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Phenolics of *Convolvulus arvensis* L. and Their Related Pharmacological Activity

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The aerial parts, roots and flowers of *Convolvulus arvensis* L. were investigated for their secondary metabolites. Eleven flavonoids were detected, namely kaempferol and its 3-*O*-β-D-glucoside, 7-*O*-β-D-glucoside, 3-*O*-α-L rhamnosyl, 7-*O*-β-D-glucoside, 3-*O*-rutinoside, 7-*O*-rutinoside, 3-*O*-α-L-rhamnoside and 3-*O*-β-D-galactorhamnoside as well as quercetin and its 3-*O*-α-L-rhamnoside and 3-*O*-rutinoside; four coumarins, namely 7-hydroxycoumarin (umbelliferone); 6,7-dihydroxycoumarin (esculetin); 6-methoxy-7-hydroxycoumarin (scopoletin) and 6-methoxycoumarin 7-*O*-glucoside (scopoletin 7-*O*-glucoside); the alkaloids tropine and its chloride salt; seventeen amino acids and eleven free sugars were also detected. The compounds were all identified through chemical and spectroscopic data including IR, UV, EI and CIMS, ^{1}H and ^{13}C NMR. The extracts showed moderate diuretic, tranquillizing, hypoglycemic, antihemorrhagic activity, in addition to antibacterial and antifungal effects. The extracts showed negative antiasthmatic effects and were found to be safe for liver and kidney functions; they also act to relieve intestinal and uterine pain.

Key Words: ***Convolvulus arvensis*, Convolvulaceae, Kaempferol and quercetin glycosides, Coumarins, Antimicrobial, Pharmacological activities.**

INTRODUCTION

The family Convolvulaceae comprises 55 genera and 1600–1700 species[1]. *Convolvulus arvensis* L. is widely distributed in Egypt[2]. Previous reports described for extracts of *C. arvensis* showed purgative activity[3], antihemorrhagic effects[4]. In folk medicine these extracts have been used to treat catharsis, asthma and cough[5]; as a treatment for jaundice[6], headache, constipation, rheumatism, diabetes and skin diseases[7, 8].

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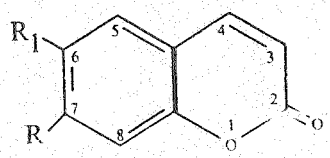
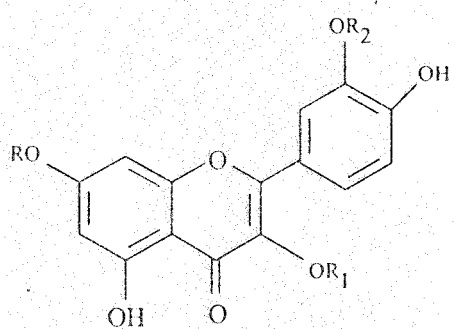
Previously isolated and identified secondary metabolites from *Convolvulus* species include tropane and pyrrolizidine alkaloids[9, 10], sugars[11, 12], phenolic compounds and coumarins[13–15], sterols and fatty acids[16, 17], resins[18, 19], quercetin and kaempferol aglycones[20]and simple sterols[4]. We report here the isolation and identification of many known compounds including eleven flavonoids, four coumarins, two alkaloids, eleven free sugars and seventeen amino acids. The protein content of the plant was found to be 45%. The different plant extracts were examined for their pharmacological, antibacterial and antifungal activities. The compounds were identified by both chemical data and spectroscopic analysis including UV, IR, EIMS, CIMS, ^{1}H and ^{13}C NMR.



	R	R_1
1. Scopoletin	OH	OCH_3
2. Scopolin	Glucose	OCH_3
3. Umbelliferon	OH	OH
4. Esculetin	OH	



	R	R_1	R_2
5. Quercetin	H	H	OH
6. Quercetin 7-0-rhamnoside	rh	H	OH
7. Quercetin-3-O-rutninoside (Rutin)	H	rh gl	OH
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-rhamnoside	H	rh	H

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

EXPERIMENTAL

The whole plants (aerial parts, root and flowers) were collected from EI-Arish, North Sinai in 1999 and were identified by Prof. Nabil EI-Hadidi, Professor of Botany, Faculty of Science, Cairo University. A plant specimen has been deposited in the Herbarium of the Desert Research Institute, Cairo, Egypt.

