

PHENOLIC COMPOUNDS OF *Convolvulus arvensis* L. AND THEIR RELATED PHARMACOLOGICAL ACTIVITY

Awaad, Amani S.; Nawal H. Mohamed; N. H. El-Sayed *;
G.A. Soliman** and T.J. Mabry***

Medicinal and Aromatic Plants Dept., Desert Research Center, El Matareya,
Cairo, Egypt.

*Protein and Leather Staining Dept., National Research Center, El Dokky,
Cairo, Egypt.

**Pharmacology Dept., Faculty of Veterinary, Cairo University, Egypt.

*** Molecular Cell and Developmental Biology Dept., Texas University,
Austin, TX.78713. U.S.A.

E-mail: amaniawaad@hotmail.com

The three organs of *Convolvulus arvensis* L. (flower, green parts and root) were phytochemically studied for their phenolic compounds content. Four coumarin compounds were isolated and identified as umbeliferone, scopoletin, asculetin and scopoline for the first time from the plant. Eleven flavonoidal compounds were isolated for the first time from different plant organs and identified by using ^1H NMR, ^{13}C NMR HMQC and UV shift reagent, these compounds were kaempferol, kaempferol-3-rutinoside, kaempferol-7-rutinoside, kaempferol-7-glucoside, kaempferol-3-glucoside, kaempferol-3-rhamnoside-7-glucoside, kaempferol-3-galactorhamnoside, quercetin, quercetin-3-rhamnoside, quercetin-3-rutinoside and kaempferol-3-rhamnoside. A quantitative estimation of the total flavonoids in methanolic extracts of the three organs of the plant were carried out by spectrophotometric method. Pharmacological screening in addition to the side effects on both liver and kidney functions were carried out for different plant organ extracts.

Keywords: Flavonoids, *Convolvulus arvensis*, Pharmacological activity, phenolic compounds

From the earliest times, it is well known that prehistoric man used to depend completely on natural resources for treating diseases, often based on guess work, which of course could lead to recovery or death. However, with modern technology of today, 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.

Thus the change of orientation in the philosophy of therapeutic medicine i.e, from the use of synthetic compounds to compounds of natural origin could be interpreted, therefore, as a progress forward in the backward direction.

The Arab world and Egypt, in particular, has not heritage in the field of medicinal plants. In fact, this heritage goes back as far as the Chinese heritage, if not even older. However, in China as well as in Japan, they were able by systematic studies and applying modern technology, to explore their natural resources of medicinal plants so that more than 80% of their diseases are now treated by what they call "traditional medicine".

So nowadays the world is intended to go back to the use of crude drugs for the treatment of various diseases but undoubtedly the plant kingdom still holds many species of plants containing substances of medicinal value, which have not yet been discovered.

Family Convolvulaceae is one of these plant kingdom, which includes a number of very important medicinal plants which can be used in treatment of many diseases such as headache, constipation, rheumatism, diabetics and skin diseases (Al Antaki, 1952, Ibn Sina 1968, Egyptian Pharmacopoeia 1984).

Convolvulus species are widely distributed all over Egypt in different localities, some of them have some medicinal activity in folk medicine as purgative, cathartic and in treatment of cough and asthma. These species are *Convolvulus althaeoides* and *Convolvulus hirtus* (Bolus, 1983 and Shahina, 1994).

A review of the current literature showed that many compounds had been previously isolated and identified from *Convolvulus* species belonging to different classes including alkaloids (Griffen and Lin, 2000 and Jenett *et al.* 1998), sugars (Son *et al.*, 1991 and El-Kashori, 1983), phenolic compounds (Kaneta *et al.* 1999; Noda *et al.* 1994 and El-Nasr 1983), sterols (Roose *et al.*, 1991 and Daulatabad *et al.*, 1992) and resins (Ono *et al.*, 1989 and Kogestu *et al.*, 1991).

The investigation of *Convolvulus arvensis* L. and its evaluation as a potential source of active constituents was therefore planned. The plant is very common in Egypt (Täckholm, 1974).

MATERIALS AND METHODS

Plant Materials

The aerial parts (leaf, stem and flower) and root of *Convolvulus arvensis* L. were collected from El-Arish (North Sinai) during 1999 and identified by Prof. N. El-Hadidi, professor of Botany, Botany Department,

Egyptian J. Desert Res., 54, No.1 (2004)

Faculty of Science, Cairo University and by comparison with plant description in flora of Egypt as well as herbarium specimens at Desert Research Center, Egypt.

Materials, Solvent Systems and Reagents for Chromatography

Adsorbents: pre-coated silica gel 60 F254 plates (E-Merck) for TLC and silica gel 60, (70-230 mesh, Merck) for column chromatography,

Solvent systems: (a) benzene-ethyl acetate (86:14), (b) ethyl acetate – methanol – water (30:5:4), (c) butanol – acetic acid – water (4:1:5), (d) ethyl acetate – methanol acetic acid – water (65:15:10:10) and (e) acetic acid – water (15:85) were used for developing the chromatoplates.

The following chromatographic reagents were prepared (Stahl, 1964):

- 1- Aluminum chloride, ammonia vapors and UV light for flavonoids.
- 2- Hydroxylamine-ferric chloride (for lactones and ester), ammonia vapors and UV light (366) for coumarins.

Apparatus

- 1- Pye Unicam pu 8800 spectrophotometer for UV spectral analysis.
- 2- Varian 500 MHz spectrometer for ^1H NMR (TMS as int. st.).
- 3- Kofflor's hot stage apparatus for melting points (uncorrected).
- 4- Mass spectra were recorded using EI mass (Chro N29 MY 5526) Ver. Ion Uie
- 5- Kymograph (B-Braun Melsungen AG. 86106).
- 8 - Apparatus for histamine-induced asthma in guinea pig.

Procedures

Extraction and Isolation of the Phenolic Compounds

One kg of each powdered organ under investigation (flower, green parts and root) was defatted then extracted in a soxhlet apparatus with 90% ethanol. The concentrated ethanolic extracts (119.1, 118.9 and 90.2 g) for flower, green parts and root respectively, were separately diluted with water (200 ml) then successively shaken with ether, chloroform, ethyl acetate, and n-butanol. Each extract was dried over anhydrous sodium sulphate and concentrated to yield the following dry extracts (6.2, 9.5, 12.7 and 30 g), (7.1, 14.2, 16.1 and 33.6g), (5.1, 7.4, 9.2 and 20 g) for flower, green parts and root respectively. The rest of the ethanolic extract was only a mixture of different salts, pectin's, proteins and resinous materials.

