ANTIOXIDANT ACTIVITY OF *COVOLVULUS HYSTRIX* VAHL AND ITS CHEMICAL CONSTITUENTS

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ABSTRACT

Total alcohol and successive extracts (diethyl ether, chloroform, ethyl acetate and n-butanol) of *Covolvulus hystrix* Vahl were screened for their antioxidant activity using free radical 2-2- diphenyl-1-picrylhydrazyl (DPPH) and Trolox as reference substance. The tested extracts showed variable antioxidant activities. The highest antioxidant activity was achieved in total alcohol (48.32%) followed by ethyl acetate (37. 8 %) and *n*-butanol (32.96 %) extracts. From the ethyl acetate extract four compounds were isolated and identified as quercetin, quercetin 3-0-rutinoside and quercetin-7-O-rhamnoside in addition to ferulic acid using different spectroscopic analysis such as, ^{1}HNMR, ^{13}CNMR, COSY, HMBC and HMQC.

Keywords: Antioxidant activity, *Convolvulus hystrix,* flavonoids, ferulic acid, total phenolics.

INTRODUCTION

From the earliest times it is well known that prehistoric man used to depend completely on natural resources for treating diseases often based on guesswork which of course could lead to recovery or death. However with modern technology safety and efficacy are secured for any compound from any source. Cost as well is a very important parameter which adds to the importance of such natural compounds particularly in the third world (Awaad, 2009).

Plants are good sources of natural antioxidants and some of these have significant antioxidative properties (Exarchou *et al*., 2002). The antioxidant activity is mainly due to phenolic contents such as flavonoids, phenolic acids, tannins and phenolic diterpenes (Awaad and Al-Jaber, 2010).

The Convolvulaceae family includes a large number of important plants which have the properties of treatment of many diseases such as headache constipation rheumatism diabetes and skin diseases (Ibn Sina, 1968 and Al Antaki, 1952).

C*onvolvulus* species are widely distributed all over the world in different localities; some of them have medicinal activity as purgatives and in the treatment of coughs and asthma.

Convolvulus pluricalus, C. althaoieds and *C. pleuricauas* showed potent antioxidant activity capable of scavenging singlet oxygen hydroxyl radicals as well as the radicals ABTS and DPPH (Tawaha *et al.,* 2008, Parihar *&* Hemnanit, 2003, Dini, 2006).

Many other pharmacological activates were reported on some Convolvulaceae species such as, the use of *C. pluricaulis* as an antidepressant in mice (Dhingra and Valecha, 2007) *Convolvulus betonisifolius* showed antiproliferative effects on a breast cancer (Abu-Dahab and Afifi, 2007). *C. arvensis* was evaluated as a potential new source of antioxidant activity (Borchardt *et al.,* 2008). Oral administration of an extract of *Convolvulus althaeoides* to normoglycemic rats produced a persistent hypoglyaemic effect compared with Daonil (Bnouham *et al.,* 2006).

Convolvulus hystrix Vahl. is used in traditional medicine Some chemical constituents have been investigated (Dawidar *et al.,* 2000) but no reports were noted concerning its biological activity. This study was carried out to evaluate its antioxidant activity and isolation of some biologically active compounds.

MATERIALS AND METHODS

Plant material

*Convolvulus hystri*x Vahl. was collected in the summer season 2007 from the South East corner of Egypt. The collected plant was identified by Prof. Dr. Ahmed Morsy Ahmed Plant Ecophysiology Desert Research Center, Cairo, Egypt and by comparison with that in Flora of Egypt (Bolus, 2000 and Tackholm, 1974). A specimen from this plant has been deposited in the Herbarium of the Desert Research Center Cairo Egypt The plant samples were air-dried under shade and reduced to a fine powder.

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Chemicals and solvents

The antioxidant assay kit was purchased from Randox Laboratories United Kingdom. Aluminium sheets 20x20 cm Silica gel G 60 F254 Merck Germany were used for thin layer chromatography. Silica gel 60 (70-230 mesh) was used in columns. Solvent systems: chloroform – methanol (9:1) ethyl acetate-methanol-water (30:5:4) and ethyl acetate-methanol-acetic acid-water (65:15:10:10) were used for the chromatoplates.

