Australian Journal of Basic and Applied Sciences, 6(10): 257-265, 2012
ISSN 1991-8178

Antihyperglycemic and Antioxidant Activities and Chemical Composition of *Conyza dioscoridis* (L.) Desf. DC. growing in Egypt

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Abstract: The antihyperglycemic activity of total EtOH 70% extracts of leaf, flower and root of *Conyza dioscoridis* (L.) Desf. was tested on Streptozotocin – induced diabetic rats using 50mg/kg orally and 10mg/kg of Gliclazide. The root extract exerted the most significant decrease in serum glucose (106.45±8.25 nmol/ml) and malondialdehyde (2.20±0.38 nmol/ml) and the most significant increase in serum insulin (6.53±0.83 ng/ml) and blood glutathione (44.25±4.58mg %) levels, (P<0.05), results were comparable to the standard. The antioxidant activity of the roots showed 76.5% of inhibition versus 88.2% of the standard. Thirteen compounds were isolated and identified using spectroscopic techniques (UV, ^{1}H NMR, ^{13}C NMR, DEPT and 2D NMR) to be: cholesterol (**1**), β-sitosterol (**2**), α-amyrin (**3**), conyzin (**4**), lupeol acetate (**5**), β- sitosterol glucoside (**6**), gallic acid (**7**), *p*-caffeic acid (**8**), syringic acid (**9**), rutin (**10**), quercitrin (**11**), quercitin (**12**) and kaempferol (**13**). Compounds (**6**) and (**8**) are newly reported in the species.

Key words: *Conyza dioscoridis*, LD_{50}, antihyperglycemic, antioxidant, constituents.

INTRODUCTION

Conyza dioscoridis (L.) Desf. (Asteraceae) is a wild growing highly branched shrub that attains a height of one to three meters and is characterized by being hairy and glandular. The plant is widely distributed in the Middle East and surrounding African countries. In Egypt, it occurs mainly in Nile region, Western and Eastern Deserts, Sinai Peninsula and Oases of the Mediterranean coastal strip **(**Boulos, 2002).

Conyza dioscoridis is reputed in folk medicine as a popular remedy to relieve rheumatic pains (Boulos and E1-Hadidi, 1989), as well as, carminative and in treatment of epilepsy in children, colic, ulcer and cold (Ibn El Bitar, 1890). Farmers in Egypt call *C. dioscoridis* "mosquito tree" due to its insect repellent effect (Shaltout and Slima, 2007). Scientifically-based bioactivity studies proved that *C. dioscoridis* extract exhibited (among those of other Egyptian medicinal plants) a significant anti-diarrheal activity (Atta, 2004), in addition to a diuretic effect (Atta, 2010); furthermore, the volatile constituents of *C. dioscoridis* showed promising antimicrobial activities (El-Hamouly and Ibrahim, 2003) and the extract of the combined aerial parts possessed anti-inflammatory activity (Awaad *et al.,* 2011). However, nothing could be traced concerning either the constituents of the roots or its antihyperglycemic activity.

The present work was planned in order to evaluate the antihyperglycemic and antioxidant potentials of the different organs of *Conyza dioscoridis* (leaves, flowers and roots) and to select the relatively most potent for further isolation and identification of its components.

Experimental:

Plant Material:

Conyza dioscoridis was collected from El-Fayoum, Egypt in 2009. The plant was identified by Prof. Dr. Mounir M. Abd Elghani, Botany Department, Faculty of Science, Cairo University, Egypt. A voucher specimen (no. C1-2012) is deposited in Pharmacognosy Department, Faculty of Pharmacy, Beni Suef University, Egypt.

Material and Methods for Biological Study:

Plant Extracts:

Samples (0.5 kg, each) of the powdered plant organs under investigation (leaves, flowers and roots) were, separately, extracted with ethanol 70%, by cold maceration, untill exhaustion (yielded 4.97, 3.66 and 3.11%, respectively).

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Experimental Animals:

Albino mice (25 – 30 g) were used for Determination of LD_{50} and adult male albino rats (120 - 150g) were used for the antihyperglycemic activity. All the animals were obtained from a breeding colony at Faculty of Veterinary Medicine, Beni Suef University, Egypt. Animals were kept under the same hygienic conditions, fed with well balanced normal diet.