TLC examination of the different extractives adopting solvent systems (a and b) and visualizing reagents (1 and 2) suggested the presence of coumarins in ether and chloroform extracts and flavonoids in ethyl acetate extract. The n-butanol extract was found to have a lot of resinous material.

Accordingly, coumarins were isolated from the combined ether and chloroform extracts using column chromatography over silica gel and eluted gradually with benzene – ethyl acetate, where compounds 1- 4 were isolated and reapplied on preparative layer chromatography (system a).

The ethyl acetate extract was subjected to preparative layer chromatography (system b) followed by repeated preparative paper chromatography (systems c and e). Bands corresponding to each flavonoid were separately extracted with methanol, concentrated and submitted to a column of Sephadex LH-20 eluted with methanol – water where compounds 5-16 were isolated.

Identification of the Isolated compounds

Acid Hydrolysis

Five milligram of each glycoside was refluxed with methanolic H_2SO_4 (5 ml MeOH, 5 ml H_2O , 1 ml H_2SO_4) for 3 hr. The reaction mixture was diluted with water and the released aglycone extracted with ether. The aqueous layer was neutralized, extracted with pyridine and the concentrated pure sugar residue was dissolved in 10 % isopropanol for identification using systems d and e.

Compound 1: (30 mg) white crystals, $R_f = 0.33$ (system a); mp. 203-204°C. UV λ_{max} (MeOH): (nm) 228, 252, 260, 295, 344; (NaOAc) 245, 277, 391. ^1H NMR (DMSO-d_6): δ 7.9 (1H, J=9, H-4); δ 7.2 and 6.75 (2H, 2S, H-5 and H-8, respectively); δ 6.2 (1H, d, J=9 Hz, H-3) and δ 3.8 (3H, S, OCH_3). This compound was identified as Scopoletin.

Compound 2: (25 mg), white crystals, $R_f = 0.45$ (system b); mp. 214-216 °C. UV λ_{max} (MeOH): (nm) 276, 347; (NaOAc) 258, 389. ^1H NMR (DMSO-d_6): δ 7.95 (1H, d, J=9 Hz, H-4); δ 6.7 (1H, d, J=8 Hz, H-1 glucose); sugar protons at 3.33- 3.78. H-5); δ 7.1 (1H, S, H-8); δ 6.31 (1H, d, J=9 Hz, H-3) and δ 3.9 (3H, S, OCH_3). ^{13}C NMR (DMSO): δ 160.40 (C-2) 112.14 (C-3), 145.88 (C-4), 109.60 (C-5), 113.19 (C-6), 149.80 (C-7), 102.91 (C-8), 153.67 (C-9), 112.23 (C-10), 55.90 (O-CH_3), 100.00 (C-1'), 75.8 (C-2'), 76.64 (C-3'), 71.70 (C-4'), 77.01 (C-5'), 69.99 (C-6'). This compound was identified as Scopolin.

Compound 3: (25 mg), white crystals; $R_f = 0.75$ (system a); mp. 272- 274 °C. UV λ_{max} , (MeOH): (nm) 244, 257, 320, 324. EI-MS m/z (% rel. int.): 162 (M^+) (100), 134 (15), 106 (10), 78 (9), 77 (10), 51 (5). ^1H NMR (DMSO-d_6): δ 7.79 (1H, d, J=9.5 Hz, H-4); δ 7.42 (1H, d, J=8.4 Hz, H-5); δ 6.77 (1H, dd, J=8.4, J=2.2 Hz, H-6); δ 6.71 (1H, d, J=2.2 Hz, H-8) and δ 6.1 (1H, d, J=9.5 Hz, H-3). ^{13}C NMR (DMSO) 161.101 (C-2'), 121.40 (C-3'), 145.10 (C-4'), 129.78 (C-5'), 113.42 (C-6'), 162.11 (C-7'), 103.10 (C-8'), 146.5 (C-9'), 112.34 (C-10'). This compound was identified as Umbelliferone.

Compound 4: (20 mg), needle crystals; $R_f = 0.12$ (system a); m.p. 272-274 °C. UV λ_{max} (MeOH): (nm), 226, 257, 293 and 346 nm. EI-MS m/z (% rel. int.): showed M^+ at 179 (100), 150 (20), 132 (3), 122 (22), 121 (18), 94 (20) and 69 (10). ^1H NMR (DMSO-d_6): δ 7.9 (1H, d, J=9.5 Hz, H-4); δ 7.1 (1H, S, H-5); δ 6.79 (1H, S, H-8) and δ 6.2 (1H, d, J=9.5 Hz, H-3). ^{13}C NMR (DMSO) 161.28 (C-2'), 110.77 (C-3'), 144.60 (C-4'), 112.25 (C-5'), 148.40

(C-6'), 150.33 (C-7'), 102.53 (C-8'), 142.84 (C-9'), 110.77(C-10'). This compound was identified as Esculetin.

Compound 5: (20 mg), yellow needle crystals, R_f = 0.7 (system b), mp. 313-315 °C, UV λ_{max} , (MeOH): (nm) 255, 269 (sh), 370; (AlCl₃): 270, 290 (sh), 455; (AlCl₃/HCl): 270, 357, 426; (NaOAc): 274 (Dec.), 325; (NaOAc/H₃BO₃): 261, 385; (NaOMe): 246, 330, 398. ¹H NMR (DMSO-d₆): δ 8.16 (1H, d, J=2Hz, H-6); δ 7.67 (1H, d, J=2Hz, H2'); δ 7.6 (1H, dd, J_{6,2'} = 8Hz, H-6'); δ 6.80 (1H, d, J = 8 Hz, H-5') and δ 6.3 (1H, d, J=2Hz, H-8). This compound was identified as Quercetin.