Instruments

A Hitachi U-200 spectrometer was used for determination of total phenolics and total flavonoids Mass spectral data were obtained using an Esquire-LC_00142 mass spectrometer (Polymer Science Dept. University of Massachusetts USA). UV spectra were determined using a Shimadzu 1201 spectrophotometer. Mp. were measured on a Kofler hot-stage apparatus, mass spectra samples were dissolved in acetonitrile and injected into a mass spectrometer. ^{1}H and ^{13}C NMR spectra were determined at 600 or 150 MHz respectively with a JEOL ECA 600 spectrometer, Carbon atom types were identified in the ^{13}C NMR spectrum by combination of broad- and proton-decoupled, ^{1}H-^{13}C correlations were done by using HMQC and HMBC, ^{1}H-^{1}H correlations were done by DQF COSY. HPLC analysis was performed on a Shimadzu HLPC system with system controller SCL – 10 Agvp.

Extraction

The defatted powdered plant aerial parts (750 g) were extracted in a soxhlet with ethanol. The ethanol extract was concentrated to yield 68 g dry extract diluted with water (200 mL) and then successively extracted with the solvents diethyl ether, chloroform, ethyl acetate and n-butanol. Each extract was dried and reconcentrated to yield 8, 14, 17 and 15g dry extracts respectively.

Toxicological studies

LD_{50} values of the extracts were done by a known method (Finney 1964). Abino mice used in this study were divided into groups of five animal in each group. Experiments were done to measure the minimum dose that killed all animals and the maximum dose that failed to kill any mice. Several doses were administrated orally to animals Animals were kept under observation for one day in which toxicity and rate of mortality were observed and recorded from which the LD_{50} was calculated.

Estimation of total phenolics

An estimation of the total phenolic of the extracts was carried out using the Folin-Ciocalteu method. Two hundred microliters of sample and 0.5 mL of Folin-Ciocalteu reagent were added to 10 mL of distilled water in a 25 mL flask. After 3 min 1 mL of 5% Na_2CO_3 was added the volume adjusted to 25 mL. The samples were left for 1 h in the dark and then the absorbance was measured at 725 nm against a blank. A calibration curve with caffeic acid was established where 25-200 µg of caffeic acid in 100 µL of distilled was used instead of the sample and the total phenolic content was expressed in milligrams of caffeic acid per gram of dried plant material (Zhisen *et al*., 1999).

Antioxidant activity

Antioxidant activity was measured using 2-2- diphenyl-1-picrylhydrazyl (DPPH) and Trolox (2 5 mM in methanol) as a reference substance. The presence of antioxidative substances in the assay leads to the reductive decoloration of the DPPH radical Depending on the content of antioxidative substances 50 µl of the sample was adjusted to 1 mL with 50% methanol and then added to 1 mL of DPPH reagent (7 5 mg in 50 mL of methanol). After 0 5 h in the dark at room temperature the absorbance was measured against a blank at 515 nm. The blank was a solution where 500 µL of Trolox and 500 µL of methanol reacted with 1 mL of DPPH reagent to obtain the complete decoloration of that radical. For the calibration curve 0 5-3 mM of Trolox in 1 mL of methanol was used and results were expressed as Trolox equivalent antioxidant capacity (TEAC) (Liu *et al*., 2002).

$$\text{Percent (\%) inhibition of DPPH activity} = \frac{A - B}{A} \times 100$$

Where A is the optical density of the blank and B is the optical density of the sample

