1′), 38.81 (C-3′), 30.44 (C-2′), 28.99 (C-4′), 23.05 (C-5′), 23.82 (C-7′), 14.10 (C-6′) and 11.02 (C-8′).

Compound 2 was obtained as pale yellow, its m.p. 348-350 ˚C, R_f values = 0.17 (15% Acetic acid, PC), 0.8 (*n*-BuOH:AcOH:H_2O; 4:1:5, PC). UV λ_{max} (MeOH) 268, 295, 339; (MeOH+NaOMe) 274, 330, 391; (MeOH+$AlCl_3$) 275, 298, 386; (MeOH+ $AlCl_3$+HCl) 276, 343, 385; (MeOH+NaOAc) 275, 321, 393; (Me+NaOAc +H_3BO_3) 270, 299, 357.

Compound 3 was isolated as yellow powder, m.p. 328-330 °C, R_f values = 0.09 (15% AcOH, PC), 0.76 (*n*-BuOH:AcOH:H_2O; 4:1:5, PC) and 0.26 ($CHCl_3$:MeOH:H_2O; 7:3:0.5, TLC). UV λ_{max}(MeOH) 267, 296, 355; (MeOH+NaOMe) 270, 347, 409; (MeOH+$AlCl_3$) 272, 314, 418; (MeOH+ $AlCl_3$+HCl) 275, 324, 382; (MeOH+ NaOAc) 273, 321, 395; (Me+ NaOAc+H_3BO_3) 268, 306, 370.

2.3.2. Chromatographic Isolation of EtOAc Fraction

Ethyl acetate extract of *C. glauca* (30 g) was subjected to column chromatography (120×6 cm) packed with silica gel 60 (70-230 mesh, Merck) as stationary phase. Elution started with $CHCl_3$ and then $CHCl_3$/MeOH gradient and ending with pure methanol. Fractions 250 ml each were collected, concentrated and examined by TLC (silica gel, solvent system $CHCl_3$:MeOH:H_2O; 7:3:0.5) and by PC (Whatmann No. 1, using solvent system 15% AcOH). Chromatograms were visualized under UV before and after exposure to NH_3 and spraying with $AlCl_3$. Three major fractions were obtained. Fractions eluted with 95% $CHCl_3$ were concentrated and purified with preparative PC using solvent system 15% AcOH to give compound **4**. Fractions eluted with 85% $CHCl_3$ re-chromatography over Sephadex LH-20 column using H_2O/MeOH as eluent to give compound **5**. Fractions eluted by 70% $CHCl_3$ were subjected to re-chromatography on silica gel column, elution started with $CHCl_3$, mixture $CHCl_3$:MeOH and ending with pure methanol. Compound **6** was obtained from fractions eluted with 80% $CHCl_3$ by purification on preparative PC (solvent system 15% AcOH)

Compound 4 was obtained as yellow powder, m.p. 314-316 ˚C, R_f values = 0.08 (15% AcOH, and 0.66 (*n*-BuOH:AcOH:H_2O; 4:1:5, PC). UV λ_{max} (MeOH) 256, 269, 371; (MeOH+NaOMe) 273, 330, 415; (MeOH+$AlCl_3$) 274, 305, 456; (MeOH+$AlCl_3$+ HCl) 269, 354, 426; (MeOH+NaOAc) 276, 319, 391; (Me+NaOAc+H_3BO_3) 260, 302, 387.

Compound 5 was obtained as yellow powder, m.p. 235-238 ˚C, R_f values = 0.56 (15% AcOH, PC), 0.47 (*n*-BuOH:AcOH:H_2O; 4:1:5, PC) and 0.5 ($CHCl_3$: MeOH:H_2O; 7:3:0.5, TLC). UV λ_{max} (MeOH) 256, 292, 360; (MeOH+NaOMe) 274, 327, 405; (MeOH+$AlCl_3$) 272, 305, 420; (MeOH+ $AlCl_3$+HCl) 269, 361, 389; (MeOH+NaOAc) 270, 328, 395; (Me+NaOAc+H_3BO_3) 262, 298, 385. ^{1}H-NMR δ: 12.60, 7.53 (2H, dd, *J* = 2.1, 8.4 Hz, H-2$^/$, H-6$^/$), 6.81(1H, d, *J* = 8.5 Hz, H-5$^/$), 6.36 (1H, d, *J* = 1.75 Hz, H-8), 6.15 (1H, *J* = 1.9 Hz, H-6) and 5.42 (1H, d, *J* = 7.5, H-1$^{//}$).

Compound 6 was isolated as yellow amorphous powder m.p. (163-164 ˚C), R_f value = 0.66 (15% AcOH) and 0.35 (*n*-BuOH:AcOH: H_2O; 4:1:5). UV λ_{max} (MeOH) 258, 321, 357; (MeOH+NaOMe) 273, 345, 410; (MeOH+$AlCl_3$) 274, 328, 405; (MeOH+ $AlCl_3$+HCl) 270, 303, 400; (MeOH+NaOAc) 273, 310, 392; (Me+NaOAc+H_3BO_3) 262, 307, 376. ^{1}H-NMR δ: 12.55 (1H, s, 5-OH), 6.22 (1H, d, *J* = 2.1 Hz, H-6), 6.43 (1H, d, *J* = 2.0 Hz, H-8), 7.99 (2H, d, *J* = 8.75 Hz, H-2$^/$,H-6$^/$), 6.88 (2H, d, *J* = 8.9 Hz, H-3$^/$,H-5$^/$), 5.32 (1H, d, *J* = 6.90 Hz, H-1$^{//}$, Glc), 4.30 (1H, d, *J* = 1.24 Hz, H-1$^{///}$, Rha) and 1.03 (3H, d, *J* = 6.25 Hz, Rha-6$^{///}$). ^{13}C-NMR δ: 177.75 (C-4), 164.53 (C-7), 161.54 (C-5), 160.28 (C-4$^/$), 157.20 (C-9), 156.84 (C-2), 133.59 (C-3), 131.21 (C-2$^/$, C-6$^/$), 121.23 (C-1$^/$), 115.46 (C-3$^/$, C-5$^/$), 104.34 (C-10), 101.70 (C-1$^{//}$), 101.12 (C-1$^{///}$), 99.09 (C-6), 94.11 (C-8), 74.54 (C-2$^{//}$), 76.11 (C-3$^{//}$), 72.20 (C-4$^{//}$), 76.75 (C-5$^{//}$), 67.25 (C-6$^{//}$), 70.70 (C-2$^{///}$), 70.30 (C-3$^{///}$), 70.98 (C-4$^{///}$), 68.59 (C-5$^{///}$), 18.07 (C-6$^{///}$).

