Egyptian J. Desert Res., 57, No.2, 333-349 (2007)

FLAVONOIDS, PHARMACOLOGICAL AND ANTITUMOR ACTIVITIES OF *Convolvulus althaeoides* L. EXTRACTS

Emad, M. Atta
Aromatic and Medicinal Plants Department, Desert Research Center, El-Matariya, Cairo, Egypt.

Eight flavonoid compounds were isolated from the alcoholic extract of *Convolvulus althaeoides* L. They are Kaempferol, kaempferol-3-rhamnoside 7- glycoside, quercetin, rutin (quercetin-3-rhamnoglucoside), kaempferol-3- rhamnoside, quercetin-3-glycoside, kaempferol-3,7-di-rhamnoside and kaempferol-3-rhamnoside-7-rhamnoxyloside. Their structures were elucidated by spectroscopic methods including Ultraviolet (UV), Electron impact mass (EI mass), 2 Dimensional Nuclear Magnetic Resonance for Hydrogen Proton and Carbon thirteen (^{1}H and ^{13}C 2D NMR), DEPT, Hetro nuclear Multiplier Bonding Correlation (HMBC) and Hetro nuclear Multiplier Quantum Correlation (HMQC) experiments. The butanol extract of the plant have high cytotoxic activity against Ehrlich tumor cell line, while other extracts and some isolated compounds showed a significant activity. Pharmacological screening was also performed which showed that repeated oral administration of rutin, aqueous and butanol extracts reduced the severity of gastric damage while rutin and aqueous extract in a dose of 300 mg/kg b.wt. increased the time required for blood coagulation. The volume of urine was significantly increased in rats administered with rutin, also the aqueous and butanol extracts produced 20% protection against writhing after 3-4 h of their oral administration.

Keywords: *Convolvulus althaeoides*, phytochemistry, Flavonoid, pharmacology, cytotoxic activity.

Family Convolvulaceae contains large number of species some of them were used as a source of human food and others as medicinal plants. In folk medicine there are many uses of these plants such as, a tonic (*Ipomoea digitata*) and (*Cressa cretica*); toothache (*Convolvulus hidentatus*) (Walter and Memary, 1977), purgative (*Merremia alata, Argyreia capitata* and *Ipomoea pedicellaris*), laxative (*Ipomoea indet*), for headache (*Ipomoea gracilis*) (Sirivon, 1973). *Ipomoea pes caprae* was used for rheumatoid and to treat dermatitis caused by the stink of jelly fish (Perry and Metzger, 1980

Scanned with CamScanner

and Hostettmann *et al.* 1995). Another important plant in this family is *Ipomoea batatas*, which is cultivated as a vegetable crop for the production of sweet potato tubers, it is very rich in vitamins B, C, D and G, and its leaves contain insulin like compounds, so it is used as an antidiabetic (Fawzy, 1985).

Flavonoid sulfate in family Convolvulaceae was detected by (Petra *et al.* 1999), they isolated three new flavonol compounds; quercetin-7-methyl ether-3,3′-disulfate, quercetin-3,7- dimethyl ether-4′-sulfate and quercetin-3′,4′,7-trimethyl ether-3-sulfate with three known compounds; quercetin-7-methyl ether-3-sulfate, kaempferol-7-methyl ether-3-sulfate and kaempferol-4′,7dimethyl ether-3-sulfate from *Argyria mollis* and *Ipomoea regnellii*.

Sundaresan *et al.* (1999) isolated two flavonoids; kaempferol-3-glucoside and kaempferol-7-rutinoside from *Evolvulus alsinoides*. Quercetin and kaempferol were determined by paper chromatography (PC) and UV spectroscopy in the unused parts of sweet potato (Kaneta *et al.* 1999).

Hilal *et al.* (1983) reported that *Convolvulus lanatus* showed a purgative activity on animals when its root and resins were added to the animal food, while Shabana *et al.* (1986), stated that: *Convolvulus lanatus* showed a significant hypoglycemic effect in fasted rats 1 hr. after administration and returned to normal after 3 hrs. whereas *Convolvulus althaeoides* showed persistent hypoglycemic effect 1 h. after administration. In China; the roots of *Marremia hungaiensis* are used to treat chronic hepatitis and children's hernia (Noda *et al.* 1994). Villasenov *et al.* (1998) tested the methanolic and aqueous extracts of both *Ipomoea aquatica* and sweet potato leaves for their antidiabetic activity, both plants gave positive activity. Malalavidhane *et al.* (2000) stated that *Ipomoea aquatica* leaf possess an insulin-like activity in both single (33%) and repeated (25%) oral administration.

Husu *et al.* (2000); showed that both green and purple leaves of sweet potato have high antioxidative activity. Sairam *et al.* (2001) evaluated the potential anti-ulcerogenic activity of *Convolvulus pluricaulis* (fresh juice) against various gastric ulcer models induced by ethanol and aspirin using two doses; it was found that both of two doses showed anti-ulcerogenic activity. It was worthy to carry out phytochemical and pharmacological study on this plant because there were no previous studies on it.

MATERIALS AND METHODS

The aerial part of *Convolvulus althaeoides* L., was collected from the Egyptian desert in 2002 and identified by Prof. Dr. N. El-Hadidi, Botany Department, Faculty of Science, Cairo University and by comparison with herbarium specimens at Desert Research Center. Dried in the shade, ground to fine powder and kept for phytochemical and pharmacological investigations.