Compound 6 : (20mg), yellow crystals, R_f = 0.79(system b). UV λ max in MeOH : (nm) 260, 300 (sh.), 358 (AlCl₃) 272, 300 (sh.), 440 (AlCl₃/ HCl) 272, 300 (sh.), 420 (NaOAc) 275, 325 (sh.), 395 (NaOAc/H₃BO₃) 260, 325 (sh.), 375 (NaOMe) 272, 320 (sh.), 430. ¹H-NMR (DMSO- d₆) : δ (ppm) 7.7 (1H, d, J=2.5 Hz, H2'), δ 7.5 (1H, dd, J = 8.5, 2.5 Hz, H6); δ 6.8 (1H, d, J = 8.5 Hz, H5'); δ 6.5 (1H, d, J = 2.5 Hz, H6); δ 6.2 (1H, d, J = 2.5 Hz, H8); δ 5.4 (1H, d, J=2 Hz, H1'' rhamnose); δ 3.2-3.9 (m, remaining sugar protons) and δ 1.2 (3 H, J=6 Hz, CH₃ rhamnose). This compound was identified as quercetin-3-O- α -L-rhamnoside.

Compound 7: (600mg), yellow crystals, R_f = 0.5 (system b), m.p 190 °C. UV λ_{max} , (MeOH) : (nm) 256, 265(sh.), 290, 355; (AlCl₃) : 274, 302(sh.), 330(sh.), 432; (AlCl₃ /HCl): 270, 298, 359, 399; (NaOAc): 272, 324, 398; (NaOAc/H₃BO₃): 263, 292(sh.), 368; (NaOMe): 272, 310, 410. ¹H NMR (DMSO -d₆): δ 8.10 (1H, d, J= 2Hz , H2') ; δ 7.86 (1H, d, J=8Hz, H-6'); δ 6.89 (1H, d, J=8Hz, H-5') ; δ 6.65 (1H, d ,J=2Hz, H-8); δ 6.5 (1H, d, J=2Hz H6); δ 5.13 (1H, d, J=7.50Hz H1''); δ 4.55 (1H, d, J=1.3Hz, H1'''); δ 3.82 (1H, dd, J=10Hz, J=2Hz H6''); δ 3.65 (1H, dd, J=3.5, H2'''); δ 3.47-3.87 (6H, m, Sugar protons) and δ 1.23 (3H, d, J=6 CH₃). This compound was identified as Quercetin -3-O - rutinoside (Rutin).

Compound 8 : (20 mg) yellow crystals, R_f = 0.91(system b), m.p. 277° C, UV, λ max in MeOH : nm 367, 268 ;(AlCl₃): 265, 350, 420 ;(AlCl₃/ HCl): 265, 350, 420; (NaOA) : 275, 300(sh), 380 ;(NaOAc/H₃BO₃) : 267, 319(sh), 380;(NaOMe) : 285, 322, 430. ¹H-NMR (DMSO-d₆): δ 8.0 (2H, d, J = 8 Hz, H2' and H6'); δ 6.9 (2H, d, J = 8 Hz, H3' and H5'); δ 6.4 (1H, d, J = 2.5 Hz, H8) and δ 6.2 (1H, d, J = 2.5 Hz, H6). EI-MS m/z (% re. lent): 285(M⁺) (100), 258 (15), 229(16), 184(8), 121(22) and 93(10). This compound was identified as Kaempferol (Mabry *et al.*, 1970).

Compound 9: (15 mg), yellow crystals, R_f = 0.47(system b), UV λ max in MeOH : (nm) 267, 320, 353 : (AlCl₃) : 275, 355 (sh), 410; (AlCl₃/HCl): 270, 298(sh), 410; (NaOAc): 270, 310(sh), 385; (NaOAc/H₃BO₃) 270, 380 (NaOMe) 275, 320(sh), 400. ¹H-NMR (DMSO- d₆) : δ 8.1 (2H, d, J = 8.7 Hz, H2' and H6'); δ 6.8 (2H, d, J = 8.7 Hz, H3' and H5'); δ 6.4 (1H, d, J =

2.5 Hz, H8); δ 6.2 (1H, d, $J = 2.5$ Hz, H6); δ 4.4 (1H, d, $J = 2$ Hz, H1'' rhamnose); δ 5.4 (1H, anomeric proton, d, $J = 7$ Hz, H1'' glucose); δ 3-4 (m, remaining sugar protons) and δ 0.9 (3H, d, $J = 6$ Hz, CH₃ rhamnose). This compound was identified as kaempferol-3-rutinoside.

Compound 10: (23 mg), yellow crystals $R_f = 0.41$ (system b) UV λ max in MeOH : (nm) 260, 322, 366(AlCl₃) : 262, 300 (sh), 352, 423(AlCl₃/HCl): 260, 300(sh), 345, 420 ;(NaOAc): 260, 322, 380, 4199sh); (NaOAc/H₃BO₃): 260, 321(sh), 375 ; (NaOMe): 268, 430. ¹H-NMR (DMSO- d₆) : δ 7.8 (2H, d, $J = 9$ Hz, H2' and H6'), δ 7.2 (2H, d, $J = 9$ Hz, H3' and H5'), δ 6.8 (1H, d, $J = 2.5$ Hz, H8), δ 6.7 (1H, d, $J = 2.5$ Hz, H6), δ 4.5 (1H, d, $J = 2$ Hz, H1'' rhamnose), δ 5.1 (1H, d, $J = 7$ Hz, anomeric protons, H1'' glucose), δ 3-4 (m, remaining sugar protons) and δ 1.0 (3H, d, $J = 6$ Hz, CH₃ rhamnose). This compound was identified as kaempferol-7-rutinoside.

Compound 11 : (19 mg), yellow crystals $R_f = 0.86$ (system b) UV λ max in MeOH : (nm) 215, 330(sh), 360; (AlCl₃) : 270, 300(sh), 423; (AlCl₃/ HCl): 270, 300(sh), 420; (NaOAc) :275, 360; (NaOAc/H₃BO₃) :275, 330(sh), 390; (NaOMe) :275, 330(sh), 390. ¹H-NMR (DMSO-d₆): δ 8 (2H, d, $J = 8$ Hz, H2' and H6'), δ 6.9 (2H, d, $J = 8$ Hz, H3' and H5'), δ 6.3 (1H, d, $J = 2.5$ Hz, H8), δ 6.1 (1H, d, $J = 2.5$ Hz, H6), δ 5.3 (1H, d, $J = 7$ Hz, H1'' glucose, anomeric proton) and δ 3-4 (m, remaining sugar protons). This compound was identified as kaempferol-7-O- β -D-glucoside.