2.3.3. Chromatographic Isolation of Butanolic Extract

Butanolic extract of *C. glauca* (35g) was chromatographed on Silica gel 60 (70-230 mesh, Merck) column (6×120 cm). Elution was started with $CHCl_3$ and then with $CHCl_3$/MeOH gradient. Fractions 250 ml each were collected, concentrated and examined by PC (Whatmann No. 1 and 3) with solvent systems (*n*-BuOH:AcOH:H_2O; 4:1:5 and 15% AcOH). Chromatograms were visualized under UV before and after exposure to NH_3 and spraying $AlCl_3$. Fractions eluted with pure $CHCl_3$ were purified on preparative silica gel TLC using solvent system ($CHCl_3$:MeOH; 7:3) to give compound **7**. Fractions eluted with $CHCl_3$:MeOH (95:5) were re-chromatographed on silica gel column. Elution started with $CHCl_3$ followed by $CHCl_3$:MeOH mixtures and finished with pure MeOH. Two major fractions I ($CHCl_3$:MeOH 70: 30) and II ($CHCl_3$:MeOH; 60:40) were obtained. Fraction I was subjected on column Sephadex LH-20 and eluting with aqueous methanol to give compound **8** whereas fraction II was subjected to preparative PC using solvent system (*n*-BuOH: AcOH:H_2O; 4:1:5 and 15 % AcOH) to give compound **9.**

Compound (7) was obtained as a white crystal with m.p. 185-186 ˚C, R_f values = 0.42 ($CHCl_3$: MeOH; 7:3, TLC) and 0.61(*n*-BuOH:AcOH:H_2O; 4:1:5, PC). IR υ_{max}(KBr): 3405.07, 2907.10, 1511.92, 1465.03, 1382.71, 1343.19, 1280.50, 1132.97, 860.09, 750.17, 572.75 cm^{-1}. ^{1}H-NMR δ: 4.69 (1H, m), 4.59 (1H, m, H-2), 4.47 (1H, t, *J* = 9.3 Hz, H-4), 4.43 (1H, t, *J* = 9 Hz, H-3) and 3.57 (3H, s, OCH_3). ^{13}C-NMR δ: 84.28 (C-3), 73.09 (C-1), 72.91 (C-5), 72.45 (C-6), 71.42 (C-4, C-2), 60.09 (O-CH_3).

Compound (8) was isolated as yellow powder, m.p 198-200 ˚C, R_f values = 0.68 (15% ACOH, PC), 0.32 (*n*-BuOH:AcOH:H_2O; 4:1:5, PC) and 0.38 (*n*-BuOH:AcOH:H_2O; 4:1:1, TLC). UV λ_{max} (MeOH) 254, 259, 356; (MeOH+NaOMe) 268, 334, 410; (MeOH+$AlCl_3$) 270, 312, 420; (MeOH+$AlCl_3$+ HCl) 269, 352, 409; (MeOH+NaOAc) 271, 312, 399; (Me+NaOAc+H_3BO_3) 265, 296, 380. ^{1}H-NMR δ: 12.55 (1H, s, 5-OH), 7.51(1H, d, *J* = 2.0 Hz, H-2$^/$), 7.49 (1H, dd, *J* = 8.4, 2.1 Hz, H-6$^/$), 6.81 (1H, d, *J* = 8.4 Hz, H-5$^/$), 6.35 (1H, d, *J* = 2.1 Hz, H-8), 6.16 (1H, d, *J* = 2.1 Hz, H-6), 5.31(1H, d, *J* = 6.85 Hz, H-1$^{//}$, Glc), 4.34 (1H, d, *J* = 1.25 Hz, H-1$^{///}$, Rha), 0.95 (3H, d, *J* = 6.1 Hz, Rha-6$^{///}$). ^{13}C-NMR δ: 177.88 (C-4), 164.61 (C-7), 157.14 (C-5), 156.94 (C-9, C-2), 148.92 (C-4$^/$), 145.25 (C-3$^/$), 122.11 (C-1$^/$), 121.69 (C-6$^/$), 116.77 (C-5$^/$), 115.74 (C-2$^/$), 104.47 (C-10), 101.67 (C-1$^{//}$), 99.21 (C-6), 94.13 (C-8), 76.53 (C-5$^{//}$), 76.39 (C-3$^{//}$), 74.58 (C-2$^{//}$), 72.35 (C-4$^{//}$), 71.06 (C-4$^{///}$), 70.88 (C-2$^{///}$), 70.50 (C-3$^{///}$), 68.76 (C-5$^{///}$), 67.85 (C-6$^{///}$), 18.24 (C-6$^{///}$).

Compound (9) was obtained as yellow amorphous powder, m.p. 227- 229 ˚C, R_f values = 0.89 and 0.24 (solvent systems; (15% AcOH, PC) and (*n*-BuOH: MeOH:H_2O; 4:1:5, PC) respectively. UV λ_{max} (MeOH) 258, 276, 360; (MeOH+NaOMe) 279, 331, 408; (MeOH+$AlCl_3$) 271, 304, 428; (MeOH+ $AlCl_3$+HCl) 277, 305, 389; (MeOH+NaOAc) 274, 327, 388; (Me+NaOAc+H_3BO_3) 266, 304, 379. ^{1}H-NMR δ: 12.63 (1H, s, 5-OH), 7.55 (1H, d, *J* = 2.3 Hz, H-2$^/$), 7.49 (1H, dd, *J* = 8.4, 2.2 Hz, H-6$^/$), 6.85 (1H, d, *J* = 8.4 Hz, H-5$^/$), 6.39 (1H, d, *J* = 2.1 Hz, H-8), 6.19 (1H, d, *J* = 2.1 Hz, H-6), 5.54 (1H, d, *J* = 7.7 Hz, H-1$^{//}$, Glc), 5.14 (1H, d, *J* = 1.5 Hz, H-1$^{///}$, Rha), 4.37 (1H, d, *J* = 1.55 Hz, H-1$^{////}$, Rha), 0.98 (3H, d, *J* = 6.1 Hz, CH_3), 0.82 (3H, d, *J* = 6.15 Hz, CH_3). ^{13}C-NMR δ: 177.69 (C-4), 164.63 (C-7), 161.70 (C-5), 157.15 (C-9), 156.85 (C-2), 148.81 (C-4$^/$), 145.27 (C-3$^/$), 133.18 (C-3), 122.06 (C-6$^/$), 121.69 (C-1$^/$), 116.59 (C-5$^/$), 115.66 (C-2$^/$), 101.27 (C-1$^{//}$), 100.99 (C-1$^{///}$), 99.17 (C-1$^{////}$), 77.69 (C-2$^{//}$), 77.63 (C-3$^{//}$), 76.21 (C-5$^{//}$), 68.72 (C-5$^{///}$), 67.56 (C-6$^{//}$), 72.36 (C-4$^{////}$), 72.31 (C-4$^{///}$),71.05 (C-3$^{///}$), 70.84 (C-2$^{////}$), 68.72 (C-5$^{////}$), 18.17 (C-6$^{////}$), 17.70 (C-6$^{///}$).