Scanned with CamScanner

I- Phytochemical investigation

1- Determination of some active constituents

Quantitative estimation of total alkaloids

Total alkaloid of *Convolvulus althaeoides* was determined by two methods; acid-base titration and gravimetric, (Egyptian Pharmacoepia 1953; Woo *et al*. 1977 and Balbaa 1986).

Quantitative estimation of total flavonoid

The method adapted was based on measuring the intensity of the colour developed when flavonoids make a complex with aluminum chloride (Karawya and Aboutable 1982). The flavonoid content was calculated as rutin.

Quantitative estimation of total Tannins

The percentage of total tannin was carried out gravimetrically using cupper acetate method according to Makkar and Goodchild (1996).

Quantitative estimation of total Saponins

Total saponin contents were determined by quantitative method according to Balbaa (1986).

2- Isolation of flavonoid compounds

Extraction and purification

Two kg of the air-dried powder of *Convolvulus althaeoides* L. were extracted with 90% alcohol by percolation till exhaustion. For purification and salt removal the alcoholic extract was dried under reduced pressure at a temperature not exceeding 45°C. Residue lifted was dissolved in hot water (500 ml) and filtered in cotton pool to remove the chlorophyll and fatty matters, the aqueous extract then reconcentrated under reduced pressure and pooled drop wise on excess methanol with continuous stirring. The solution was then filtered, concentrated and re-dissolved in alcohol. This process was repeated several times till no further salt precipitated.

II- Chromatographic investigation

For investigation of the flavonoids, paper and thin layer chromatography were used.

1- Paper chromatography (PC):

One and two dimensional paper chromatography was preformed on a Whatman No 1 filter paper using the following systems. Stahl, E. (1969):

a- n-butanol- acetic acid- water (4:1:5 v:v) organic phase (BAW).

b- acetic acid- water (15: 85 v:v) one phase (15% AcOH).

2- Thin layer chromatography (TLC) Stahl, E. (1969):

Thin layer chromatography was done using the following systems:

a- ethyl acetate- methanol- water (30: 5: 4 v:v:v) one phase.

b- n-butanol- acetic acid- water (4: 1: 5 v:v:) organic phase.

Both PC and TLC chromatograms were air dried at room temperature, localization of zones or spots were carried out by examination under UV

Scanned with CamScanner

light before and after exposure to ammonia vapors or by spraying with $AlCl_3$.

III- Isolation and identification

The purified alcoholic extract was dissolved in 40 ml methanol and mixed with 10 gm silica gel G for column. The solvent was evaporated on water bath to form a free flowing dry powder. The powder was introduced on the top of a glass column (100 x 5 cm) containing 500 gm. of silica gel G for column chromatography packed by dry method using chloroform then ethyl acetate and methanol in a gradual increasing. Elution was done at a rate of 30 drops/ minute; each fraction (200 ml) was concentrated under reduced pressure at 45°C, and then subjected to TLC using system (c).

Six main collective fractions were collected from the column. These fractions were containing eight flavonoid compounds and subjected to preparative TLC using system (a) the bands corresponding to the flavonoid compounds were visualized under UV, eluted with methanol and water. The eluted bands were purified on column Sephadex LH20 using methanol and water as eluting system. Identification of the purified flavonoid compounds was done by, R_f values in systems BAW and 15% AcOH, melting point and spectral data.

IV- Biological activity

1- Pharmacological studies

Plant materials

The main flavonoid compound (rutin), butanol fraction and aqueous extract of the plant aerial part were subjected to pharmacological studies.

a- Antiulcerogenic effect

Twenty five male Sprague-Dawley rats, 220-250 g, were used. They were starved for 48 h before use (Grag *et al.* 1993) to ensure an empty stomach. To avoid dehydration during the period of fasting, rats were supplied with sucrose 8% (w/v) solution in NaCl 0.2% (w/v), which was removed one hour before experimentation (Glavin and Mikhail 1976). A control group was given distilled water (10 ml/kg) and 3 treatment groups administered one of the plant extracts in a dose of 300 mg/kg orally via a stomach tube. Two doses were given on the first day at 08:00 h and 16:00 h; a third dose was given on the second day 1.5 h. Rats of the positive control and the treatment groups were given ethanol 50% (v/v) (in distilled water) in a dose of 10 ml/kg orally to induce gastric ulceration. Another group of rats was kept as normal control. One hour after ethanol administration, all rats were killed by an overdose of chloroform and the stomachs were rapidly removed, opened along their greater curvature and gently rinsed under running tap water. Lesions in the glandular part of the stomach were measured under an illuminated magnifying microscope (10 x). Long lesions were counted and their lengths were measured. Petechial lesions were counted, and then each five petechial lesions were taken as 1 mm of ulcer