Compound 12 : (23 mg), yellow crystals $R_f = 0.82$ (system b) UV λ max in MeOH : (nm) 265, 325(sh), 350;(AlCl₃): 265, 290(sh), 420;(AlCl₃/ HCl) 265, 300, 420 (NaOAc) 215, 300, 365 (NaOAc/H₃BO₃) 270, 360 (NaOMe)275, 325, 400. ¹H-NMR (DMSO- d₆) : δ 7.9 (2H, d, $J = 9$ Hz, H2' and H6'); δ 6.7 (2H, d, $J = 9$ Hz, H3' and H5'); δ 5.6 (1H, d, $J = 2.5$ Hz, H8); δ 5.5 (1H, d, $J = 2.5$ Hz, H6); δ 5.1 (d, $J = 7$ Hz, H1'' glucose) and δ 3.1-3.8 (m, remaining sugar protons). EI-MS m/z (% re. lent): 447 (M⁺) (100), 285(95), 315(18), 448(68). ¹³C NMR (DMSO) δ : 153.3 (C-2); 132.6 (C-3); 174.3 (C-4); 159.6 (C-5); 98.6(C-6); 160.4(C-7); 95.5(C-8); 153.2(C-9); 103.4(C-10); 121.1(C-1'); 130.3(C-2'); 114.8(C-3'); 157.4(C-4'); 114.8(C-5'); 130.3(C-6'); 102.2 (C-1''); 75.4(C-2''); 79.0(C-3''); 71.2(C-4''); 79.0 (C-5''); 59.8(C-6''). This compound was identified as kaempferol-3-O- β -D-glucoside.

Compound 13: (25mg) yellow crystals m.p. 220-221, $R_f = 0.49$ (system b), UV λ max in MeOH : (nm) 257, 350; (AlCl₃): 257, 350, 400; (AlCl₃/HCl): 257, 350, 400; (NaOAc): 257, 260(sh), 355; (NaOAc/H₃BO₃): 257, 260(sh), 355; (NaOMe):270, 420. ¹H-NMR (DMSO- d₆): δ 7.2 (2H, d, $J = 8$ Hz, H2' and H6'), δ 6.8 (2H, d, $J = 8$ Hz, H3' and H5'), δ 5.8 (1H, d, $J = 2.5$ Hz, H8), δ 5.7 (1H, d, $J = 2.5$ Hz, H6), δ 5.4 (1 H, d, $J = 2$ Hz, H1'' rhamnose anomeric sugar proton), δ 5.1 (1 H, d, $J = 7$ Hz, H1'' anomeric glucose proton), δ 3-4 (m, remaining sugar protons) and δ 1.1 (3 H, d, $J = 6$ Hz, CH₃

rhamnose). This compound was identified as Kaempferol-3-O- α -L-rhamnosyl-7-O- β -D-glucoside.

Compound 14 : (21mg) yellow crystals, $R_f = 0.40$ (system b) . UV λ max in MeOH : (nm) 265, 350; ($AlCl_3$)265, 300(sh), 410 ($AlCl_3/HCl$) 265, 350, 410 (NaOAc)275, 300, 380 (NaOAc/ H_3BO_3) 270, 310, 375 (NaOMe)275, 330, 400. 1H -NMR (DMSO- d_6) : δ 7.9 (2H, d, $J = 8$ Hz, H2' and H6'), δ 6.8 (2H, d, $J = 8$ Hz, H3' and H5'), δ 5.8 (1H, d, $J = 2.5$ Hz, H8), δ 5.6 (1H, d, $J = 2.5$ Hz, H6), δ 5.4 (1 H, d, anomeric proton), δ 5.2 (1 H, d, $J=2$ Hz, H1''' rhamnose), δ 3-4 (m, remaining sugar protons) and δ 1.2 (3 H, d, $J=6$ Hz, CH_3 rhamnose). ^{13}C NMR (DMSO) : δ 153.2 (C-2); 132.6(C-3); 174.3(C-4); 159.6 (C-5); 98.8(C-6); 160.4(C-7); 93.9(C-8); 153.2(C-9); 103.4(C-10); 121.1(C-1'); 130.3(C-2'); 114.8(C-3'); 157.4(C-4') 114.7(C-5'); 130.3(C-6'); 102.5(1''); 71.2(2''); 73.2(3''); 68.5(4''); 75.5(5''); 59.8(6''); 101.3 (1''') δ 70.6 (C-2'''), 70.4 (C-3'''), 71.9(C-4'''), 70 (C-5'''), and δ 17.6 (C-6'''). This compound was identified as kaempferol-3-galactorhamnoside.

Compound 15: 23mg yellow crystals; its mp. 228-230 $^{\circ}C$, $R_f = 0.80$ (system b). UV λ max in MeOH: 265, 350 $AlCl_3$, 265, 300(sh), 405 $AlCl_3/HCl$ 265, 350, 405 NaOAc 270, 300, 380 NaOAc/ H_3BO_3 270, 310, 373 NaOMe 275, 330, 400. 1H -NMR (DMSO- d_6): δ 7.2 (2H, d, $J = 8$ Hz, H2' and H6'), δ 6.8 (2H, d, $J = 8$ Hz, H3' and H5'), δ 5.8 (1H, d, $J = 2.5$ Hz, H8), δ 5.7 (1H, d, $J = 2.5$ Hz, H6), δ 5.4 (1 H, d, $J=2$ Hz, H1'' rhamnose anomeric sugar proton), and δ 1.1 (3 H, d, $J=6$ Hz, CH_3 rhamnose). EI-MS m/z (% re. lent): 433(M^+), 303(25), 287(95), 275(45), 257(85), and 135(80). This compound was identified as kaempferol-3-O- α -L-rhamnosid.

Quantitative Estimation of Total Flavonoids

For quantitative estimation the method adopted was based on measuring the intensity of the colour developed when flavonoids were treated with aluminium chloride reagent, percentage was calculated as quercetin with reference to a pre-established standard calibration curve (Mabry *et al.*, 1970 and Balbaa *et al.*, 1981).

Pharmacological Study

Pharmacological Screening

Preparation of extracts

The air-dried powder, of the three plant organs (100 g each) were extracted with ethanol 95% (Soxhlet). The concentrated ethanolic extract was suspended in water (pH 6-7) in different concentrations and used for the *in vivo* experiments.

In vivo Experiments

Determination of Median Lethal Dose (LD₅₀)

The LD₅₀ of investigated extract was determined following Finney method (Finney, 1964) from which the therapeutic index was also calculated. The different organ extracts were subjected to the following experiments:

Anti-asthmatic effect (Laurence and Bacharach, 1964), antipyretic activity (Alpermann, 1972), the analgesic effect (Okun *et al.*, 1963), anti-inflammatory effect (Domenjoz *et al.*, 1955), diuretic effect, anti-ulcerogenic effect (Garge *et al.*, 1993), anti convulsing effect (Kerley *et al.*, 1956), tranquilizing activity (Robert, 1965), Anticoagulant activity (Dacie and Lewis 1984), effect on blood glucose level (Saleh *et al.*, 1974). Results obtained are shown in tables 1 -10.