Isolation

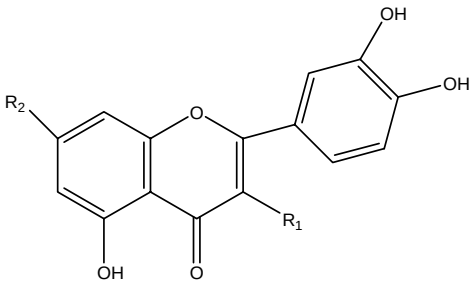
TLC examination of the different extracts using solvent systems chloroform –methanol (9:1) (ethyl acetate-methanol-water (30:5:4) and visualizing reagent. Ethyl acetate and n-butanol extracts showed similarity in No. of spots and colors using visualizing reagent and showed the highest antioxidant activities. Accordingly the ethyl acetate and butanol extracts were collected (30 g) and applied to column chromatography over silica gel (900 g) and eluted gradually with ethyl acetate –methanol. A total of 150 fractions were achieved and combined into three main fractions (according to number colour and R_f of the spots). Each fraction was concentrated to yield of 3. 22, 2. 87 and 3.11 respectively. Each fraction was separated by preparative paper chromatography using system ethyl acetate-methanol-acetic acid-water (65:15:10:10). Bands of each flavonoid were extracted with methanol then concentrated and re-purified on a Sephadex LH-20 column and eluted with methanol – water from which compunds (**1-4)** were isolated.

Acid hydrolysis

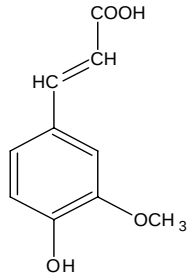
Five milligrams of each compounds were refluxed with ethanolic H_2SO_4 (5 ml EtOH 5 ml H_2O and 1 mL H_2SO_4) for two hours. The reaction mixture was diluted with water and the released aglycone was extracted with diethyl ether. The aqueous layer (sugar moiety) was

Table 1: Antioxidant activity and total phenolics of *Convolvulus hystrix* extracts and the isolated compounds.

Different extract	Inhibition %	TEAC	Total phenolics (mg/g DW)
Aqueous extract	48.32 ±2.1	1.82±0.08	12.6 ± 0.9
Butanol extract	32. 96±2.7	1 27±0.03	13.2 ±1.1
Ethyl acetate extract	37.80± 1.3	1.45±0.4	14.6 ± 1.2
Chloroform extract	22.30 ± 1.5	0.86±0.03	10.82 ± 0.8
Diethyl extract	11.53 ±1.2	0.52±0. 02	8.4 ± 0.7
Compound 1	66.43± 2.3	2.41±0.09	
Compound 2	74.2±2.8	2.68±0.07	
Compound 3	68 2±2.2	2.57±0.01	
Compound 4	18.11± 1.1	0.73±0.02	



Compounds 1-3



Compound 4: Ferulic acid

Compound 1: R_1 = OH R_2= OH
Compound 2: R_1= rutinoside R_2= OH
Compound 3: R_1= OH R_2 = rhamnose

Fig. 1: Isolated compounds from *Convolvulus hystrix*..

neutralized using barium carbonate and filtered off the filtered aqueous layer was dissolved in 10 % aqueous isopropanol and applied on TLC | (silica gel G 60) using the system acetic acid - water (15-85) and sprayed with naphthoresorcinol sulphuric acid (Stahl, 1969).

RESULTS

Toxicological studies

The plant is safe for human use since the LD_{50} was found to be over 6 g/kg b. wt.

Isolated compounds

Quercetin (1): yellow needles, R_f= 0.7 (system ethyl acetate-methanol-water (30:5:4) mp. 313-315°C, UV λmax in MeOH: (nm) 255,269, ($AlCl_3$): 270,290, by using $AlCl_3$/HCl: 270, 357 426 , in case of NaOA: 274, 325 , ($NaOAc/H_3BO_3$): 261, 385 , (NaOMe): 246, 330, 398 . ^{1}H NMR using DMSO-d6: δ 7.73 (1H d *J* = 2.5 Hz H-2'), 7.63 (1H dd *J* = 8.5 & 2.5 Hz H-6`), 6.88 (1H d *J* = 8.5 Hz H-5'), 6.39 (1H d *J* = 2.5 Hz H-6) and 6 18 (1H d *J* = 2.5 Hz H-8). ^{13}C NMR (DMSO-d6): δ ppm 177.4 (C-4), 165.5 (C-7), 162.8 (C-5), 158.2 (C-2), 149.1 (C-9), 148.1 (C-4'), 146.2 (C-3'), 138.2 (C-3), 124 1 (C-6'), 121.6 (C-1'), 116.3(C-2'), 115.9 (C-5'), 104.5 (C-10), 99.1 and 94.2 (C-6 and C-8 respectively).