Scanned with CamScanner

(Cho and Ogle 1979). To calculate the ulcer index (mm), the sum of the total length of long ulcers and petechial lesions in each group of rats was divided by its number. The curative ratio was determined according to the formula:

$$\text{Curative ratio} = \frac{\text{(Control ulcer index)} - \text{(Test ulcer index)}}{\text{(Control ulcer index)}} \times 100$$

b- Anticoagulant activity

In this experiment, 20 rats (150-200 g) of both sexes were used. They were divided into 4 equal groups. The first group was kept as a control, whereas the 2nd, 3rd and 4th groups were orally administered rutin, aqueous and butanol extracts of the plant respectively in a dose of 300 mg/kg b.wt for 10 successive days. Blood samples were collected from the orbital plexus of rats through a heparinized capillary tube into glass tubes containing sodium citrate as anticoagulant. Blood samples were centrifuged at 3000 rpm for 15 min to obtain plasma. PT (Prothrombin Time) and APTT (Activated Partial Prothrombin Time) were determined in plasma according to the method of Dacie and Lewis (1984) using the spectrophotometer.

c- Effect on urine volume

Four groups of 5 rats were used. The 1st group was kept as a control, while the 2nd, 3rd and 4th groups were orally administered rutin, aqueous and butanol extracts of the plant, respectively in a dose of 300 mg/kg b.wt. Each rat was placed in a wire cage with a bottom of narrow mesh and hanged over a suitable funnel. The urine voided from each rat was collected within 24 h of administration in a test tube and measured in ml.

d- Analgesic activity

The analgesic effect of the tested extracts was evaluated using the writhing method as described by El-Hakim and Ain-Shoka (1995). Four groups of 5 mice each (20-25 g) were used. The first group was kept as a control, whereas the 2nd, 3rd and 4th groups were orally administered rutin, aqueous and butanol extracts of the plant respectively in a dose of 300 mg/kg b.wt. After 30 min, each mouse was injected intraperitonealy with 0.25 ml of p-benzoquinone aqueous solution (0.1 mg/ml). Thereafter, mice of all groups were observed for writhing every one hour for 5 h. animals devoid of writhing in each group were counted and the analgesic potency of the tested extracts was determined as % protection against writhing.

Statistical analysis

The results obtained were statistically analyzed using "t" test (Snedecor and Cochran 1976).

2- Antitumor activity (cytotoxic activity)

It was mentioned that *Convolvulus althaeoides* and *Convolvulus scammonia* were used in folk medicine for treatment of certain cancer tumor (El-Sayeda 1983).

Tumor cells: Ehrlich tumor cell lines

Scanned with CamScanner

Measurement of Potential Cytotoxic by SRB Assay

Potential Cytotoxicity of *Convolvulus althaeoides* extracts were tested using the method of Skehan *et al.* (1990).

Cells were plated in 96-multiwell plate (10^4 cells) for 24 hrs before treatment with the extracts to allow attachment of cells to the wall of the plate. Different concentrations of the different plant extracts (0, 1, 2.5, 5 and 10 µg/ml) were separately added to the cell mono-layer. Triplicates was prepared for each individual dose.

Mono-layer cells were incubated with the extracts for 48 hr at 37 °C and in atmosphere of 5% CO_2. After 48 hr. cells were fixed, washed and stained with sulfurhodamine β stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Colour intensity was measured in an enzyme linked immunasorbent assay (ELISA) reader, then the relation between surviving fraction and extracts concentration was plotted to get the survival curve of each tumor cell line after specified extract.

RESULTS AND DISCUSSION

Phytochemical studies

I- Determination of some active constituents

Some biologically active constituents were determined in table (1); which indicates that the plant contains a high percentage of flavonoids, tannins and saponins while the alkaloid percentage is higher in the green part extract than the flower extract.

Table (1). Some active constituents' percent of *Convolvulus althaeoides* L.

Sample	Total alkaloids%	Total flavonoid%	Total tannins%	Total saponins%
Flower	0.099	2.9	3.6	2.4
Green part	0.17	2.7	4.1	3.7

II- Isolation of flavonoid compounds

Eight flavonoid compounds were isolated, purified by column, PC and TLC chromatography and identified through UV shift reagent, ^1H and ^{13}C-NMR. These compounds were named E_1- E_8.

1- Kaempferol

Compound E_1 was obtained as yellow crystals, soluble in methanol. m.p. 277°C, R_f -values and colour reactions are outlined in table (2).

Scanned with CamScanner

Table (2). R_f values and colour reaction of flavonoids compounds isolated from *Convolvulus althaeoides*.

Compound	R_f X 100 in		Colour		
	BAW	AcOH	Vis.	UV	UV/NH_3
E_1	85	40	y	y	y
E_2	51	70	br	y	y
E_3	73	29	br	d.p.	y
E_4	49	54	y	d.p.	y
E_5	87	50	-	d.p	y
E_6	80	60	-	br	br
E_7	50	80	d.p	y	y
E_8	69	50	br	br	fl.y

Where y: yellow, fl.y.: floreseence yellow, d.p.: deep purple br.: brown, p.: purple and vis.: visible..

a- UV spectrum

UV spectral data, λ max in MeOH and shift reagents showed that:

1- Band I, II appeared at 367 and 268 nm respectively, which indicates that it is a flavonol type with free OH at position 3.

2- Addition of NaOH resulted a bathochromic shift in band I (+63 nm) which proved the presence of free OH group at position 4`.

3- Bathochromic shift in band II (+7 nm) occurred on addition of NaOAc, which indicates the presence of free OH at position 7. Addition of boric acid gave no shift indicating the absence of orthodihydroxy group at B-ring.