2.4. Cytotoxic Assay

The cytotoxic activity of the defatted methanolic extract of C. *gluaca* and three isolated compounds toward human liver carcinoma cell lines (HepG2) were carried out according to the reported method by **Skehan *et al.* (1990)** at National Cancer Institute, Cairo University, Egypt**.** The cells were cultured in RPMI-160 media supplemented with 10% heat inactivated fetal bovine serum (FBS), 10% (w/v) Penicillin/ Streptomycin and 1 % glutamine at 37 $^{\circ}$C under 5 % CO_2 in air in a humidified incubator at 37 $^{\circ}$C with 5% CO_2. Briefly, cells were seeded at a density of 5×104 cells/well in 96-well plates. After 24 h, serial dilutions of samples and standard drug (Methotrexate) solutions were added for different concentrations (0, 10, 25, 50 and 100 µg/ml) and incubation of the plate was continued for 24, 48 and 72 hours left without treatment. For cell fixation, the culture medium was removed and trichloroacetic acid (50%, 50 µL) was added in each plate. Then the plates were air-dried and 0.4% SRB (Sigma) in 1% acetic acid was added for 30 min and unbound dye was washed out with 1% acetic acid. After air-drying, SRB dye within cells were dissolved with 100 µL solution of tris-base 10mM (pH 10.5). The optical density of the extracted SRB dye was measured with a microplate reader at 564nm with an ELIZA micro-plate reader. The experiment was repeated three times for each cell line. The 50% inhibitory concentration (IC_{50}) of the test samples was calculated from the prism program obtained by plotting the percentage of surviving cells versus the concentrations interpolated by cubic spine.

3. Results and Discussion

According to the American National Cancer Institute (NCI) guidelines, an extract with IC_{50} values less than 20 µg/ml is considered active **(Boyed, 1997).** In the present study, the 85% methanol extract of the leaves of *Cassia glauca* exhibited high cytotoxic potential with IC_{50} value of 17 µg/ml against HepG2 using SRB assay. This result fall within the NCI criteria, thus this extract is considered as of promising anticancer agent. Thus, fractionation of the methanolic extract of *C. glauca* with chloroform, ethyl acetate and *n*-butanol was carried out. Each of these fractions was submitted to chromatographic isolation. Three compounds **1-3** were isolated from the chloroform fraction, and compounds **4-6** were isolated from the ethyl acetate fraction whereas compounds **7-9** were isolated from the butanolic fraction. The chemical structures of the isolated compounds (Figure 1) were elucidated as follows:-

Compound (1) was obtained as a colorless oily liquid. It is soluble in chloroform, ethyl acetate and ethanol but in soluble in water. The IR spectrum of this compound showed a characteristic peak of the carbonyl group (C=O) at 1729.83 cm^{-1}. Other peaks appeared at 2930.31 cm^{-1} (-CH_2 stretch), 2866.67 cm^{-1} (-CH_3 stretch), 1461.78 and 743.42 cm^{-1} (methylene C-H bend and $(CH_2)_n$-rocking) respectively and 1126.22 cm^{-1} (C-O stretch) **(Rao *et al.*, 2000; Lyuts-Kanova *et al.*, 2009).** The ^{1}H-N MR spectrum of the compound showed the aromatic protons at δ: 7.67 and 7.48, two protons at δ: 4.21, one proton at δ: 1.66 and two methyl resonances at δ: 0.89 **(Rao *et al.*, 2000**; **Al-Bon *et al.*, 2006).** Also the characteristic signals of the methylene groups, the existence of only two aromatic protons in the molecule were showed in the ^{1}H-NMR spectrum. This suggested that the compound must has an ortho-disubstituted benzene ring bearing the same substituted in both protons (**Al-Bon *et al.*, 2006).** The ^{13}C-NMR spectrum of compound **1** confirmed the symmetry of the molecule and exhibited the expected 12 carbon resonances including two quaternary, three methine and five methylene carbons with two methyl groups **(Rao *et al.*, 2000**). ESI mass spectrum of compound **1** displayed pseudo-molecular ions at m/z 413.26 $[M+Na]^+$ and 803.54 $[2M+Na]^+$. ESI-HRMS due to m/z 391.28 **(Rao *et al.*, 2000**). By comparison of ^{1}H-NMR and ^{13}C-NMR data to those published in literatures **(Al-Bon *et al.*, 2006; Lyuts-Kanova *et al.*, 2009**) compound **1** was identified as Di-(2-ethylhexyl) phthalate (DEHP).

Compound (2) was obtained as pale yellow. UV spectrum of methanolic solution of the compound **2** showed characteristic bands of flavone structure at 268 nm and 339 nm. Bathochromic shift of band I (52 nm) in presence of NaOMe indicated to the presence of free OH at $4^{/}$. Also, a bathochromic shift of band II (7 nm) after addition of NaOAc indicated the presence of OH group in position 7. Bathochromic shift of band II with $AlCl_3$ (7 nm) reflected the presence of free OH at position 5. Addition of NaOAc+H_3BO_3 led to shift of band I (18 nm) indicating the absence of OH group in position $3^{/}$. Its UV data was in good agreement with that of apigenin **(Seida *et al.*, 1990; Fathiazl *et al.*, 2006**; **Moussaui *et al.*, 2010).** This compound was identified as apigenin by direct comparing its m.p. and Co-PC behavior with authentic apigenin.