Drugs and Kits:

Streptozotocin (STZ), used for induction of Diabetes, was obtained from Sigma (St. Louis, MO, USA), Gliclazide (Diamicron®), Servier Laboratories, France, was obtained from local pharmacies and served as standard. Glucose kit (BIOLABO SA, France) was used for the enzymatic determination of glucose adopting glucose – oxidase method. Rat insulin ELISA kit (H type) Biovendor, Czech Republic, was used for determination of the concentration of insulin in samples.

Determination of LD_{50}:

LD_{50} of the total ethanolic 70% extracts of the leaf, flower and root of *Conyza dioscoridis* was estimated according to Spearman and Karber, (1978) procedure. Thirty six male albino mice (25-30g) body weight were divided into six groups each of six animals. Preliminary experiments were carried out to determine the minimal dose that kills all animals (LD_{100}) and the maximal dose that failed to kill any animal. Several doses at equal logarithmic intervals were chosen in between these two doses. Each dose was injected in a group of six animals by subcutaneous injection. All groups of animals were observed for 24 hours and symptoms of toxicity and mortality rates in each group were recorded and the LD_{50} calculated.

Radical Scavenging Activity:

The free radical scavenging capacity of total EtOH 70% extracts of the three organs (leaf, flower and root) of *C. dioscoridis* were evaluated using 2, 2-Diphenyl-1-picrylhydrazyl (Amic *et al.,* 2003). Absorbance readings were taken at 520 nm against methanol as a blank and the scavenging activity was calculated.

Antihyperglycemic Acitivity:

The rats (36 adult male albino rats) were divided into six groups (6 rats for each group). The first group served as a negative control, receiving normal saline orally. Rats in groups of 2-6 were all fasted for 12 hours before the induction of the diabetes with STZ. They were injected intraperitoneally with freshly prepared solution of STZ/kg body weight, (b.wt); STZ was dissolved in 0.05 M sodium citrate buffer, pH 4.5 (Montilla *et al.,* 2004). Blood samples were collected after 72 hours of induction of diabetes for measuring blood glucose, insulin, and MDA and GSH levels. Group 2 served as STZ-diabetic control, and groups 3, 4 and 5 STZ diabetic rats treated orally by an intragastric tube with total alcohol extracts of leaf, flower and root respectively in doses of 50mg/kg b.wt for each, daily for two weeks. Group 6 was treated with a standard drug Gliclazide in a dose of 10 mg/kg b.wt. At the end of the experiment blood samples were collected for estimation of the biochemical parameters.

Biochemical Analysis:

Determination of blood Glutathione (GSH) level by the method of (Beutler *et al.,* 1963), lipid peroxidation in liver tissues was evaluated by measuring the formation of thiobarbituric acid reactive substance (TBARS) as an index of malondialdehyde (MDA) production level according to the method of (Mihara & Uchiyama, 1978). Fasting Serum glucose level was determined according to the method described by (Trinder, 1969). Insulin concentrations in samples were determined by Enzyme Linked – Immunosorbent Assay (ELISA) (King *et al.,* 2002).

Statistical Analysis:

Analysis of the data was performed by one way Analysis of Variance (ANOVA) and subsequent analysis was performed using Tukey test. The *p*-values < 0.05 were selected to indicate statistical significance between the groups. Statistical analysis of results, was done using named SPSS statistics 17.0, release (Aug. 23, 2008), Chicago, USA as analytical software.

Ethics:

All animal procedures were performed after approval from the Ethics Committee of Beni Suef University and in accordance with the recommendations of the proper care and use of laboratory animals.

Material and Equipment for Phytochemical Study:

Plant Extracts:

70% ethanol extract (as under plant extracts of the biological study).

Successive Extracts:

The most bioactive plant organ (1kg powder) was subjected to continuous successive extraction by maceration, on cold, using petroleum ether (40-60^{o}C), dichloromethane, EtOAc and EtOH. The solvent in each case was evaporated and the residues (yielded 1.17, 0.24, 0.74 and 1.09% respectively), kept for further isolation of the bioactive constituents.