4- Bathochromic shift in band I (+53 nm) occurred on addition of $AlCl_3$ indicating the presence of free OH group at C-3 and C-5, this shift was not affected after addition of HCl indicating the absence of orthodihydroxy group at B- ring.

b- ^{1}H-NMR

The spectrum of compound E_1 in dimethyl sulfoxide acid DMSO-d6, showed signals at:

δ (ppm) 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 = 1.5 Hz, H8) and δ 6.2 (1H, d, J = 1.5 Hz, H6).

From UV analysis, compound E_1 is probably kaempferol. The structure of this compound was further confirmed by ^{1}H NMR spectrum in DMSO which showed the signals characteristic for kaempferol (Mabry *et al.* 1970).

2- kaempferol-3-O-α-L-rhamnosyl-7-O-β-D-glucoside

Compound E_2 was obtained as yellow crystals, soluble in methanol, its m.p. 220-221°C; R_f -values and colour reactions are recorded in table (2).

a- UV spectrum

UV spectral data of compound E_2 showed that:

1. The band I at 350 nm and band II at 265 nm showed that it might be flavonol in nature.

Scanned with CamScanner

2. The bathochromic shift in band I with addition of NaOMe (39 nm) with increase of intensity indicated the presence of free OH group at 4' positions.
3. With addition of NaOAc no significant shift appears which indicated the substitution of H in 7 positions.
4. Addition of NaOAc/ H_3BO_3, no bathochromic shift in bands I or II, which indicated the absence of orthodihydroxy groups at B-ring especially at 3' and 4' positions.
5. Addition of $AlCl_3$, no bathochromic shift in band I indicated the absence of orthodihydroxy groups also the presence of substitution at position 3.
6. After the addition of HCl no hypsochromic shift in band I in $AlCl_3$ spectrum indicated the absence of orthodihydroxy groups.

From colour reaction and UV spectral data compound (E_2) may be kaempferol with substitution at 3 and 7 positions (Mabry *et al.* 1970).

b- **Acid hydrolysis**

1- Complete acid hydrolysis:

Compound E_2 by complete acid hydrolysis with 2N HCl, yielded kaempferol in the ethyl acetate extract, the sugars released after hydrolysis was glucose and rhamnose.

2- Mild acid hydrolysis:

Compound E_2 was subjected to mild acid hydrolysis using 0.1 N HCl at 100 °C for 15 min. the reaction mixture was examined at definite intervals by CoPC. An intermediate was observed which has the same R_f -value and colour reaction of kaempferol-7-glucoside. After 45 min. kaempferol was detected.

c- ^{1}H-NMR

Spectrum of compound E_6 in DMSO- d6, showed the following signals:

δ (ppm) 8.1 (2H, d, J = 8.5 Hz, H2 and H6), δ 6.92 (2H, d, J = 8 Hz, H3 and H5), δ 6.2 (1H, d, J = 1.5 Hz, H8), δ 5.9 (1H, d, J = 1.5 Hz, H6), δ 5.4 (1 H, d, J=2 Hz, H 1'' 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 (3H,d, J=6 Hz, CH_3 rhamnose).

From UV compound E_2 may be kaempferol with substitution at position 3 and 7, the structure was confirmed by ^{1}H-NMR which showed the presence of rhamnose which is directly attached to the ring at position 3 and presence of glucose which directly attached to the ring at position 7 (Markham 1982; Mabry *et al.* 1970 and Markham 1989).

From above data E_2 identified as kaempferol-3- rhamnosyl-7-glucoside.

Scanned with CamScanner

3- Quercetin

This compound (E_3) was obtained as yellow crystals, soluble in methanol, its m.p. 322-324 °C, R_f -values and colour reactions are recorded in table (2).

a- UV spectrum

Compound E_3 was subjected to UV spectral analysis in methanol and shift reagents. The obtained result showed that:

1-Band I in methanol appear at 370 nm, indicating that the compound is a flavonol type with free OH at position 3.

2- The bathochromic shift in band I (+70 nm) with an increase of intensity by addition of NaOMe indicating the presence of free OH at position 4`.

3- The bathochromic shift occurred in band I (+11 nm) on addition of NaOAc indicating the presence of free OH at position 7, which deleted by H_3BO_3 addition, indicates the presence of orthodihydroxy group (3`,4` position).

4- The bathochromic shift in band I (+75 nm) appeared on addition of $AlCl_3$ indicated the presence of 3 and 5-OH free groups.

5- The hypthochromic shift of $AlCl_3$ spectrum in band I (-20 nm) after the addition of HCl indicates the presence of orthodihydroxy group in B-ring (3`,4` position).

Thus from UV and R_f -values compound E_3 may be quercetin, this was confirmed by ^{1}H-NMR.

b- ^{1}H-NMR spectrum

Spectrum of compound E_3 in DMSO- d6, showed the following signals:

δ (ppm) 7.7 (1H, d, J = 8.5 Hz, H2), δ 7.5 (1H, dd, J = 8.5, J=2.5 Hz, H6`), δ 6.8 (1H, d, J = 8.5 Hz, H5`), δ 6.5 (1H, d, J = 1.5 Hz, H6), and δ 6.2 (1 H, d, J=1.5 Hz, H-8).

From the previously mentioned data and by comparing with published data, (Crowford and Mabry 1978) compound E_3 identified as quercetin.