In vitro Experiment

All total and successive plant extracts were used to study the following: effect on isolated rabbit's intestine (Staff of Pharmacology dept., University of Edinburgh 1970), effect on the uterine motility of rats (Finney, 1964), effect on the isolated heart of rabbits (Burn, 1952), effect on isolated tracheal strips of guinea pigs (Dungan and Lish, 1962).

Antitumor Activity (Cytotoxic Activity)

Tumor Cells

Human tumor cell lines U251 and MCF7 (brain tumor and breast carcinoma cell lines respectively). Measurement of Potential Cytotoxicity by SRB Assay (El- Kashori, 1983).

Effect on Liver and Kidney Functions

Single Administration

Twenty mature albino rats of 150-180 gm b. wt. were divided into 5 equal groups. The 1st group was left as a control, while the 2nd and 3rd groups were administered a single oral dose of the plant extracts (400 mg/kg), blood samples were collected from the orbital plexus of rats, 6 hr after medication. Samples were left to clot at room temperature for 20 min. The obtained sera were collected and used to determine the activity of (AST) aspartate aminotransferase and (ALT) alanine aminotransferase (Reitman and Frankel, 1957). Levels of urea (Fawcett and Scott 1960), creatinine were also estimated (Husdan and Rapoport, 1968)

Repeated Administration

Mature rats of 150-180 gm b. wt. were divided into 4 equal groups. The 1st group was left as a control, while the 2nd, 3rd and 4th groups (6 rats each) were orally given the plant extracts in a dose (400 mg/kg) for 15 days. Blood samples were collected from each rat and sera were separated. Both the activity of AST and ALT and concentration of urea and creatinine were estimated as mentioned before. Results are shown in tables 11 and 12.

RESULTS AND DISCUSSION

Isolated Compounds

Compounds 1, 3, 4, 5, and 8 were identified by comparing their TLC chromatograms, UV spectram in methanol and with different shift reagents, EI-MS, ^1H NMR and ^{13}C NMR spectra with authentic samples and published data. Compound 5 and 8 migrated with quercetin and kaempferol and their spectral data were in agreement with those published for these compounds (Mabry *et al.*, 1970). Compounds 1, 3, 4 were found to be Co-chromatographically identical with scopoletin (Murray *et al.*, 1970), umbelliferone (Murray *et al.*, 1982) and esculetin, respectively (Fig. 1). Acid hydrolysis of compound 2 released glucose, which has been identified as before, and its aglycon was found to be similar to scopoletin when co-migrated with the authentic on TLC (system f).

Acid hydrolysis (Harborne *et al.*, 1975) of compounds 6 and 7 revealed the sugars rhamnose and rhamnose glucose respectively which identified by PC and TLC (system c and f), and an aglycon which was found to be identical with compound 5 when compared with its TLC, UV shift reagents. They are substituted at position 3 as indicated by their UV spectra upon addition of diagnostic shift reagent, so from data given and by comparison with published data (Geissman, 1962, Mabry *et al.*, 1970 and Harborne *et al.*, 1975), these compounds were identified as quercetin 3-O-rhamnoside and Quercetin-3-O-rutinoside (Rutin).

Upon acid hydrolyses(compound 15) revealed the sugars rhamnose, (compound 11 and 12) glucose and rhamnose, (compond 9,10, 13 and 14) galactose and rhamnose. their sugars were identified by PC and TLC (system c and d), and the aglycon was found to be identical with compound 8 when compared with its TLC, UV shift reagents. The sugar was linked at C_1 for compouns 9,12,13 and 14 and at C_7 for compound 10 and 11 as included from their UV spectra upon addition of diagnostic shift reagent, so from data given and by comparison with published data these compounds were identified as kaempferol-3-rutinoside, kaempferol-7-rutinoside, kaempferol-7-O- β -D-glucoside, kaempferol-3-O- β -D-glucoside, kaempferol-3-O- α -L-rhamnosyl-7-O- β -D-glucoside, kaempferol-3-galactorhamnoside, kaempferol-3-O- α -L-rhamnosid.

Total flavonoid estimation by spectrophotometer yielded 3.2, 1.0 and 0.5 % for flower, green parts and root respectively calculated as quercetin.

Pharmacological Activity

In vivo Experiments

Determination of Median Lethal Dose (LD_{50})

The ethanolic extract of *Convolvulus arvensis* was non-toxic (LD_{50} over 5 g/kg b. wt.), this indicate that the plant is highly safe for human use. This was proved by its use as a source of food for goat (Shahina, 1994).

The results of pharmacological study proved that there has no anti-asthmatic effect of the plant as stated in folk medicine (Bolus, 1983). The plant has neither antipyretic (Table 1), analgesic (Table 2), anti-inflammatory (Table 3), anti-ulcerogenic (Table 4) nor anticonvulsant activity (Table 5).

The present work revealed the moderate diuretic (Table 6) and tranquilizing activity (Tables 7 and 8) of both root and flower extracts.

Both leaves and root are used as anti-hemorrhagic, but the results obtained shows that both green parts and flower have anticoagulant (Table 9) activity at a dose of 400 mg/kg body wt., this may be due to the presence of coumarins. Successive extracts of both green part and root also exert the same effect as the total alcoholic extracts

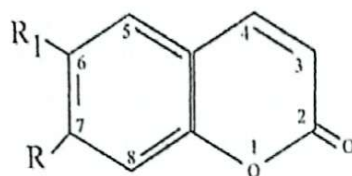
Concerning hypoglycemic effect, it was observed that green part has significant effect, which may be attributed to the presence of flavonoids. It was also observed that green part extract has hypoglycemic effect (Table 10) on diabetic rats only and not normal one which prove the safety of the plant.