General Experimental Procedures:

Precoated silica gel 60 F_{254} plates for thin layer chromatography (TLC) and silica gel 60 for column chromatography (CC) were obtained from E. Merck, Darmstadt, Germany. Sephadex LH_{20} for CC was purchased from Amersham Pharmacia Biotech B, Uppsala, Sweden. Silica gel H (E-Merck, Darmstadt, Germany) was used for vacuum liquid chromatography (VLC). Solvent systems were prepared from analytical grade solvents as follows: S1 (n- hexane/ acetone; 8:2); S_1 (n- hexane/ acetone; 8:2); S_2 (n-hexane/ petroleum ether; 8:2); S_3 (n-hexane/ EtOAc; 9:1); S_4 (EtOAc /formic acid/acetic acid/H_2O; 26:2.3:2.3:15), S_5 ($CHCl_3$/acetic acid; 8:2) ; S_6 ($CHCl_3$/acetic acid 9:1); S_7 (butanone/toluene/MeOH/acetic acid/H_2O; 8:1:0.4:0.2:0.9) ; S_8 ($CHCl_3$/MeOH/H_2O; 15:6:2). The chromatograms were visualized under UV light (at 254 and 366 nm) before and after exposure to ammonia vapor, as well as spraying with spray reagents. The spray reagents (Stahl, 1969) used for spot location were: *p*-anisaldehyde – sulphuric acid and H_2SO_4 20% in MeOH (for sterols and triterpenes); $AlCl_3$ 1% (for flavonoids), and aniline hydrogen phthalate (for sugars). Reference materials for flavonoids, sterols, triterpenes and sugars were obtained from E. Merck, Darmstadt, Germany. UV-visible spectrophotometer Shimadzu UV 240 (P/N204-58000), USA, was used for recording UV spectra and measuring the absorbance in UV range, NMR Jeol GNM: ^{1}HNMR (300 and 400 MHz) and ^{13}C NMR (125 MHz). The NMR spectra were recorded in DMSO and $CDCl_3$ using TMS as an internal standard and chemical shift values were recorded in δ (ppm) relative to TMS.

Isolation of the Major Constituents of the Petroleum Ether Extract of the Roots:

The petroleum ether extract (5g) of the roots (Fig. 1), was subjected to saponification with potassium hydroxide (10%) and the unsaponifiable (USM) and the saponifiable fractions were prepared according to a reported procedure (Finar, 1973). A portion of USM was subjected to a CC of silica gel and gradient elution with solvents in increasing polarity was adopted using: [P.E. (40-60^{o}C), P.E./$CHCl_3$, $CHCl_3$ and $CHCl_3$/EtOAc]. Fractions (50 ml, each) were collected and monitored by TLC (S_1, spray reagent) and pooled together to give two collective fractions (A and B). The two fractions were rechromatographed on silica gel columns using gradient mixtures of benzene/EtOAc to afford three compounds: **1** (71mg), **2** (32mg), and **3** (28mg).

Isolation of Constituents of the Chloroform Extract of the Roots:

An aliquot (4 g) of the $CHCl_3$ extract was fractionated over a silica gel vacuum liquid chromatography (VLC), (120g). Elution was carried out with benzene followed by 5% increments of EtOAc in benzene. Fractions (100 ml each) were collected and monitored by TLC analysis (systems S_2 and S_3) and pooled into three fractions (A, B and C). Repeated column chromatography of the three fractions on silica gel followed by Sephadex LH_{20} allowed the isolation of three compounds **4**, **5** and **6**.

Isolation of the Polar Constituents of the Roots:

The residue left after fractionation of the total ethanol 70% with $CHCl_3$ (5g) was suspended in H_2O and fractionated over a reversed polyamide column (50g, 100×3 cm). Elution was carried with distilled H_2O followed by distilled H_2O with 5% of increasing amount of MeOH and finally with MeOH. Fractions (100ml each) were collected to yield 32 fractions; these fractions were monitored by TLC using $\mathbf{S_4}$ and pooled into 2 similar fractions. The latter were subjected to preparative paper chromatography and Sephadex LH_{20} column which allowed the isolation of seven compounds **7** – **13**. All the isolated compounds were identified by physical, chemical and spectral analyses (^{1}H NMR, ^{13}C NMR, DEPT and 2D NMR).