4- Rutin (quercetin-3-O-α-L-rhamnoside (1-6) β-D-glucoside)

Compound E_4 was obtained as yellow crystals; its mp. 190 °C, R_f -values and colour reactions are recorded in table (2).

a- UV spectrum

1-The absorption maxima in methanol, band I at 350 nm, indicates that it is a flavonol with 3-OH substitution.

2- The remaining UV spectral data were found to be similar to that of quercetin type compound.

b- ^{1}H-NMR spectrum

^{1}H-NMR spectrum give the following signals: δ (ppm) 7.6 (1H, d, J=2.5Hz, H2), δ 7.5 (1H, dd,J=8.5, 2.5Hz, H6`),δ 6.8 (1H, d, J= 8Hz, H5), δ 6.4 (1H, d, J= 1.5Hz, H8), δ 6.2 (1H, d, J= 1.5Hz, H6), δ 5.3 (1H, d, J=

Scanned with CamScanner

8Hz, H1`` glucose), δ 4.5 (1H, d, J=2.5Hz, H-1``` rhamnose), δ 3.4 (m, remaining sugar protons) and δ 0.8 (3 H, d, J=6 Hz, CH_3 rhamnose).

c- ^{13}C-NMR spectrum

^{13}C-NMR of E_4 gave the following peaks in DMSO-d6:

1- 174 for ketonic carbon C-4, at 164 for C-7, at 161 for C-5 at 156.7 and 156.4 for C-2 and C-9 respectively, at 148.5 for C-4` and 144.8 for C-3`.
2- 133.3 for C-3 appeared more up field indicating the presence of substitution at this carbon, followed by C-6` at 121.6, C-1` at 121.2, C-2` at 116.2 and C-5` at 115.2. The most non-effected carbon is C-10 at 103.8
3- Anomeric carbons appeared at 100.7 for C-1``` rhamnose and 101.3 for C-1``` glucose indicating the presence of two sugar moieties.
4- The lower affecting aromatic carbon C-6 and C-8 appeared at 98.8 and 93.7 respectively.
5- The remaining sugar carbons appeared at 76.5 (C-3``), 75.9 (C-5``), 74.1(C-2``), 71.9 (C-4```), 70.6 (C-2```), 70.4 (C-3```), 70 (C-5```), 62.8 (C-6``) and δ 17.6 (C-6```).

C6`` of glucose appeared shifted more down field at 62.8 ppm, so the linkage is 1-6 (Harborn *et al*. 1975).

d- Hetero nuclear multiple quantum correlation (HMQC)

The HMQC spectrum of compound E_4 in DMSO showed:

Correlation between δ 116.2 (C-2`); δ 7.6 (CH2`); δ 121.6 (C-6`); δ 7.5 (CH6`); δ 115.2 (C-5`); δ 6.8 (CH5`); δ 93.7 (C-8); δ 6.4 (CH8); δ 98.8 (C-6); δ 6.2 (CH6); δ 101.3 (C-1``); δ 5.3 (CH1`` glucose); δ 100.7(C-1```); δ4.5 (H1``` rhamnose); δ 133.3 (C-3 quercetin); δ 5.3 (H1`` glucose) which confirmed the presence of glucose at C-3.

From above data and by comparison with those published by Harborn *et al*. (1975), compound E_4 was identified as rutin (quercetin-3-O-α-L-rhamnoside (1-6) β-D-glucoside.

5- kaempferol-3-O-α-L-rhamnoside

Compound E_5 was obtained as yellow crystals; its m.p. 228-230 °C, R_f -values and colour reactions are recorded in table (2).

a- UV spectrum

UV spectral data of compound E_5 in methanol and shift reagent, from which it can be, concluded that:

Compound E_5 may be kaempferol with substitution at position 3, as it gave band I with methanol at 350 nm and band II at 265 nm, the remaining UV spectral data were found to be similar to that of kaempferol type.

b- Acid hydrolysis

A known weight of this compound was subjected to partial and complete acid hydrolysis using 0.1 N and 2 N HCl, this afforded kaempferol as the aglycone moiety and rhamnose as the sugar moiety.

Scanned with CamScanner

c- Mass spectrum

EI mass spectrum revealed the presence of molecular ion peak M^+ at m/e 433 and other important ions m/e 303, 287 kaempferol.

From UV, acid hydrolysis, EI mass spectrum and by comparison with authentic sample, compound E_5 could be kaempferol-3-O-α-L-rhamnoside.

6- quercetin-3-glucoside

This compound (E_6) was found to be yellow crystals, its mp. 228-230°C, R_f-values and colour reactions are recorded in table (2).

a- UV spectrum

UV spectral data of compound E_6 in methanol and shift reagent, from which it can be concluded that: Compound E_6 may be quercetin with substitution at position 3. The remaining UV spectral data were found to be similar to that of quercetin type.

b- ^{1}H-NMR spectrum

^{1}H-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, H 1`` glucose).

From UV, ^{1}H-NMR spectrum and by comparison with authentic sample, compound E_6 could be identified as quercetin-3-glucoside.