UV spectrum of Compound 3 showed two characteristic bands at 267 nm and 355 nm reflecting the compound belongs to flavonoid group **(Moussaui *et al.*, 2010).** Bathochromic shift in band I by addition of $AlCl_3$ (63 nm) indicated the presence of free ortho-dihydroxy groups at position $3^{/}$,$4^{/}$ and a bathochromic shift in band II (5 nm) indicated to presence of free OH group in position 5. By addition NaOAc, a bathochromic shift observed with band II at (6 nm) reflecting the presence of OH group in position 7. Bathochromic shift in band in band I (15 nm) by addition of NaOAc+H_3BO_3 indicated the presence of OH groups in position $3^{/}$ and $4^{/}$ and absence of OH

group in position 3 **(Moussaui *et al.*, 2010).** This compound was identified as luteolin by direct comparison of m.p, Co-PC with authentic luteolin.

UV spectrum of the compound 4 in methanolic solution showed two major absorption bands at 371 nm and 256 nm which confirmed the flavonol structure. Observation of bathochromic shift (17 nm) of band II with NaOMe indicated the presence of free 7-OH and 5-OH groups. Bathochromic shift in band I with $AlCl_3$ (85 nm) reflected the presence of *O*-dihydroxy group in B-ring at $3^{/}$ and $4^{/}$ positions and band II with (18 nm) indicated of free 5-OH group. Hypsochromic shift in band I with $AlCl_3$/HCl (30 nm) indicated the presence of $3^{/}$, $4^{/}$-OH groups. Bathochromic shift (16 nm) in band I with $NaOAc/H_3BO_3$ also indicated the presence of *O*-dihydroxy groups in B-ring at $3^{/}$ and $4^{/}$ positions **(Lee *et al.*, 2004; Awaad *et al.*, 2008).** From this data of compound **4** and by direct comparing its m.p. and Co-PC behavior with authentic quercetin compound **4** was elucidated as quercetin.

UV spectra of compound 5 showed a characteristic two major absorptions bands of flavonoid compounds at 256 nm and 360 nm. Bathochromic shift in band I (45 nm) was appeared by addition of NaOMe reflected the presence of OH group at position $3^{/}$, $4^{/}$. Bathochromic shift in band I (25 nm) was appeared by addition $NaOAc+H_3BO_3$ reflecting the presence of free OH group at position $3^{/}$ and $4^{/}$. By addition of NaOAc, a bathochromic shift (14 nm) in band II was clear due to presence of OH group at position 7. Bathochromic shift (60 nm) in band I upon the addition of $AlCl_3$ indicated the free ortho dihydroxy groups at position $3^{//}$ and $4^{/}$. **(Sikorska & Matlawska 2000; Awaad *et al.*, 2008; El-Sawi & Sleem 2010**). ^{1}H-NMR spectrum of compound **5** showed a characteristic signals of quercetin skeleton at δ: 12.60 (s, 5-OH), 7.53 (2 H, dd, J = 2.1, 8.4 Hz, H2$^{/}$, H-6$^{/}$), 6.81 (1 H, d, J = 8.5 Hz, H-5$^{/}$), 6.36 (1 H, d, J = 1.75 Hz, H-8), 6.15 (1 H, d, J = 1.9 Hz, H6) beside the anomeric proton signal of glucose unit at δ: 5.42 (1 H, d, J = 7.5, H-1$^{//}$). Acid hydrolysis of compound **5** gave quercetin as aglycone and glucose as sugar moiety. Both the aglycone and glucose was identified by comparison with authentic quercetin and glucose. Therefore, the structure of compound **5** was characterized as quercetin-3-*O*-β-D-gluco-pyranoside.

Compound (6) UV absorption spectrum of this compound in methanol exhibited two absorption maxima at 357 nm and 258 nm indicating it's a flavonol structure. A bathochromic shift of band I (53 nm) with addition NaOMe indicated the presence of $3^{/}$, $4^{/}$-dihydroxyflavone and a bathochromic shift of band II (15 nm) indicated the presence of free 7-hydroxy group. A bathochromic shift (48 nm) in band I with addition $AlCl_3$ indicated the existence of 5-hydroxy group **(Han *et al.*, 2004**; **Hashimoto *et al.*, 2008).** The ^{1}H-NMR spectrum of the compound showed the expected signals of 4-disubstituted B-ring protons and two ortho-coupled resonances at δ: 7.99 (2H, d, J = 8.9 Hz) and δ: 6.88 (2H, d, J = 8.7 Hz) for H-2$^{/}$, H-6$^{/}$, H-3$^{/}$ and, H-5$^{/}$, respectively **(Song *et al.*, 2007; Mu *et al.*, 2009).** The presence of a 5, 7-dihydroxy A-ring was deduced from the typical meta-coupled resonances of H-8 and H-6 at δ: 6.43 (1H, d, J = 2.0 Hz) and δ: 6.22 (1H, d, J = 2.1 Hz) respectively. The presence of a β-glucopyronosyl moiety directly attached to the aglycone was detected from the relatively downfield β-anomeric proton H-1$^{//}$ resonance at δ: 5.32 (1H, d, J = 6.90 Hz). The terminal attachment of α-rhamno-pyronosyl moiety to C-6$^{//}$ of the inner glucose was evidenced from the resonance of the anomeric proton H-1$^{///}$ at δ: 4.30 (1H, J = 1.24 Hz) and CH_3-resonance at δ: 1.03 (3H, d, J = 6.25 Hz, Rha-6$^{///}$) **(Moustafa *et al.*, 2009; Mu *et al.*, 2009).** Comparison of the ^{13}C-NMR spectral data of compound **6** with its aglycone spectrum of kaempferol showed that there is a down field of 2.6 ppm for C-3 signal which confirm the glycosylation at position C-3 **(Song *et al.*, 2007; Mu *et al.*, 2009).** The (1$^{///}$→6$^{//}$)-*O*-glycosidic linkage of the rhamnopyranosyl on the glucoside moiety was evidenced from the fact that the C-6$^{//}$ signal at 67.25 ppm was shifted downfield (6.65 ppm) **(Song *et al.*, 2007; Mu *et al.*, 2009).** This related to the 67.25 ppm chemical shift of the corresponding carbon (C-6$^{//}$) of the inner glucose. Two anomeric carbon signals were observed at δ: 101.70 and 101.12 ppm in ^{13}C-NMR spectrum **(Moustafa *et al.*, 2009**; **Mu *et al.*, 2009**). ESI-Ms exhibited a molecular ion peak at m/z 617.14 and fragment at m/z 287.05 which corresponding to $[M+Na]^+$ and $[M-Na-146-163]^-$ suggesting the presence of two glycosyl moieties; hexose and deoxy-hexose (glucose and rhamnose). Acid hydrolysis of compound **6** gave kaempferol as aglycone and D-glucose and L-rhamnose as sugar moiety. Both of aglycone and sugar was identified by comparison with authentic samples. Therefore, compound **6** was concluded as kaempferol-3-*O*-rutinoside.