Acid Hydrolysis:

Hydrolysis of the isolated glycosides was performed according to (Mabry *et al.*, 1970), by refluxing with 10% H_2SO_4 for 3 hrs. The aglycone was extracted with $CHCl_3$, purified and subjected to TLC. The produced sugars were identified by TLC using silica gel G and system $\mathbf{S_8}$.

Results:

Determination of LD_{50}:

All extracts were safe up to 5g/kg b. wt.

Radical Scavenging Activity:

The three organs of *C. dioscoridis* showed prominent percentage of radical scavenging capacity in the following order: flower: 87.5%; roots: 76.5% and leaf: 70.5% when compared to the standard: 88.2%.

Antihyperglycemic Acitivity:

The antihyperglycemic acitivity on treatment with 50mg/kg of ethanol 70% extracts showed significant increase in GSH level of the three organs and the most significant results were for leaves (45.14 ± 3.24 mg%), Table 1. For MDA results were significant for both roots and leaves only, as they showed significant decrease in MDA level (2.20 ± 0.38; 2.02 ± 0.31 nmol/ml, respectively), and a weak effect for flowers (5.43 ± 1.07 nmol/ml), (Table 1). Regarding glucose and insulin levels, the most significant results were for roots followed by leaves (Table 2). A significant decrease in serum glucose levels for roots and leaves (106.45 ± 8.25; 109.36 ± 9.83 nmol/ml, respectively) when compared to Gliclazide standard (109.28 ± 8.00 nmol/ml) and the root extract was more significant than the standard itself. A significant increase in insulin levels for roots and leaves (6.53 ± 0.83; 6.17 ± 0.68 ng/ml, respectively) were showed when compared to the standard (6.26 ± 0.66 ng/ml).

Table 1: Effect of administration of 50 mg/kg of ethanolic 70% extracts of three organs (leaves, flowers and roots) of *C. dioscoridis* on blood Glutathione (GSH) and Serum Malondialdehyde TBARS (MDA) levels in Streptozotocin (STZ) –induced diabetic male rats.

Treated groups	GSH	MDA
	$\bar{x}$ ± SE (mg %)	$\bar{x}$ ± SE (nmol/ml)
gp1: Normal control	46.86 ± 3.88	2.26 ± 0.46
gp2: STZ-diabetic control	27.58 ± 2.84*	5.83 ± 1.08*
gp3: STZ-alcoholic extract of leaf	45.14 ± 3.24 [a]	2.02 ± 0.31 [a]
gp4: STZ-alcoholic extract of flower	44.14 ± 4.11[a]	5.43 ± 1.07*
gp5: STZ-alcoholic extract of root	44.25 ± 4.58 [a]	2.20 ± 0.38 [a]

Data was expressed as mean± SE (n=6 rats per group).
Values are statistically significant at P <0.05
Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test.
∗: *Significantly different from the normal control group at P <0.05.*
a*: Significantly different from the diabetic control group at P < 0.05.*

Table 2: Effect of administration of 50 mg/kg of ethanolic 70% extracts of three organs (leaves, flower and roots) of *C. dioscoridis* on serum glucose and insulin levels of streptozotocin (STZ) – induced diabetic male rats.

Treated groups	Serum glucose level $\bar{x}$ ± SE (nmol/ml)	Serum insulin level $\bar{x}$ ± SE (ng/ml)
gp1: Normal control	94.80 ± 7.60	7.05 ± 0.76
gp2: STZ-diabetic control	333.77 ± 28.00 *	2.51 ± 0.43*
gp3: STZ-alcoholic extract of leaf	109.36 ± 9.83 [a]	6.17 ± 0.68 [a]
gp4: STZ-alcoholic extract of flower	284.81 ± 39.82*	2.55 ± 0.57*
gp5: STZ-alcoholic extract of root	106.45 ± 8.25 [a]	6.53 ± 0.83 [a]
gp6: STZ-Gliclazide (10 mg/kg)	109.28 ± 8.00 [a]	6.26 ± 0.66 [a]