The result of *in vitro* experiments revealed that the plant extracts contain a mixture of pharmacologically active compounds that would exert various effects on various isolated organs. From this it can be concluded that the plant can be used to relieve intestinal and uterine pain since it decreases their motility. The plant also inhibits the force of heart contraction. Furthermore on investigation of both liver and kidney functions, it was observed that the different plant extracts significantly decrease both liver (AST and ALT) and kidney (Urea and Creatinine) functions. It was reported that *convolvulus arvensis* plant is used to treat Jaundice due to the presence of convolvuline resinous glycosides and so more studies are needed to decide whether the plant can be used to treat liver diseases or not. Results are reported in tables (11 and 12). It was also found that different plant extracts have no anti-cancer (brain and breast cancer) activity.

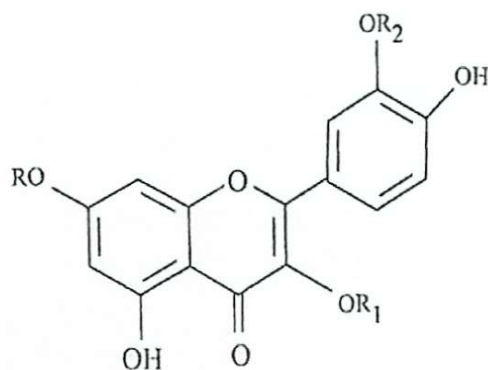
TABLE (1). Antipyretic effect of different extracts of *Convolvulus arvensis* in rats (n=5).

Group	Dose (mg/kg b.wt.)	Mean rectal temperature (°C) after			
		1 hr	2 hr	3 hr	4 hr
Control	0	38.7±0.7	38.8±0.7	38.8±0.6	38.6±0.7
Paracetamol	50	36.5±0.7*	36.3±0.7*	36.4±0.6*	36.8±0.7*
Flower	400	38.3±0.6	38.2±0.7	37.8±0.7	38.2±0.7
Green part	400	38.1±0.6	38.1±0.6	38.1±0.7	38.1±0.6
Root	400	38.0±0.6	38.1±0.6	38.2±0.6	37.9±0.6

*Significant at $p \leq 0.05$



	R	R ₁
1. Scopoletin	OH	OCH ₃
2. Scopolin	glucose	OCH ₃
3. Umbelliferon	OH	H
4. Esculetin	OH	OH



	R	R ₁	R ₂
5. Quercetin.	H	H	H
6. Quercetin 7-O-rhamnoside .	rh.	H	H
7. Quercetin -3-O - rutinoside (Rutin).	H	rh.gl	H
8. kaempferol.	H	H	H
9. kaempferol-3-rutinoside .	H	rh.gl.	H
10. kaempferol-7-rutinoside.	rh.gl.	H	H
11. kaempferol-7-O-β-D-glucoside.	Gl.	H	H
12.kaempferol-3-O-β-D-glucoside.	H	gl.	H
13.kaempferol-3-O-α-L-rhamnosyl-7-O-β-D-glucoside.	gl.	rh.	H
14. kaempferol-3-galactorhamnoside.	H	gal.rh.	H
15. kaempferol-3-O-α-L-rhamnosid.	H	rh.	H

Figure 1. The isolated compounds of *Convolvulus arvensis*.

TABLE (2). Analgesic effect of the extracts of *Convolvulus arvensis* in mice (n=5).

Group	Dose (mg/kg b.wt.)	% Protection against writhing after			
		1 hr	2 hr	3 hr	4 hr
Control	0	0	0	0	0
Paracetamol	50	100	80	60	40
Flower	400	60	40	20	0
Green part	400	40	20	0	0
Root	400	40	20	20	0

TABLE (3). Anti-inflammatory effect of the extracts of *Convolvulus arvensis* in mice (n=5).

Group	Dose (mg/kg b.wt.)	Thickness of paw in mm after			
		1 hr	2 hr	3 hr	4 hr
Control	0	7.5±0.21	7.6±0.24	7.5±0.25	7.5±0.25
Paracetamol	50	6.7±0.25*	6.6±0.21**	6.2±0.26*	6.4±0.23*
Flower	400	7.8±0.24	7.4±0.24	7.3±0.21	7.7±0.25
Green part	400	7.4±0.27	7.1±0.26	7.2±0.25	7.5±0.24
Root	400	7.3±0.24	7.4±0.27	7.5±0.24	7.1±0.26

*, ** Significant at $p \leq 0.05$ and $p \leq 0.01$, respectivelyTABLE (4). Anti-ulcerogenic effect of the extracts of *Convolvulus arvensis* in mice (n=5).

Group	Dose (mg/kg b. wt.)	Number of gastric ulcer
Control	0	8.16±0.26
Flower	400	7.83±0.25
Green part	400	7.39±0.26
Root	400	7.37±0.29

TABLE (5). Anticonvulsant effect of the extracts of *Convolvulus arvensis* in mice (n=5).

Group	Dose (mg/kg b. wt.)	Time of death after injection of strychnine
Control	0	7.83±0.16
Na Phenobarbital	40	102.87±7.89***
Flower	400	7.49±0.18
Green part	400	7.62±0.19
Root	400	7.32±0.15

*** Significant at $p \leq 0.001$

TABLE (6). Effect of *Convolvulus arvensis* extracts on urine volume of rats (n=5).

Group	Dose (mg/kg b.wt.)	Volume of urine (ml) within 24 hrs
Control	0	7.42±0.27
Furesemide	20	13.86±0.86**
Flower	400	7.22±0.24
Green part	400	7.16±0.25
Root	400	8.25±0.24*

*, **Significant at p ≤ 0.05 and p ≤ 0.01, respectively

TABLE (7). Tranquilizing activity of *Convolvulus arvensis* extracts in mice using rotating rod test (n=5).

Group	Dose (mg/kg)	Time (sec) required by mice to fall			
		1 hr	2 hr	3 hr	4 hr
Control	0	138.6±11.6	139.5±11.4	138.6±11.6	140.6±12.5
Chlorpr-Omazin	4	20.5***±1.3	20.2***±1.3	36.6***±1.9	52.4***±2.8
Flower	400	99.6*±7.3	98.9±8.2	106.8*±9.7	137.5±10.3
Green part	400	129.4±9.4	124.7±10.1	127.6±10.5	135.5±11.5
Root	400	117.5±8.3	125.2±7.4	130.4±9.9	138.6±11.6

*, ***Significant at p ≤ 0.05 and p ≤ 0.001, respectively

TABLE (8). Tranquilizing activity of *Convolvulus arvensis* extracts in mice using traction test (n=5).