Quercetin 3-*0*-rutinoside (rutin) (2): (45 mg) yellow crystals, R_f = 0 5 (ethyl acetate-methanol-water (30:5:4) m p 190°C. UV λmax in MeOH: (nm) 256, 290 355. ($ALCL_3$): 274, 432. ($ALCl_3$ /HCl): 270, 298, 359, 399. (NaOAc): 272, 324 398, ($NaOAc/H_3BO_3$): 263, 292, (NaOMe): 272,310, 410. ^{1}H NMR (DMSO –d $_6$): δ 8.10 (1H d *J* = 2 Hz H2\` ), 7.86 (1H d *J* = 8 Hz H-6\`), 6.89 (1H d *J* = 8 Hz H-5` ), 6.65 (1H d *J* = 2 Hz H-8) , 6.5 (1H d *J* = 2 Hz H6 ), 5.13 (1H d *J* = 7.50 Hz H1\`\` ), 4.55 (1H d *J* = 1.3Hz H1\`\`\`), 3.82 (1H dd *J* = 10 Hz *J* =2 Hz H6\`\`), 3.65 (1H dd *J* =3.5 H2\`\`\`), δ 3.47-3.87 (6H m for sugar protons) and δ 1.23 (3H d *J* = 6 Hz CH_3). ^{13}C NMR (DMSO –d $_6$): δ ppm 178 (C-4) 164. 65 (C-7) 161.57 (C-5) 158.92 (C-2) 148.4 (C-9) 148.1 (C-4') 144.8 (C-3'), 134.23 (C-3), 123.1 (C-6'), 122.6 (C-1'), 116.3 (C-2') 114.64 (C-5'), 104.2 (C- 10), 98.55 and 93.47 (C-6 and C-8 respectively), 102.3 (C-1``), 75.1 (C-2``), 78.1 (C-3``), 72 (C-4``), 77.2 (C-5``), 61.3 (C-6``), 100.1 (C-1```), 71.8 (C-2```), 71.3(C-3```), 72.8 (C-4```), 70.1 (C-5```), 17.4 (C-6```).

Quercetin-7-*O*-rhamnoside (3): yellow amorphous, R_f = 0.34 (in ethyl acetate-methanol-water (30:5:4). UV λ_{max} in MeOH: nm 254, 266, using $AlCl_3$: 270, 301 408, ($AlCl_3$/HCl): 269, 297 sh 255, 402. (NaOAc): 254, 266 sh, 346. ($NaOAc/H_3BO_3$): 254, 266. (NaOMe): 271, 331. ^{1}H NMR (DMSO-d_6): δ 7.4 (1H d *J* = 2 Hz H-2`), 7.3 (1H

dd $J_{6`5`}$ = 8.5 $J_{6`2`}$ = 2.1 Hz H-6`), 6.8 (1H d *J* = 8.5 Hz H-5`), 6.4 (1H d *J* = 2.5 Hz H-8), 6.2 (1H d *J* = 2.5 Hz H-6), 5.2 (1H d *J* = 2.5 Hz H-1 rhamnose), 1.2 (3H d *J* = 6 Hz CH_3) , sugar protons at δ 3.2 – 3.9 . ^{13}C NMR (DMSO-d_6) δ 149 (C-2), 134.80 (C-3), 163.9 (C-4), 159 (C-5), 99.80 (C-6), 163.20 (C-7), 92.50 (C-8), 155.20 (C-9), 103.20.(C-10), 121.30 (C-1`), 114.80 (C-2`), 144.80 (C-3`), 146.70 (C-4`), 115.60 (C-5`), 119.70 (C-6`), 100.70 (C-1`` sugar), 68.10 (C-2``) 70 30(C-3``), 70.60(C-4``), 75.20 (C-5``), and 17.60 (-CH_3).