Data was expressed as mean± SE (n=6 rats per group).
Values are statistically significant at P <0.05
Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test.
∗: *Significantly different from the normal control group at P <0.05.*
a*: Significantly different from the diabetic control group at P < 0.05.*

Phytochemical Study:

A total of thirteen compounds were isolated and identified from all the fractions as follows:

Compound 1:

71 mg, white powder, m.p. 148°C, soluble in n-hexane, positive test for sterol and/or triterpenes. (R_f = 0.69 in S_1), violet color in *p*-anisaldehyde/H_2SO_4. ^{1}H NMR and ^{13}C NMR data are in agreement with literature (Goad *et al.*, 1997). From the above mentioned data compound **1** could be identified as **cholesterol**. This compound was previously isolated from the leaves of this species (Hammouda *et al.*, 1978; Sayed *et al.*, 1991)

Compound 2:

32 mg, white needle crystals, m.p. 140-142°C, freely soluble in n-hexane, positive test for sterol and/or triterpenes**;** R_f = 0.42 in S_1; violet color with p-anisaldehyde/H_2SO_4. ^{1}H NMR and ^{13}C NMR are in agreement with literature (Ghazala and Shameel, 2005; Abdul Munit *et al.*, 2010). From the above mentioned data, compound **2** could be identified as **β-sitosterol**. This compound was previously isolated from the leaves of this species (Sayed *et al.*, 1991).

Compound 3:

28 mg, white powder, m.p. 197-199°C, freely soluble in CH_2Cl_2, positive test for sterol and/or triterpenes; R_f = 0.38, S_1; violet color in *p*-anisaldehyde/H_2SO_4. From m.p and by co-TLC with authentic sample using different solvent systems, in addition to direct comparison with published data (Dekebo *et al.,* 2002), compound **3** could be identified as **α- Amyrin.** This compound was previously isolated from the leaves of this species (Sayed *et al.,* 1991).

Compound 4:

5 mg, white fine needles, m.p. 151°C, soluble in CH_2Cl_2; R_f = 0.59, S_2; pink color in *p*-anisaldehyde/H_2SO_4. By comparison with published data (El Hefnawy, 1990) compound **4** could be identified as **Conyzin** but it needs more data for confirmation.

Compound 5:

21 mg, white needles, m.p. 218°C, soluble in CH_2Cl_2. (R_f = 0.44, S_2); violet color in *p*-anisaldehyde/H_2SO_4. From the above data and by comparison with the reported spectral data (Jamal *et al.,* 2008), compound **5** could be identified as **Lupeol acetate**. This compound was previously isolated from the leaves of this species (Sayed *et al.,* 1991).

Compound 6:

7mg, white amorphous powder, m.p. 273°C, soluble in CH_2Cl_2, positive tests for sterol and/or triterpenes and carbohydrates and/or glycosides. (R_f = 0.17, S_2); violet color in *p*-anisaldehyde/H_2SO_4. Hydrolysis of compound **6,** (Ruchi, *et al.,* 1992) proved sugar moiety. From the above mentioned properties, m.p and by co-TLC with an authentic sample using different solvent systems, in addition to direct comparison with published data (Mizanur *et al.,* 2009), compound **6** could be identified as **β- sitosterol glucoside**. This is the first report from *C. dioscoridis*.

Compound 7:

18 mg, Yellowish white needles, m.p. 251°C, soluble in hot water and methanol. (R_f = 0.22, S_5); dark violet color with $FeCl_3$. By comparison with the reported spectral data (Eldahshan, 2011), compound **7** could be identified as **Gallic acid.** which was previously reported from *C. dioscoridis* leaves and roots (Fahmy *et al.,* 2012).