Group	Dose (mg/kg)	% of mice incapable to touch the wire after			
		1 hr	2 hr	3 hr	4 hr
Control	0	0	0	0	0
Chlorpr-Omazin	4	100	100	80	80
Flower	400	60	40	40	0
Green part	400	0	0	0	0
Root	400	0	0	0	0

TABLE (9). The effect of *Convolvulus arvensis* extracts on prothrombin time (PT) and partial prothrombin time (PTT) in rats (n=5).

Organ	Group	PT (in sec)	PTT (in sec)
	Control	21.4±1.08	32.4±1.7
Flower	Pet. ether	22.0±0.32	31.6±1.83
	Ether	20.0±0.0	20.1±2.1**
	Chloroform	21.0±1.1	23.4±1.2**
	Ethyl acetate	20.4±1.09	20.0±0.0**
	Alcohol	19.2±0.2	26.2±2.1*
	Total alcohol	21.2±1.16	26.3±1.5**
Green part	Pet. ether	22.0±0.32	22.0±0.81**
	Ether	20.0±0.0	18.6±0.93**
	Chloroform	20.1±0.01	19.2±0.2**
	Ethyl acetate	22.0±0.31	14.0±0.63***
	Alcohol	23.2±1.10	20.1±0.01**
	Total alcohol	23.5±1.2	21.2±1.16**
Root	Total alcohol	23.6±1.14	31.4±0.81

*, **and *** Significant at p ≤ 0.05, p ≤ 0.01 and p ≤ 0.001, respectively.

TABLE (10). Effect of single and repeated oral administration of different extracts on blood glucose level in normal and alloxan diabetic rats (n=5).

Group	Dose (mg/kg body wt.)	Blood glucose level (mg/dl)			
		normal		Alloxan	
		Single dose	Repeated dose	Single dose	Repeated dose
Control	0.0	122.5±6.22	118.2±5.81	357.7±10.9	314.4±12.5
Tolbutamide	100	64.1***±3.14	52.7***±3.06	197.5***±6.4	188.2***±6.7
Flower	400	120.1±6.12	124.5±6.2	316.4±8.25	399.7±9.1
Green part	400	117.2±4.90	126.5±4.5	314.0±7.5	248.4**±10.1
Root	400	123.2±6.5	120.5±5.31	318.0±4.7	354.7±10.1

, * Significant at $p \leq 0.01$ and $p \leq 0.001$, respectively

TABLE (11). Effect of single and repeated oral administration of different extracts on liver function activity in rats (n=5).

Group	Dose (mg/kg b. wt.)	Single		Repeated	
		AST u/l	ALT u/l	AST u/l	ALT u/l
Control	0	36.72 ±0.82	40.47 ±0.83	36.81 ±0.92	41.17 ±0.85
Flower	400	36.10 ±0.94	40.33 ±0.82	37.0 ±0.85	40.76 ±0.88
Green Part	400	35.28 ±0.84	39.32 ±0.72	31.65*** ±0.80	37.02** ±0.84
Root	400	35.12 ±0.64	40.28 ±0.57	29.12*** ±0.73	30.16*** ±0.99

, * Significant at $p \leq 0.01$ and $p \leq 0.001$, respectively

TABLE (12). Effect of single and repeated oral administration of *Convolvulus arvensis* extracts on kidney function test in rats (n=5).

Group	Dose (mg/kg b. wt.)	Single		Repeated	
		Urea mg%	Creatinine mg%	Urea mg%	Creatinine mg%
Control	0	52.65±1.34	0.66±0.04	54.62±1.48	0.65±0.03
Flower	400	51.46±2.55	0.64±0.04	49.0*±1.54	0.61±0.03
Green Part	400	51.82±1.22	0.65±0.02	50.67±1.36	0.64±0.02
Root	400	51.61±1.93	0.65±0.04	45.48**±1.89	0.55*±0.03

* and ** Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

RECOMMENDATION

From the present study, it was found that, after investigation of the safety limits of the plant extract on liver and kidney functions, *Convolvulus arvensis* could be used as a natural anticoagulant and in treatment of the liver

enzymatic disorders. Preparation of the crude methanolic extract in a proper pharmaceutical form is rather more suitable and agreeable than the ether or chloroformic extracts which may contain any remnants of harmful organic residues.

ACKNOWLEDGEMENT

This research was supported by a grant from the Robert A. Welch Foundation (F-130), Texas University, Austin, U.S.A.

REFERENCES

- Al-Antaki, D. (1952). In "*Tazkarit Oli El-Albab*". Vol. 1, Elhalaby Co., Cairo, p.193.(in Arabic)
- Alpermann, H. (1972). Berichuber pharmakologische untersuchungen mit fenbendazol. *Anteilung fur Pharmakologic*. 1: 863.
- Balbaa, S.I; S.H. Hilal and A.Y. Zaki (1981). In "*Medicinal plant constituents*". 3rd ed., General Organization of University Books, Cairo, Egypt, 644 pp.
- Bolus, L. (2000). In "*Flora of Egypt*". Vol. II, al Hadara Publishing, Cairo, Egypt, 249 pp.
- Bolus, L. (1983). In "*Medicinal plant of North Africa*". Reference Publications Inc. 68
- Burn, J.H. (1952). In "*Practical pharmacology*". Black Well Scientific Publication Ltd., Oxford, 250 pp.
- Dacie, J. and S. Lewis (1984). In "*Practical hematology*". 6th ed., Churchill Livingstone, Edinburgh, London and New York p. 60-70.
- Daulatabad, C.D; V.A. Desai; K.M. Hosamani and V. B. Hiremath (1992). Epoxy oleic acid in Quamoclit seed oils. *J. of The American Oil Chemistry Society*, 69(2):190-191.
- Domenjoz, R; W. Theobald and K. Morzdorf (1955). Inflammation inhibiting effect of sodium salicylate. *Arzmeim Forsch*, 5: 488-89.
- Dungan, K.W. and T.M. Lish (1962). Amidophrina (1) pharmacological characterization of a simpathomimetic alkyl- sulphonamido-phenethanolamini. *J. Allergi*, 32: 139.
- Egyptian Pharmacopoeia (1984). General Organization for Governmental Printing Affairs, Cairo, 483 pp.
- El-Kashori, Sayeda, A. (1983). Pharmacognostical study of certain *Convolvulus* species. *Ph.D. Thesis*, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