Compound (7) was obtained as a white crystal. Its IR spectrum showed characteristic bands of hydroxyl groups at 3405 cm^{-1} and other bands at 2907, 1455, 1132 and 1061 cm^{-1} **(Chaubal *et al.*, 2005**; **Sureshan *et al.*, 2009**). ^{1}H-NMR spectrum of the compound showed a multiplet at δ: 4.69 for H-2 and H-4, a multiplet at δ: 4.59 for H-1 and H-5, a triplet at δ: 4.47, a triplet at δ: 4.43 for H-6 and H3 and a singlet at δ: 3.57 for OMe, 3H atoms **(Abdoulaye *et al.*, 2004**; **Jain *et al.*, 2007**). The ^{13}C-NMR spectrum of compound **7** showed a characteristic signals of C-3 attached to OMe group appeared at δ: 84.28, a signals of C-2 and C-4 at δ: 71.42, a signal of C-1 at 73.09, a

signal of C-5 at 72.91, a signal of C-6 at 72.45, and OMe appeared at δ: 60.09 **(Narayanan *et al.,* 1987**; **Boven *et al.,* 2001**; **Abreu & Relva 2002**; **Yu *et al.,* 2005**). ESI-MS showed two peaks at 217.06 and 411.14 corresponding to $[M+Na]^+$ and $[2M+Na]^+$. From the above data, compound **7** was elucidated as D(+)-pinitol. This compound had been isolated from the leaves of *bongainvillea spectabilis*, *Lespedeza cuneata* and the root bark of *Tamarindus indica* **(Narayanan *et al.,* 1987**; **Jain *et al.,* 2007).** Pinitol is known for its antidiabetic, anti-inflammatory, and feeding stimulating activities **(Narayanan *et al.,* 1987**). This is the first time for the isolation of this compound from *C. glauca.*

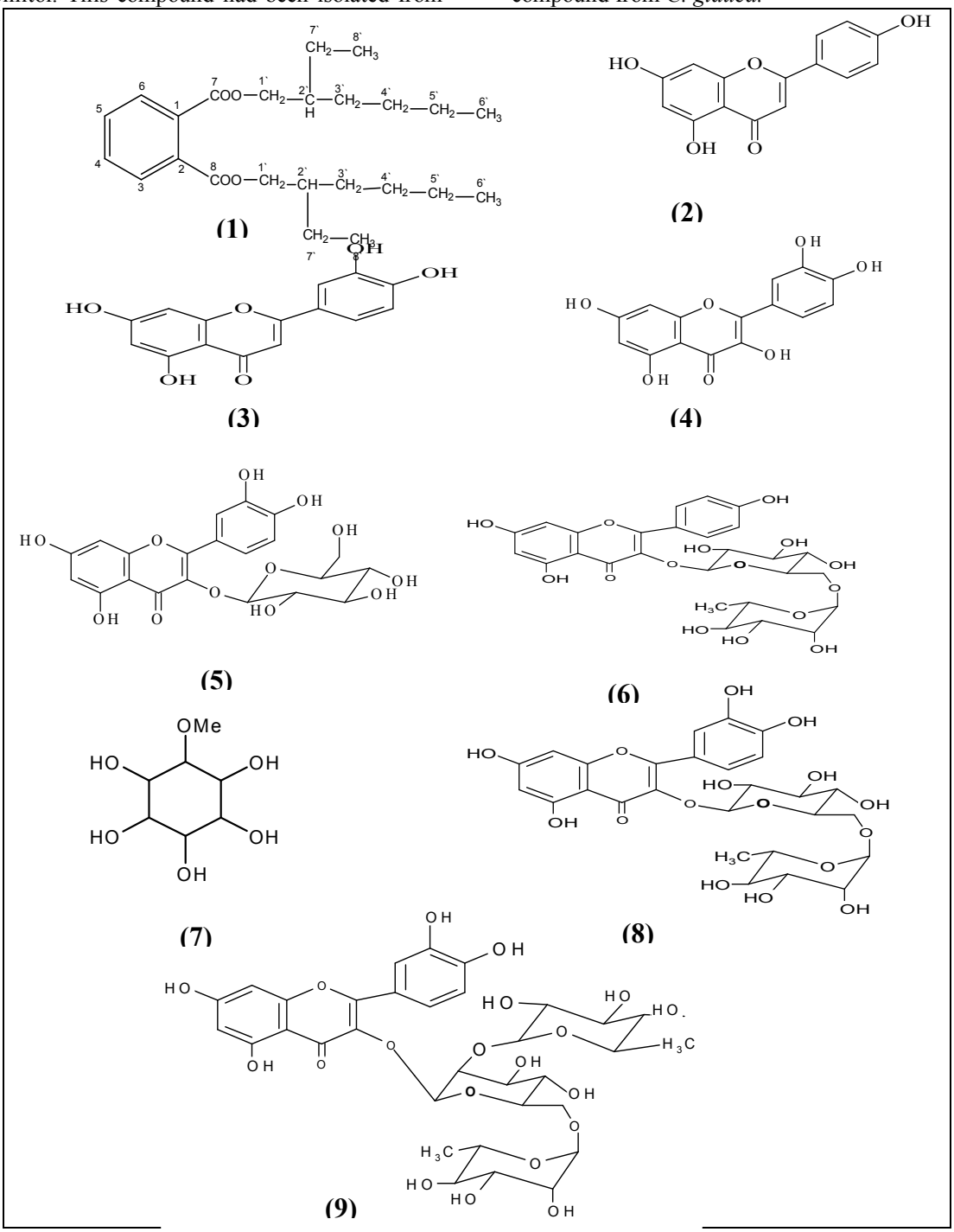

Figure 1: Chemical structures of compounds **1-9** isolated from the leaves of *Cassia glauca.*