Ferulic acid (4): (24 mg) white crystals R_f = 0.73 in system (a) (m p 228 ^oC) UV max (MeOH): nm 219, 231 and 420 nm ^{1}H NMR 400 MHz (MeOD): 8.83 (1H S COOH) ,7.52 (1H d *J* = 20 Hz H-C=C), 7.15 (1H d *J* = 2.5 Hz H-a). 7.1(1H d *J* = 2.5 Hz & *J* = 2 Hz H-C), 6.82 (1H d *J* = 9 Hz H-b), 6.23(1H d *J* = 20 Hz H-1), 3.82 (3H s OCH_3), ^{13}C NMR (MeOD) δ 167.6 (C-9), 149.7(C-4), 146.6 (C-3), 144.9 (C-7), 127.4 (C-1),123.8 (C-6), 116.9 (C-5), 112.6 (C-2), 56.78 (CH3). Mass: m/z 194, 151, 133, 105 and 77.

Total phenolics

Total phenolic measurement (Table 1) showed variation in the amount for each extract (diethyl ether, chloroform, ethyl acetate and butanol). The highest concentration was recorded in the n-butanol extract (13.2 mg/g DW) followed by ethyl acetate and total alcohol extracts (12.6 10.82 mg/g DW respectively). The chloroform and diethyl ether extracts showed the lowest concentration.

Antioxidant activity

The scavenging activity of crude extract and different extracts (diethyl ether, chloroform, ethyl acetate and n-butanol extracts) were determined. As shown in table 1 the highest activity was observed in the crude extract (48.32 %) followed by the ethyl acetate and butanol extracts (37.8 % and 32.97 % respectively). The diethyl ether and chloroform extracts showed the lowest antioxidant activity (11.53 % and 22.30 % respectively).

DISCUSSION

Four compounds were isolated from *Covolvulus hystrix* Vahl. Compounds were identified by comparing their EI-MS, ^{1}H NMR, ^{13}C NMR and UV spectrum in methanol and with different shift reagents with published data (Mabry *et al*., 1970, Harborne *et al.,* 1975)

Compound **1** was identified by comparing its TLC chromatograms UV spectrum in methanol and with different shift reagents, ^{1}H NMR and ^{13}C NMR spectra with authentic samples and published data. It compared with quercetin and its data were in agreement with this published for this compound (Mabry *et al.,* 1970).

Antioxidant activity was measured by the DPPH assay by using it we can do many samples in a short period and it is sensitive to determine active components at low concentrations (Sa´nchez-Moreno, 2002).

Acid hydrolysis of compounds **2** and **3** revealed that, sugars were rhamnose and glucose from **2** and rhamnose from **3** which were identified by TLC (ethyl acetate - methanol - acetic acid - water (65:15:10:10). The aglycone in both cases was identified as compound**1** based on comparison by TLC and UV shift reagents. Compound **2** was substituted at position 3 Compound **3** was substituted at position 7 as indicated by their UV spectra. From these data and published data (Geissman, 1962 and Mabry *et al.,* 1970 and Harborne *et al.,* 1975) identified these compounds as Quercetin-3-O–rutninoside (rutin) and quercetin 7-*O*-rhamnoside.

The flavonoids with the highest inhibition of oxidation of beta-carotene and lipid peroxidation by the ammonium thiocyanate method were also found to be rutin and apigenin. Methoxylated flavonoids exhibited a lesser antioxidant activity (Sharififar *et al.,* 2009).

Rutin showed strong DPPH radical scavenging activity. At the concentration of 0.05 mg/ml, rutin also had inhibition of lipid peroxidation (Yang *et al.,* 2008). From these results, *Covolvulus hystrix* can be used as a source of antioxidant compounds.

REFERENCES

Abu-Dahab R and Afifi F (2007). Antiproliferative activity of selected medicinal plants of Jordan against a breast adenocarcinoma cell line (MCF7). *Scientia Pharmaceutica,* **75**: 121-136.

Al-Antaki D (1952). *In:* "*Tazkarit Oli El-Albab*" Elhalaby Co Cairo, **1**: p 193 (in Arabic).