Compound 8:

12 mg, Yellowish white needles, m.p. 213 °C, soluble in hot H_2O and MeOH, (R_f = 0.34, S_5); dark blue color with $FeCl_3$. From the above data and by comparison with the reported data (Durust *et al.,* 1999), compound **8** could be identified as **Caffeic acid.** It was previously reported from *C. blinii* (Xu *et al.,* 1998) and this is the first report from *C. dioscoridis***.**

Compound 9:

11 mg, white needles, m.p. 207 °C, soluble in diethyl ether, hot H_2O and MeOH; R_f = 0.8, S_6. From the above data and by comparison with the reported spectral data (Bao K *et al.,* 2009), compound **9** could be identified as **Syringic acid** which was previously reported from *C. blinii* (Xu *et al.,* 1998) and it was previously reported from *C. dioscoridis* leaves and roots (Fahmy *et al.,* 2012).

Compound 10:

23mg, yellow powder, m.p. 193°C, soluble in MeOH, positive Molisch´s test indicating its glycosidic nature. (R_f = 0.14, S_7); purple color in UV 365 nm which turning to green fluorescence upon exposure to NH_3 vapor and after spraying with $AlCl_3$ reagent, **UV** (λmax, nm, MeOH): 258, 301, 358; NaOMe: 268, 328sh, 410; $AlCl_3$: 270, 306, 426; $AlCl_3$/ HCl: 268, 287, 366, 400; NaOAc: 265, 302, 382; NaOAc/ H_3BO_3: 261, 308, 378. From the above mentioned data and comparing with the reported spectral data (Mabry *et al.,* 1970), compound **10** could be identified as **Rutin.** It was previously reported from *C. dioscoridis* leaves and roots (Fahmy *et al.,* 2012).

Compound 11:

6mg, yellow powder, m.p. 316°C, soluble in MeOH, positive Molisch's test; (R_f = 0.60, S_7); deep purple color in long UV which convert to bright yellow green upon exposure to NH_3 vapor and after spraying with $AlCl_3$. **UV** (λmax, nm, MeOH): 256, 265sh, 301sh, 350; NaOMe: 270, 326, 393; $AlCl_3$: 276, 304sh, 333, 430; $AlCl_3$/ HCl: 272, 303sh, 353, 401; NaOAc: 272, 322sh, 372; NaOAc/ H_3BO_3: 260, 300sh, 367. From the above mentioned data and by co-TLC with authentic sample using different solvent systems in addition to direct

comparison with published data (Mabry *et al.*, 1970), compound **11** could be identified as **Quercetrin.** This compound was previously from the leaves of this species (Ismail *et al.*, 1979).

Compound 12:

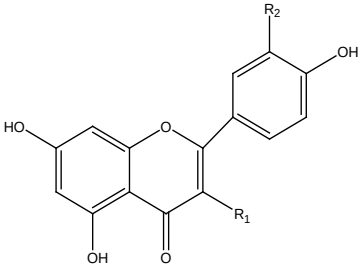
32mg, yellow powder, m.p. 316°C, soluble in MeOH; R_f value (0.74, $\mathbf{S_7}$); yellow color in U.V 365 nm which intensified upon exposure to ammonia vapor and after spraying with $AlCl_3$, **UV** (λmax, nm, MeOH): 255, 268, 302sh, 370; NaOMe: 275, 318sh, 415; $AlCl_3$: 276, 307sh, 333, 458; $AlCl_3$/ HCl: 273, 303sh, 328, 452; NaOAc: 257, 273sh, 327, 390; NaOAc/ H_3BO_3: 261, 303, 389. UV, ^{1}H NMR and ^{13}C-NMR were in accordance with the published data (Ismail *et al.*, 1979; Guyenalp and Demirezer, 2005), compound **12** was identified as **Quercetin** and it was previously isolated from three species of *Conyza* (*dioscoridis, linifolia and blinii*), (Batanouny *et al.*, 1999, El Sherei *et al.*, 1975 and Xu *et al.*, 1998) respectively.