Compound (8) was isolated as yellow powder. Its UV spectrum exhibited characteristic absorption bands of flavonoid structure at 254 and 356 nm **(El-Sawi & Sleem 2010**; **Noyeem & Karnekar 2010**). Bathochromic shift in band II (14 nm) was clear by addition NaOMe reflected the presence of free OH at C-7. Also, shifts was obtained by band I (43nm) with NaOAc indicate the presence of OH groups at position $3^{/}$ and $4^{/}$. Bathochromic shift (64 nm) in band I by addition of $AlCl_3$ reflecting the presence of free OH groups at position $3^{/}$ and $4^{/}$. Bathochromic shift band I (24 nm) with NaOAc+H_3BO_3 indicating the presence of OH group at position $3^{/}$ and $4^{/}$ **(Noyeem & Karnekar 2010).** In its ^{1}H-NMR spectrum a sharp singlet was appeared at δ: 12.55 for 5-OH group. Other five protons of the aglycone moiety was appeared at δ: 7.51 (1H, d, J = 2.0 Hz), 7.49 (1H, dd, J = 8.4 Hz and 2.1 Hz), 6.81 (1H, d, J = 8.4 Hz), 6.35 (1H, d, J = 2.1 Hz) and 6.16 (1H, d, J = 2.1 Hz) corresponding to H-$2^{/}$, H-$6^{/}$, H-$5^{/}$ and H-8 and H-6 respectively. Also two anomeric proton signals were appeared at δ: 5.31(1H, d, J = 6.85 Hz, H-$1^{//}$ Glc) and δ: 4.34 (1H, d, J = 1.25 Hz, H-$1^{///}$ Rha), and methyl group signal at δ: 0.95 **(Hueilin & Tzelin 1999; Sikorska & Matlawska, 2000; El-Sawi & Sleem 2010).** This confirmed by the presence of twenty-seven carbon signals in ^{13}C-NMR of compound **8**. Fifteen of them assigned to the carbon signals the aglycone part where as the remaining carbon signals for the two sugar moiety; D-glucose and L-rhamnose were obtained by complete acid hydrolysis of compound **8**. Both of the aglycone part and of sugar part was identified by comparison with authentic sample. Therefore, compound **8** was determined as quercetin-3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (Rutin).

Compound (9) was obtained as yellow amorphous powder. Its UV spectra of this compound in methanol as shown in gave two major spectral peaks at 258 nm (Band II) and 360 nm (Band I). The bathochromic and hypsochromic shifts of the compound in the usual shifts reagents were in a good agreement with quercetin 3-*O*-glycoside structure **(Beck & Haberlein 1999**). Free $4^{/}$-OH group was clear from the bathochromic shifts in band I (48 nm) with the increase of its intensity upon addition of NaOMe. Also a bathochromic shift (16 nm) in band II was clear by addition NaOAc reflected presence of free 7-OH. The bathochromic shift (68 nm) in Band I upon the addition $AlCl_3$ indicated to free ortho dihydroxy in position 3`and 4`. The presence of ortho-dihydroxy groups in B-ring was deduced from the hypsochromic shift in band I in $AlCl_3$/HCl spectrum (39 nm) relative to that in case of addition of $AlCl_3$ **(Beck & Haberlein 1999**; **Hossain *et al.*, 2006**). The ^{1}H-NMR spectrum indicated a 5,7-dihydroxylated pattern for ring-A (two signals at δ: 6.19 and 6.39 (J = 2.1 Hz, H-6 and H-8). For ring-B signals at δ: 6.85 (1H, d, J = 8.5 Hz), δ: 7.49 (1H, dd, J = 8.4, 2.2 Hz) and δ: 7.55 (1H, d, J = 2.3 Hz) for H-$5^{/}$, H-$6^{/}$ and H-$2^{/}$ respectively, allowing the aglycone to be recognized as quercetin **(Han *et al.*, 2001; Manguro *et al.*, 2004**; **El-Sawi & Sleem 2010**) Three anomeric signals of anomeric protons were cleared in the ^{1}H-NMR spectrum at δ: 5.54, 5.14, and 4.37 suggested the presence of a trisaccharide. Comparative analysis of ^{13}C-NMR data of compound 9 and quercetin showed downfield shift for C-3 at δ: 133.18 ppm, this suggested glycosylation site at C-3 of the aglycone. Also, the signals at δ: 77.69 and 67.56 attributed to C-$2^{//}$ and C-$6^{//}$ at the inner glucose suggested the sites of attachments of the sugar units **(Han *et al.*, 2001; Manguro *et al.*, 2004**; **El-Sawi & Sleem 2010**) The positive ESI-MS of the compound **9** exhibited a peak at m/z 779.20 that originated from $[M+Na]^+$. Other significant peaks visible at m/z 633.14 $[(M+Na)-Rha]^+$, 477.16 $[(M+Na)-2\times Rha]^+$, 331.10 $[Glc+Rha+Na]^+$, These fragments indicated the successive loss of branched two rhamnose units and inner hexose units **(Han *et al.*, 2001**; **Manguro *et al.*, 2004**; **Hossain *et al.*, 2006**; **El-Sawi & Sleem 2010**). Acid hydrolysis of compound **9** gave Quercetin as aglycone and rhamnose and glucose as sugar. Each of aglycone and sugar were identified by comparison with authentic samples. On these bases, compound **9** was established as quercetin-3-*O*-[α-L-rhamnopyronosyl-(1→2)-α-L-rhamnopyronosyl-(1→6)]-β-D-glucopyronoside.

It has been reported that, the anticancer activity of the plant extracts is associated with a variety of classes, such as polyphenols, flavonoids and catechins **(Abdel-Hady *et al.*, 2011**; **Ghasemzadeh & Neda 2011**). Flavonoids are non toxic polyphenolic compounds display a remarkable spectrum of biological activities including antioxidant and anticancer. So, flavonoid can be considered as possible chemopreventive and therapeutic agents against cancer **(Wang, 2000; Mavundza *et al.*, 2010**; **Ghasemzadeh & Neda 2011**). A number of flavonoids and polyphenols have previously been isolated from different parts of plant extracts which may be involved in their reported antioxidant and cytotoxic properties **(Moussaui *et al.*, 2010; Saeed *et al.*, 2012**; **Eman *et al.*, 2013**). In this study, the three flavonoids compounds **5, 6** and **9** showed high cytotoxic agent against human liver carcinoma cell lines HepG2 (IC_{50}= 14.3, 9 and 15.2 µg/ml) respectively.