- El- Nasr, M.M. and E.M. Seif (1983). Coumarins of *Convolvulus lanatus* and *Convolvulus arvensis*. *Fitotrepia*, 53 (5-6), 189-90.
- Fawcett, J.K. and J.E. Scott (1960). Enzymatic determination of urea. *J. Clin. Path.*, 13: 156-59.
- Finney, D.J. (1964). In "Statistical methods in biological assay". Charl Griffen & Co. Ltd., London, 97pp.
- Geissman, T. A. (1962). In "Chemistry of flavonoids Compounds". The McMillan Company Ltd., New York, 320 pp.
- Glavin, G.B, and A.A. Mikhail (1976). Stress and ulcer etiology in the rat. *Physiology and behavior*, 16: 135-39.
- Grage, G.P; S. Nigam and C.W.Ogle (1993). The gastric antiulcer effects of the leaves of the neem tree. *Planta Medica*, 59: 215-217.
- Griffen, W.J. and G.D. Lin (2000). Chemataxonomy and geographical distribution of tropane alkaloids. *Phytochem*, 53(6): 623-637.
- Harborne, T. B.; T. J. Mabry and H. Mabry (1975). In "The Flavonoids", Chapman and Hall, London, 219 pp.
- Husdan, H. and A. Rapoport (1968). Estimation of creatinine by the Jaffe reaction. *Clin. Chem.*, 14: 222-238.
- Ibn Sina, A.H. (1968). In "Alkanun fil Tibb". Vol. I, El Halaby and Co., Cairo, 385pp. (in Arabic)
- Jenett, S.K; M. Petra; M. Mack; S. Karsten; J. Jasmin; W. Ludger and E. Eckart (1998). Tropane alkaloids with an unique type of acyl moiety from two *Convolvulus* species. *Phytochem.*, 49 (5): 1449-1451.
- Kaneta, M.; A.S. Nakagawa and G.D. Yuko (1999). Investigation and identification of antioxidative flavonol, quercetin and kaempferol glycosides in the unused part of food plant commonly consumed in Japan. *Food Sci. Tech. Res.*, 5 (2): 125-128. (Chemical Abstract, 133: 119297).
- Kerley, T.L.; A.G. Richard; R.W. Begley; B.E. Abreu and L.C. Weaver (1956). In "Medical Experiment", 285 pp.
- Kiho, T: Y.A. Hui and S. Ukai (1993). In "Biological and pharmaceutical Bulletin", 16,12:1291.
- Kogistu, H; K. Naoki; M. Tashio and L. Kazumoto (1991). Scammonin III-VI, resin glycosides of *Convolvulus scammonia*. *Photochemistry*, 30(3): 957-963.
- Laurence, D.R. and A.L. Bacharach (1964). In "Evaluation of drug activities pharmacometrics". Academic Press, London and New York, Vol. 2, 492 pp.

- Mabry, T.J.; K.R. Markham and M.B. Thomas (1970). In "*The systematic identification of flavonoids*". Springer-verlag, Berlin, 198 pp.
- Markham, K.R. (1982). In "*Techniques of flavonoid Identification*". Academic Press, London, New York, 235 pp.
- Murray, R. D.; J. Mendez and S. A. Brown (1982). In "*The Natural Coumarins, Occurrence, Chemistry, and Biochemistry*". John Wiley and Sons Ltd., Chichester, 509 pp.
- Noda, N.; I. K. Tsu; K. Miyahara and C. R. Yang (1994). "Resin glycosides xxi Tugualapins I-X, the resin glycosides having long chain fatty acid group from the root of *Marremia hungaiensis*". *Chemical and Pharmaceutical Bull.*, 42(10): 2011-2016.
- Okun, R. L; S. J. Liddon and L. Lasagana (1963). In "*Screening methods in pharmacology*". Academic Press, New York and London, p114-133.
- Ono, M.; K. Kubo; K. Miyahara and T. Kawasaki (1989). "Operculin I and II, new ether soluble resin glycosides (Jalapin) with fatty acid ester groups from *Rhizoma Jalapae Braziliensis* (Roots of *Ipomoea operculata*". *Chem. And Pharm. Bull.*, 37(1): 241-244.
- Reitman, S. and S. Frankel (1957). Glutamic oxalo acetic and glutamic pyruvic transaminases. *Am. J. Path.*, 28: 56-63.
- Robert, A. T. (1965). In "*Screening methods in pharmacology*". Academic Press, New York and London, p90-91.
- Roose, A; A. T. Robert; D.A. Bakker and H.E. Jan (1991). Process for isolation of hydroxy fatty acid derivatives from *Convolvulaceae* plants. *Eur. Bot. Appl. Ep.*, 320 (Chemical Abstarct, 115:26210).
- Saleh, S.; M. T. Abdel-Aziz and H. M. Ahmed (1974). "Effect of calcium chloride or EDTA when concomitantly administrated with tolbutamide on catecholamine metabolism in normal and alloxan-diabetic rats". *Bull. Fac. Pharm.*, Cairo Univ., 8: 159-166.
- Shahina, A.G. (1994). In "*Hand book of Arabian medicinal plants*". CRC. Press, p89-90.
- Skehan, S. (1990). Effect of anti-cancer agent on isolated cell line. *J. Nat. Cancer Inst.*, 82:1106-1112.
- Son, K.G; R.F. Severson; M.E. Snook and S.J. Kays (1991). Root carbohydrates, organic acids and phenolic chemistry in

- relation to sweet potato weevil resistance. *Hot Science*, 26 (10): 1305-1308.
- Staff of pharmacology depart. Univ. of Edinburgh (1970). In "Pharmacological experiments on isolated preparations". 2nd ed. Livingston, E. and S. Ltd., Edinburgh and London, 54pp.
- Stahl, E. (1969). In "Thin layer chromatography a laboratory handbook" 2nd ed. George Allen and Unwin Ltd London, Springer Berlin, 880 pp.
- Sundaresan, V; A.j.Britto, and A.J. De Britto (1999). Preliminary phytochemical studies on some medicinal plants of Tirunelveli Hills. *J. of Economic and Taxonomic Botany*, 23(2):377-380.
- Täckholm, V. (1974). In "Student's flora of Egypt". 2nd ed. Published by Cairo Univ. printed by Cooperative printing Co., Beirut, p425-430.
- Todd, F.G; F.R. Stermitz; P. Schultheis; A.P. Knight and D.J. Traub (1995). Tropane alkaloids and toxicity of *Convolvulus arvensis*. *Phytochemistry*, 39(2): 301-303.

Received: 06/03/2003

Accepted: 06/03/2004