Awaad AS and AL- Jaber NA (2010). Antioxidant Natural Plant*. RPMP Ethnomedicine: Source & Mechanism,* **27:** 1-35.

Awaad SA (2009). Flavonoids of *Bidens bipinnata* and their Antioxidant Activity. *J. King Saud Univ.,* **21**: 183-189.

Bnouham M, Ziyyat A, Mekhfi H, Tahri A and Legssyer A (2006). Medicinal plants with potential antidiabetic activity. *Int. J. Diabetes & Metabolism,* **14**: 1-25.

Borchardt JR, Wyse DL, Sheaffe CC, Kauppi KL, Fulcher RG, Ehlke NJ, Biesboer DD and Bey RF (2008). Antioxidant and antimicrobial activity of seed from plants of the Mississippi river basin. *J. Medicinal Plants Research,* **2**(4): 81-93.

Boulos L (2000). *Flora of Egypt*. Al Hadara Publishing Cairo, **2**: 246

Dawidar AM, Ezmiriy ST, Abdel-Mogib M, El-Dessouki Y and Angawi RF (2000). New stilbene carboxylic

acid from *Convolvulus hystrix. Pharmazie*, **55**(11): 848-849.

Dhingra D and Valecha R (2007). Screening for antidepressant-like activity of *Convolvulus pluricatlis* choisy in mice. *Pharmacologyonline,* **1**: 262-278.

Dini I, Tenore JC and Dini A (2006). New Polyphenol Derivative in *Ipomoea batatas* tubers and its Antioxidant Activity. *J. Agric. Food Chem.,* **54**(23): 8733-8737.

Exarchou V, Nenadis N, Tsimidou M, Gerothanassis IP, Troganis A and Boskou D (2002). Antioxidant activities and phenolic composition of extracts from Greek oregano Greek sage and summer savory. *J. Agric. and Food Chem.,* **50**: 5294-5299.

Finney DJ (1964). *Statistical methods in biological assay* Charles Griffin and company Ltd., London, p.97.

Geissman TA (1962). *In*: "Chemistry of flavonoids Compounds". The McMillan Company Ltd., New York, p.320.

Harborne TB, Mabry TJ and Mabry H (1975). *The Flavonoids* Chapman and Hall, London, p.219.

Ibn Sina AH (1968). *In*: "*Alkanun fil Tibb*" Vol. I, El Halaby and Co., Cairo, p.385 (in Arabic).

Liu M, Li XQ, Weber C, Lee CY, Brown J and Liu RH (2002). Antioxidant and antiproliferative activities of raspberries. *J. Agric. Food Chem.* **50**: 2926-2930.

Mabry TJ, Markham KR and Thomas MB (1970) *Systematic identification of flavonoids* Springer-Verlag, Berlin, p.198.

Parihar MS and Hemnanit T (2003). Phenolic antioxidants attenuate hippocampal neuronal cell damage against kainic acid induced excitotoxicity. *J. Biosci.* **28**: 121-128.

Sa´nchez-Moreno C (2002). Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Sci. Technol. Int.,* **8**: 121-137.

Sharififar F, Dehghn-Nudeh G and MirtaJaldini M (2009). MaJor flavonoids with antioxidant activity from *Teucrium polium* L. *Food Chem.,* **112**: 885-888

Stahl E (1969). Thin Layer Chromatography a Laboratory Handbook (2nd edn) George Allen and Unwinlid, London, Springer Berlin, p.880.

Tackholm V (1974). Students' Flora of Egypt (2nd edn.) Cairo University Press, Cairo, Egypt, pp.425-426.

Tawaha K, Alali FQ, Gharaibeh M, Mohammad M and El-Elimat T (2008). Antioxidant activity and total phenolic content of selected Jordanian plant species. *Food Chem.,* **104**: 1372-1378.

Yang J, Guoa J and Yuan J (2008). *In vitro* antioxidant properties of rutin. *LWT,* **41**: 1060-1066.

Zhisen J and Mengcheng JW (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.,* **64**: 555-559.