The results of cytotoxicity of the three isolated compounds revealed that compound **6** (kaempferol-3-*O*-rutinoside) has higher activity than the other two compounds; **5** (quercetin-3-*O*-β-D-glucopyranoside)

and **9** [quercetin-3-*O*-[α-L-rhamnopyronosyl-(1→2)-α-L-rhamnopyronosyl-(1→6)]-β-D-glucopyronoside)], then the kind of flavonoid aglycon play important role in the activity. The presence of two hydroxyl groups of the B-ring (quercetin aglycon) decrease the cytotoxic activity rather than the presence of one hydroxyl group in B-ring of the flavonoid (kaempferol aglycon). This result was previously reported by **Kathrin *et al.*, (2007)** who stated that there is inverse correlation between the cytotoxicity and the number of hydroxyl phenolic groups of flavonoids. Also, it is noticed that the number of sugar units play a role in cytotoxic activity as shown between the two compounds **5** and **9** which have the same quercetin aglycon. On the other hand the antioxidant properties of the flavonoid compounds increased by increasing the number of free phenolic hydroxyl groups **(Choi *et al.*, 2002**).

Conclusions

The present study revealed that the methanolic extract of *Cassia glauca* leaves exhibited high *in vitro* anticancer activity toward hepatocellualar carcinoma cell line (HepG2). Nine compounds including eight flavonoid compounds were isolated from the methanolic extract, three of these compounds showed high cytotoxic activity. From the above results and our previous reports, the methanol extract of the leaves of *Cassia glauca* could be used as promising antioxidant and anticancer agent after more *in vitro* and *in vivo* studies.

Conflict of interest

Authors have declared that there is no conflict of interest in this manuscript.

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References

1. **Abdel-Hady NM, Dawoud GT, El-Hela AA, Morsy TA.** Interrelation of antioxidant, anticancer and antilieshmania effects of some selected Egyptian plants and their phenolic constituents. Journal of Egypt Parasitology 2011; 41(3):785-800.
2. **Abdoulaye A, Moussa I, Keita DA, Ikhiri K.** Le D-pinitol isole de *limeum pterocarpum*. Comptes rendus-Chimie 2004; 7: 989-991.
3. **Abreu P, Relva A.** Carbohydrates from *Detarium microcarpum* bark extract. Carbohydrate Research 2002; 337: 1663-1666.
4. **Al-Bon MA, Sayed MA, Rahman MS, Mosadik MA.** Characterization and antimicrobial activities of phthalic acid derivatives produced by *Streptomyces bangladeshie* a novel species collected in Bangladesh. Research Journal of Medicine and Medical Science 2006; 1(2): 77-81.
5. **Awaad AS, Mohamed NH, Maitland DJ, Soliman GA.** Antiulcerogenic activity of extract and some isolated flavonoids from *Desmostachia bipinnala* (L.) stapf. Records of Natural Products 2008; 2(3):76-82.
6. **Beck MA, Haberlein H.** Flavonol glycosides from *Eshsholtzin califorrica*. Phytochemistry 1999; 50: 329-332.
7. **Boven MV, Busson LR, Holser R, Cokelaere M, Flo G, Decuypere E.** Identification of 4, 5-didemethyl-4-O-α-D-glucopyranosl immondsin and pinitol α-D-galactopyranoside in *Jojoba* seed meal (*Simmondsia chinensis*). Journal of Agricultures and Food Chemistry 2001; 49: 4278-4283.
8. **Boyed MR.** The NCI in vitro anticancer drug discovery screen. In: Teicher 2nd ed., Anticancer Drug Development Guide preclinical screening, Clinical trials and approval, Totowa: Humana Press 30, 1997.
9. **Chaubal R, Pawar PV, Hebbalkar GD, Tungikar VB, Puranik VG, Deshpande VH, Deshpande NR.** Larvecidal activity of *Acacia nilotica* extracts and isolation of D-pinitol a bioactive carbohydrates. Chemistry and Biodiversity 2005; 2: 684-688.
10. **Choi JS, Hae YC, Sam SK, Mee JJ, Jung WK, Jae KN, Hyun AJ.** The structure-activity relationship of flavonoids as scavengers of peroxynitrite. Phytotherapy Research 2002; 16: 232-235.
11. **El-Hashash MM, Abdel-Gawad MM, El-Sayed MM, Sabry WA, Abdel-Hameed ES, Abdel-Lateef E E.** Antioxidant properties of methanolic extracts of the leaves of seven Egyptian *Cassia* species. Acta Pharmaceutica 2010; 60: 361-367.
12. **El-Sawi SA, Sleem AA.** Flavonoids and hepatoprotective activity of leaves of *Senna Surattensis* (Burm.f.) in CCl_4 induced hepatotoxicity in Rats. Australian Journal of Basic and Applied Sciences 2010; 4(6):1326-1334.
13. **El-Sayed MM, Abo-Sabra SA, El-Nahas HA, Abdel-Gawad MM, Abdel-Aziz MM, Abdel-Hameed E S, Sabry WA, Abdel-Lateef EE.** Evaluation of antioxidant and antimicrobial activities of certain *Cassia* species. Australian Journal of Basic and Applied Sciences 2011; 5(9):344-352.
14. **Eman RE, Matloub AA, Atta EM.** Cytotoxicity of new flavonoid compound isolated from *Farsetia aegyptia*. International Journal of Pharmaceutical Science Invention 2013; 2(1): 23-27.
15. **Fathiazl F, Delazar A, Amiri R, Sarker SD.** Extraction of flavonoids and quantification of rutin from waste tobacco leaves. Iranian Journal of Pharmaceutical Research 2006; 3:222-227.
16. **Ghasemzadeh A, Neda G.** Review, Flavonoids and phenolic acids: Role and biochemical activity in plants and human. Journal of Medicinal Plants Research 2011; 5(31): 6697-6703.
17. **Han JT, Bang MH, Chun OK, Kim DO, Lee CY, Baek NI.** Flavonol glycosides from the aerial parts of *Aceriphyllun rassu* and their antioxidant activities. Archives Journal of Pharmaceutical Researches 2004; 27(4): 390-395.
18. **Han Y, Nishibe S, Noguchi Y, Jin Z.** Flavonol glycosides from the stems of *Trigonella foemum-graecum*. Phytochemistry 2001; 58: 577-580.