Compound 13:

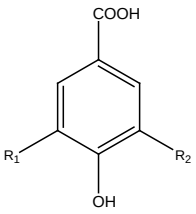
7mg, yellow powder, m.p.276–278°C, soluble in MeOH, R_f = 0.80, S_7); dull yellow color in long UV no change with NH_3 vapor and after spraying with $AlCl_3$, UV (λmax, nm, MeOH): 253sh, 266, 294sh, 322sh, 367; NaOMe: 278, 316, 416 (dec.); $AlCl_3$: 260sh, 268, 303sh, 350,424; $AlCl_3$/ HCl: 256sh, 269, 303sh, 348, 424; NaOAc: 274, 303, 387; NaOAc/ H_3BO_3: 267, 297sh, 320sh, 372. From the above mentioned data and by comparison with an authentic sample and published data (Mabry *et al.*, 1970), compound **13** could be identified as **Kampferol.** This compound was previously from the leaves of this genus (Manguro *et al*., 2010).

Fig. 1: Roots of *Conyza dioscoridis* L. (Desf.).

Fig. 2. Structures of polar compounds isolated from root of *Conyza dioscoridis*

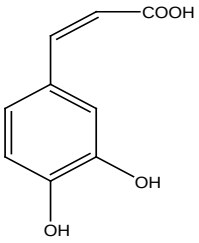


Compound no.	Compound name	R1	R2
10	Rutin	O-Glc-Rha	OH
11	Quercitrin	O-Glc	OH
12	Quercitin	OH	OH
13	Kampferol	OH	H



Compound no.	Compound name	R1	R2

7	Gallic acid	OH	OH
9	Syrinigic acid	OCH_3	OCH_3



8 P-caffeic acid

Discussion:

Hyperglycemia is related to the development of certain complications of diabetes. The latter are prevented by improving the antioxidant status which is related to the presence of phenolic compounds (Shahwar *et al*., 2012) and sterols (Alvin *et al*., 2004). The ethanol 70% extract of the roots of *C. dioscoridis* significantly decreased blood glucose level of streptozotocin diabetic rats (Tables 1 and 2) and their effects were comparable to the oral antidiabetic reference drug Diamicron®. Ethanol 70% extract of *Conyza* roots, with the best biological results, was further proceeded to isolate its antihyperglycemic principles. Thirteen compounds were isolated and identified belonging to different class of compounds [phenolic: compounds (**7** - **13**); sterols: compounds **(1**, **2** and **6**) and triterpenes: compounds (**3** - **5**)]. The attributed antihyperglycemic and antioxidant activities of this organ is due probably to a synergistic effect between its phenolic compounds, sterols and triterpenes components (Saha *et al.*, 2011, Prajapati *et al.*, 2008, Shahwar *et al.*, 2012).

Among the phenolic compounds, Rutin (compound **10**), exhibits effect via its insulinogenic activity as decreasing intestinal glucose absorption, affecting mediators of insulin resistance and improving peripheral insulin action by enhancing the glucose uptake and decreasing hepatic glucose output (Ahmed *et al*.**,** 2010). Rutin also prevents STZ-induced oxidative stress which protects pancreatic β-cells resulting in increased insulin secretion (Vessal *et al*.**,** 2003). Quercetin (compound **12**), decreased elevated blood glucose concentration and increased insulin release in STZ-induced diabetic rats. It may also protect the pancreatic β-cells by decreasing oxidative stress and preserving β-cells integrity (Coskun *et al*. 2005). Phenolic compounds as Caffeic acid, (compound **8**) is considered as as antihypergycemic agent (Jung *et al.*, 2006). Triterpenes components as lupeol acetate (compound **5**), α- amyrin (compound **3**), could be also responsible for the antihyperglycemic activity (Prajapati *et al.*, 2008). It was also reported that sterols containing drugs (as cholesterol compound (**1**), β-sitosterol compound (**2**) are responsible for antihyperglycemic and antioxidant activities (Alvin *et al*., 2004; Hatapakki *et al*., 2005) they can decrease serum glucose level (Koffi, 2009).

GSH is normally present at high concentrations in the cells and is a direct scavenger of free radicals; therefore it protects the cells against the toxic effects of oxidative stress. The ethanol 70% extract increased GSH level thus helps in avoiding the complications of free radical in diabetes mellitus.

As a conclusion, the antihyperglycemic and antioxidant activities of the root of *Conyza dioscoridis* L. could be attributed to a synergistic effect between its phenolic steroidal and triterpenoidal contents.

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