7- kaempferol-3,7-dirhamnosid

Compound E_7 was found to be yellow crystals, its mp. 233-234 °C R_f-values and colour reactions are recorded in table (2).

a- UV spectrum

UV spectral data of compound E_7 in methanol and shift reagent was coinciding with that of substituted kaempferol at 3 and 7 position (Mabry *et al.* 1970 and Markham 1989).

b- ^{1}H-NMR spectrum

^{1}H-NMR (DMSO- d_6): δ 7.8 (2H, d, J = 8.5 Hz, H2 and H6), δ 6.9 (2H, d, J = 8.5 Hz, H3 and H5), δ 6.8 (1H, d, J = 2.5 Hz, H8), δ 6.4 (1H, d, J = 2.5 Hz, H6), δ 5.55 (1 H, d, J=2.5 Hz, H 1`` rhamnose), δ 5.3 (1 H, d, J=2.5 Hz, H 1``` rhamnose), δ 3.4 (m, remaining sugar protons), δ 1.1 (3 H, d, J=6 Hz, CH_3 rhamnose), δ 0.8 (3 H, d, J=6 Hz, CH_3 rhamnose).

c- Acid hydrolysis

A known weight of this compound was subjected to partial and complete acid hydrolysis using 0.1 N and 2 N HCl, this afforded kaempferol as the aglycone moiety and rhamnose as the sugar moiety.

From UV, ^{1}H-NMR spectrum, acid hydrolysis and by comparison with previous published data, compound E_7 could be identified as kaempferol-3,7-dirhamnosid.

8- kaempferol-3-rhamnoside,7-rhamnoxyloside

Compound E_8 was found to be brownish crystals, its mp. 180-182 °C R_f-values and colour reactions are recorded in table (2).

Scanned with CamScanner

a- UV spectrum

UV spectral data of compound E_7 in methanol and shift reagent was coinciding with that of substituted kaempferol at 3 and 7 position (Mabry *et al.* 1970 and Markham 1989).

b- ^{1}H-NMR spectrum

^{1}H-NMR (DMSO- d_6): δ 7.8 (2H, d, J = 8.5 Hz, H2 and H6), δ 6.9 (2H, d, J = 8.5 Hz, H3 and H5), δ 6.8 (1H, d, J = 2.5 Hz, H8), δ 6.4 (1H, d, J = 2.5 Hz, H6), δ 5.55 (1 H, d, J=2.5 Hz, H 1`` rhamnose), δ 5.3 (1 H, d, J=2.5 Hz, H 1``` rhamnose), δ 4.25 (1 H,d,J= 8.5 Hz, H 1``` xylose), δ 2.9-3.8 (m, remaining sugar protons), δ 1.1 (3 H, d, J=6 Hz, CH_3 rhamnose), δ 0.8 (3 H, d, J=6 Hz, CH_3 rhamnose).

c- Acid hydrolysis

A known weight of this compound was subjected to partial and complete acid hydrolysis using 0.1 N and 2 N HCl, this afforded kaempferol as the aglycone (compared with authentic sample), rhamnose and xylose as the sugar moiety.

From UV, ^{1}H-NMR spectrum and acid hydrolysis, compound E_7 could be identified as kaempferol-3-rhamnoside,7-rhamnoxyloside.

III- Biological activity

I- Pharmacological studies

a) Antiulcerogenic effect

Gastric damage induced by ethanol was characterized by both long ulcers and petechial lesions. The number of ulcers and the ulcer index in control rats (received ethanol) were highly significant ($P<0.001$) when compared to normal untreated animals (received distilled water). Repeated oral administration of rutin, aqueous and butanol extract of the plant reduced the severity of gastric damage table (3).

Table (3). Effect of *Convolvulus althaeoides* (300 mg/kg b.wt) on ethanol-induced gastric damage in rats (n=5).

Treatment group	Number of ulcers (M±S.E)	Ulcer index (mm)	Curative ratio (%)
Normal control	0	0	-
Positive control	6.20±0.37[a]	8.39±0.22[a]	-
Rutin	3.60±0.24 ***	2.50±0.19 ***	70.2
aqueous extract	4.20±0.20 **	2.61±0.20 ***	68.89
butanol extract	3.40±0.24 ***	2.64±0.12 ***	68.53

[a] Compared to normal control ($P< 0.001$).

** $P< 0.01$ compared to positive control.

*** $P< 0.001$ compared to positive control.

b) Anticoagulant activity

The obtained results showed that oral administration of rutin and aqueous extract in a dose of 300 mg/kg b.wt, increased the time required for blood coagulation. This was manifested by the significant increase in PT and APTT values (Table 4).

Scanned with CamScanner

Table (4). Effect of repeated oral administration of *Convolvulus althaeoides* in a dose of 300 mg/kg b.wt on PTT and PTT in rats (n=5).

Treatment group	PT (sec)	APTT (sec)
Control	20.91±0.89	33.94±1.11
Rutin	29.43±1.23 ***	43.31±1.46 ***
aqueous extract	29.37±0.95 ***	45.08±1.24 ***
butanol extract	18.53±0.54	39.76±0.71

*** P< 0.001 compared to control.

c) Effect on urine volume

The volume of urine was significantly increased in rats administered rutin and butanol extract as compared with that of control group (Table 5).

Table (5). Effect of *Convolvulus althaeoides* (300 mg/kg b.wt) on volume of urine in rats (n=5).

Treatment group	Volume of urine (ml)
Control	2.54±0.16
Rutin	3.52±0.17 **
aqueous extract	2.62±0.18
butanol extract	3.46±0.23 *

* P< 0.05 compared to control.
** P< 0.01 compared to control.

d) Analgesic activity

Table (6) showed that 300 mg/kg b.wt of aqueous and butanol extract produced 20 % protection against writhing after 3-4 h of their oral administration.