19. **Hashimoto M, Twashina T, Kitujima J, Malsumoto S.** Flavonol glycosides from *Clematis cultivars* and Taxa and their contribution to yellow and white flower colors. Bulletin of the National Museum of Nature and Science Series B (Botany) 2008; 34(3):127-134.
20. **Hossain MA, Islasn A, Jolly YN, Kabir MJ.** A new Flavonol glycoside from seeds of *Zea mays*. Indian Journal Chemistry 2006; 45B: 1319-1321.
21. **Hueilin J, Tzelin Y.** Flavonoids from the leaves of *Loranthus Kaoi* (Chao) Kiu. Food Drug Analysis 1999; 7(3):185-190.
22. **Jain R, Jain S, Sharma A, Ito H, Hanato T.** Isolation of (+)-pinitol and other constituents from the root bark of *Tamarindus indica* Linn. Journal of Natural medicine 2007; 61: 355-356.
23. **Kathrin P, Gabriele K, Eleni K, Elke R, Peter R, Axel R, Peter S, Carsten S.** Structure–activity relationships of flavonoid-induced cytotoxicity on human leukemia cells. Archives of Biochemistry and Biophysics 2007; 460: 1–9.
24. **Khan RA, Khan NA, Khan FU, Ahmed M, Shah AS, Khan MR, Shah MS.** Phytochemical, antioxidant and cytotoxic activities of *Periploca aphyla* and *Mentha longifolia*, selected medicinal plants of District Bannu, Pakistan. African Journal of Pharmacy and Pharmacology 2012; 6(45): 3130-3135.
25. **Lee JH, Ku CH, Boek NI, Kim SH, Park HW, Kim DK.** Phytochemical constituents from *Diodia teres*. Archives Journal of Pharmaceutical Researches 2004; 27(1):40-43.
26. **Lyuts-Kanova D, Ivanova V, Stoilona-Disheva M, Kolorova M, Aleksieva K, Peltekova V.** Isolation and characterization of *Psychrotolerant streptomyces* strain from permafrost soil in spitrbergen producing phthalic acid ester. Biotechnology and Biotechnological Equipment 2009; 23: 1220-1224.
27. **Manguro LO, Ugi I, Leonen P.** Further Flavonol glycosides of *Embella schumperi* leaves. Bulletin of Chemical Society of Ethiopia 2004; 18(1):51-57.
28. **Mavundza EJ, Tshikalange TE, Lall N, Hussein AA, Mudau FN, Meyer JM.** Antioxidant activity and cytotoxicity effect of flavonoids isolated from *Athrixia phylicoides*. Journal of Medicinal Plants Research 2010; 4(23): 2584-2587.
29. **Mohsen SM, Ammar AS.** Total phenolic contents and antioxidant activity of corn tassel extracts. Food Chemistry 2009; 112(3): 595-598.
30. **Moussaui F, Zellagui A, Segueni N, Touil A, Rhouati S.** Flavonoid constituents from Algerian *Launaea resedifulia* and their antimicrobial activity. Records of Natural Products 2010; 4(1): 91-95.
31. **Moustafa AM, Khodair A, Salah MA.** Isolation, structural elucidation of flavonoid constituents from *Leptania pyrolechnica* and evaluation of their toxicity and antitumor activity. Pharmaceutical Biology 2009; 47(6): 539-552.
32. **Mu B, Liu S, Xie Y, Kano Y, Yuen D.** Flavonol glycosides and triterpenes from the leaves of *Clncasia rlynchaphylla* (Miq.) Jacks. Asian Journal of Traditional Medicine 2009; 4(3): 85-92.
33. **Namiki M.** Antioxidants/antimutagens in food. Critical Reviews in Food Science and Nutrition, **1990;** 29: 273-300.
34. **Narayanan CR, Joshi DD, Mujumdar AM, Dhekne VV.** Pinitol, a new antidiabetic compound from the leaves of *Baugaenvillea spectabilis*. Current Science 1987; 56(30):138-141.
35. **Noyeem N, Karnekar MD.** Isolation of phenolic compounds from the methanolic extract of *Tectonagradis*. Research Journal of Pharmaceutical and Biological Chemistry 2010; 1(2):221-225.
36. **Qiu S, Jiang Y, Zhang M, Chen F.** Antioxidant and anticancer activities of Wampee (*Clausena lansium* (Lour.) Skeels) peel. Journal of Biomedicine and Biotechnology 2009; 6: 1-6.
37. **Rao GN, Kumar PM, Dhandapania VS, Krishna TR, Hayashi T.** Constituents of *Cassia auriculata*. Fitoterapia 2000; 71:82-83.
38. **Saeed N**, **Khan MR**, **Shabbir M.** Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. Complementary and Alternative Medicine 2012; Doi:10.1186/1472-6882-12-221.
39. **Seida AA, El-Gohary UM, Azzam SM, El-Deeb KS.** Flavonoids of *Achillea fragrantissina* (Forsk) Bip herb. Bulletin of Faculty of Pharmacy Cairo University 1990; 28(1): 59-60.
40. **Sikorska M, Matlawska I.** Quercetin and its glycosides in the flowers of *Asclepias syriaca* L. Acta Polonia Pharmacy and Drug Research 2000; 57(4): 321-324.
41. **Skehan P, Storeng R, Scudiero D, Monks A, McMahon I, Vistica D.** New colorimetric cytotoxicity assay for anticancer drug screening. Journal of National Cancer Institute 1990; 82: 1107-1112.
42. **Song N, Xu W, Guan H, Liu GX, Wang Y, Nie X.** Several flavonoids from *Carpsella bursa-Pastoris* (L.). Medical Asian Journal of Tradition Medicines 2007; 2(5):218-222.
43. **Sureshan KM, Murakami T, Watanabe Y.** Total syntheses of cyclitol based natural products from myo-inositol: brahol and pinpollitol. Tetrahedron 2009; 65: 3998-4006.
44. Wang HK. The therapeutic potential of flavonoid. Expert Opinion on Investigational Drugs 2000; 9:2103-2119.
45. **Woraratphoka J, Intarapichet KO, Indrapichate K.** Antioxidant activity and cytotoxicity of six selected regional, Thai vegetables. American-Eurasian Journal of Toxicological Sciences 2012; 4(2): 108 -117.
46. **Yu DH, Bao YM, Wei CL, Jain A.** Studies of chemical constituents and their antioxidant activities from *Astragalus mongholicus bunge*. Biomedical and Environmental Sciences 2005; 18: 297-301.