Table (6). Analgesic effect of *Convolvulus althaeoides* (300 mg/kg b.wt) in mice using writhing method (n=5).

Treatment group	% Protection against writhing (hr)				
	1	2	3	4	5
Control	0	0	0	0	0
Rutin	0	0	0	0	0
aqueous extract	0	0	20	20	0
butanol extract	0	0	20	20	0

2- Antitumor activity

According to the results in table (7), the most effective extract of *Convolvulus althaeoides* L. against Ehrlich tumor cell lines was the butanol extract which have a cytotoxic effect 100% at different concentrations followed by flower extract while rutin have the minimum activity compared with the others.

Scanned with CamScanner

Table (7). Effect of *Convolvulus althaeoides* on Ehrlich tumor cell lines.

Sample	Inhibition of cell viability (Mg/ml).		
	100 µg/ml	50 µg/ml	25 µg/ml
Rutin	30%	20%	5%
Aqueous extract	65%	55%	50%
Flower total extract	85%	80%	75%
Butanol extract	100%	100%	100%

The anti-tumor and the pharmacological activity could be attributed to the presence of flavonoids as the plant contains many compounds with high yield. Flavonoids have been shown to have antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic, and vascodilatory activity. The potent antioxidant activity of flavonoids is their ability to scavenge hydroxyl radicals, superoxide anions, and lipid peroxy radicalsÑmay be the most important (Alan and Miller, 2001). Bioflavonoids such as Quercetin and Rutin are vital in their ability to increase the strength of the capillaries (blood vessels) and to regulate their permeability. They are essential for the proper absorption and use of vitamin C; prevent Vitamin C from being destroyed in the body by oxidation; connective tissues and builds a protective barrier against infections (Robak and Gryglewski, 1996).

REFERENCES

Alan L. Miller, N.D. (2001). In "*Antioxidant Flavonoids*" Structure, Function and Clinical Usage. Thorne Research Inc. 111 pp

Balbaa, S. I. (1986): In "*Chemistry of crude drugs*" Laboratory Manual. Fac. of Pharm. Cairo Univ. 195 pp.

Cho, C. H., and C. W. Ogle (1979). Cholinergic- mediated gastric mast cell degranulation with subsequent histamine H1- and H2- receptors activation in stress ulceration in rats. *Eu. J. of Pharmacology*, 55: 23-33.

Crawford, D. J. and T. J. Mabry (1978). In "Flavonoid chemistry of *Chemopodium fremontii*". Biochem. Syst. Ecol. 6 (3): 189-192.

Dacie, J. and S. Lewis (1984). In "Practical Haematology", 6th ed., Cheruchill Livingstone, Edinburgh, London and New York. pp 60-72.

Egyptian Pharmacoepia (1953). 1st ed., Cairo Univ. Press, Cairo, U.A.R.

El –Sayeda, A.E. (1983): Pharmacognostical study of certain *Convolvulus* species. *Ph. D. Thesis*, Fac. Of Pharmacy, Cairo Univ. Cairo, Egypt.

El-Hakim, A. E. and A. A.Ain-Shoka (1995). Synthesis and pharmacological activity of certain "N-Arylanthranilic acid esters". *Ibid*, 36 (1-6): 55-70.

Scanned with CamScanner

Fawzy, T.K. (1985). In "*Medicinal plant in Libya*" Arab Encyclopedia House.

Glavin, G. B. and A. A. Mikhail (1976). Stress and ulcer etiology in the rat. Physiology and Behavior, 16: 135-139.

Grag, G. B.; S. K. Nigam and C. W. Ogle (1993). The gastric antiulcer effects of the leaves of the neem tree. *Planta Medica*. 59: 215-217.

Harborne, J.B.; T.J. Mabry and H. Mabry (1975). "*The Flavonoids*" Chapman and Hall, London, 1204 pp

Hilal, S.H; M.Y Haggag; F.M. Soliman and A.E. El- Sayeda (1983). Phytochemical and biological screening of *Convolvulus lanatus*. *J. Pharm. Sci.* 24 (1-4): 139-148.

Hostettmann, K; A. Marston; M.Maillard and M.Hamburger (1995). In "*Phytochemistry of plant used in traditional medicine*" Oxford Univ. Press Inc. New York, 149 pp

Husu, H.F; C.L. Chang and Y.H. Chu (2000). Flavonoid contents and antioxidative activities of several vegetables. *J. of Agric. Chem. and Food Sci.* 38 (5): 377-387.

Kaneta, M; Nakagawa, and 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. (through c. a. 133: 119297).

Karawya, M.S. and E.A. Abou Tabl (1982). Phyto- constituents of *Taberna emonatana cornaria*. Jac Q. wiled and dichotoma robs. Growing in Egypt. Part IV, *The flavonoids bull. of pharm*. Cairo Univ. xxi, (1): 41-49.

Mabry, T.J.; K.R. Markham and M.B. Thomas (1970). In "*The systematic identification of flavonoids*". Springer-verlag, Berlin.

Makkar, H.P.S. and A.V. Goodchild (1996). In "*Quantification of Tannins: A laboratory Manual International Center for Agricultural Research in The Dry Areas*. Aleppo, Syria. 25 pp

Malalavidhane, T. S; S. M. Wickramasighe and E. R. Jansz (2000). Oral hypoglycemic activity of *Ipomoea aquatica*. *J. of Ethnopharmacology*, 17 (1-2): 293-298.

Markham, K.R. (1982). In "*Techniques of flavonoid Identification*" Academic Press, London, New York.

Markham, K.R. (1989). In "*Methods in plant biochemistry*" Acad. Press Ltd. 216.

Noda, N; I. K. Tsu; K.Miyahara and C. R. Yang (1994). Resin glycosides having long chain fatty acid group from the root of *Marremia hungaiensis*. *Chem. and pharm. bull.* 42 (10): 2011-2016.

Scanned with CamScanner

Perry, L.M. and J.Metzger (1980). In "*Medicinal plants of East and South East Asia attributed properties and uses*" P.107, MII Press, London.

Petra, M; T. Britta; K.Macki and E.Eckart (1999). Flavonoid sulphates from Convolvulaceae. *Phytoch.* 50 (2): 267-271 (through c. a. 130: 249470).

Robak, J. and RJ.Gryglewski (1996). Bioactivity of flavonoids. *Pol J Pharmacol* ; 48 (6): 555-64

Sairam, K; C.V. Rao and R.K.Goel (2001). Effect of *Convolvulus pluricaulis* on gastric ulceration and secretion in rats. *Indian J. of Exp. Biology*, 39 (4): 350-354.

Shabana, M.M.; E.A. Aboutable; Y.W. Mirhom; A.A. Genenah; M. Atef; S.A. Youssef and A.Shalaby (1986). Study of wild Egyptian plants of potential and medicinal activity. *Bull. of Fac. of Pharmacy*, Cairo Univ.

Sirivon, R.A. (1973). In "*Drugs and food from little known plants*". Harvard Univ. Press, 234-236.

Skehan P; R. Storeng; D. Scudiero; J. McMahon; JT. Warren; H. Bokesch; S. Kenney and MR. Boyd (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Nat. cancer inst.* 82:1106-1112.

Snedecor, G. W. and W.G. Cochran, (1976). In "*Statistical Methods*", 6th ed, p.502-503, Ames, Iowa, USA.

Stahl, E. (1969). In "*Thin layer chromatography a laboratory handbook*" 2nd ed. George Allen and Unwinlid London, Springer Berlin, 855 pp

Sundaresan, V; Britto, AJ, de and De-Britto, AJ. (1999). Preliminary phytochemical studies on some medicinal plants of Tirunelveli hills. *J. of Econ. and-Taxonomic Botany*, 23 (2): 377-380

Villasenov, I.M.; M.A. Carbera; K.B. Meneses; V.R. Rivera and R.M.Villasenov (1998). Comparative antidiabetic activities of some medicinal plants. *Philippine J. of Science*, 127 (4): 261-266.

Walter, H.L. and P.F. Memory (1977). In "*Medical botany plants affecting mans' health*" Awilley interscience publication, New York, 194,257,329.

Woo, W.S.; H.J. Chi and H.S. Yun (1977). Alkaloid screening of some Saudi Arabian plant. *Kor. J. Pharmacog.*, 8 (3): 109-113.

Received: 27/03/2006
Accepted: 06/06/2007

Scanned with CamScanner

فلافونيدات والتأثير الفارماكولوجي والمضاد للأورام لمستخلصات نبات خويتمة

عماد الدين محروس ابو النور عطا
قسم النباتات الطبية والعطرية- مركز بحوث الصحراء- المطرية- القاهرة- مصر

تم فصل ثمانية مركبات فينولية من المستخلص الكحولي لنبات خويتمة باستخدام طرق الفصل الكروماتوجرافي وتم التعرف عليها من خلال تحليل طيف الأشعة فوق البنفسجية وطيف الرنين النووي المغناطيسي لأنوية الهيدروجين والكربون ثنائي الأبعاد وكذلك مطياف الكتلة وهذه المركبات هي: كامفيرول، كامفيرول-٣-راسنوسيد-٧- جلوكوسيد، كوارستين، كوارستين-٣-جلوكوسيد، كامفيرول-٣،٧-راسنوسيد، كامفيرول ثلاثي جليكوسيد، روتين (كوارستين-٣-راسنوجلوكوسيد)، كامفيرول-٣- راسنوسيد.

مستخلص النبات بالبيوتانول كان له تأثير واضح مضاد للأكسدة على الخلايا السرطانية لحيوانات التجارب بينما المستخلصات الأخري تأثيرها اقل ولكنه ملموس.

تم عمل مسح فارماكولوجي لبعض المستخلصات والتي اوضحت ان المستخلص المائي ومستخلص البيوتانول عندما تمت تجربتهم بجرعات متتالية علي حيوانات التجارب المصابة بقرحة المعدة قد اختزلت التأثير المدمر للقرحة وقللت اعداد القرح بمعدة تلك الحيوانات، بينما المستخلص الكحولي والروتين بجرعة ٣٠٠ مجم/كجم ساعدت على سيولة الدم في الحيوانات بزيادة الزمن اللازم للتجلط . كما ان تناول الحيوانات للمستخلصات النباتية ساعد علي زيادة كمية البول بعد تناولهم العقار بفترة من ٣- ٤ ساعات مقارنة بالطبيعي.

Scanned with CamScanner
ممسوحة ضوئيا بـ CamScanner