1 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 roots, 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 33.6 g), (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, pectins, proteins and resinous materials.

Pre-coated silica gel 60 F254 plates (E. Merck) for TLC and silica gel 60, (70–230 mesh, Merck) was used for column chromatography, using 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).

TLC and UV examination of the different extracts from the solvent systems (a and b) suggested the presence of coumarins in ether and chloroform extracts and flavonoids in ethyl acetate extract. The *n*-butanol extract was found to have mostly 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 thin layer chromatography (system a).

The ethyl acetate extract was subjected to a column followed by preparative thin layer chromatography (system b), then 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, yielding compounds **5–15.**

Chloroform extraction of acidified extracts followed by adding ammonia to give a basic aqueous layer and liberating the alkaloid into the chloroform layer. Protein levels were estimated by the Micro-Kjeldahl method, free sugars and amino acids were determined chromatographically.

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.

Toxicological studies were done as reported by Kerber[21], in which 8 groups of five mice each weighing 20 g were used to determine the LD50 while groups of

male guinea pigs 250–350 g were used for investigating anti-asthmatic effect[22], antipyretic activity[23], analgesic effect[24], effect on uterine of rats[25], anti-tumour[12], anti-inflammatory effect[26], diuretic effect[27], anti-ulcerogenic effect[28, 29], anti-microbial activities were determined according to Duguid *et al.*[30]; anticonvulsing effect[31], tranquillizing activity[32], anticoagulant activity[33], effect on blood glucose level[34] as well as effects on kidney and liver functions[35, 36] and urea-related activity[37]. Repeated administration was done using 400 mg/kg body weight for oral doses for 15 days. Results obtained are presented in Tables 1–7.

TABLE-1
EFFECT OF *CONVOLVULUS ARVENSIS* EXTRACTS ON DIFFERENT BACTERIA AND FUNGI

Organism	Flower					Aerial Parts			Roots		
	Ether	Chloro-form	EtOAc	95% Alc.	50% Alc.	EtOAc	95% Alc.	50% Alc.	Ether	EtOAc	50% Alc.
B. subtilis	+	–	+	++	+	++	++	+	–	+	+
M. kristinae	++	–	++	++	+	++	++	+	++	++	+
S. maxima	++	+	++	+++	+++	++	++	+	+++	++	+
S. aureus	++	+	++	++	+	++	+	+	++	++	++
Salmonella sp.	++	–	++	++	–	+	–	+	++	++	+
M. lacunata	+	–	+	++	+	+	++	+	+	++	+
E. coli	+	–	++	++	+	++	++	+	+	++	++
C. albicans	+	–	–	–	–	–	++	–	–	+	–
A. niger	–	–	–	–	–	–	–	–	–	–	+
A. flavus	–	–	–	–	–	–	–	–	–	+	+
P. aeruginosa	–	–	–	++	+	+	–	–	–	+	+
S. cerevisiae	+	–	–	–	–	–	–	–	–	+	–
M. phely	++	–	–	++	++	++	++	–	–	++	++
P. chrysogonum	–	–	–	+	+	–	–	–	–	–	+
K. pneumonia	++	–	–	+++	++	++	+	–	++	++	++

Significant at *$p < 0.05$, †$p < 0.01$

TABLE-2
EFFECT OF *CONVOLVULUS ARVENSIS* EXTRACTS ON URINE VOLUME OF RATS

Group	Dose (mg/kg b.wt.)	Volume of urine (mL) within 24 h
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 $p \leq 0.05$ and $p \leq 0.01$

TABLE-3
TRANQUILLIZING ACTIVITY OF *CONVOLVULUS ARVENSIS* EXTRACTS IN MICE USING ROTATING ROD TEST

Group	Dose (mg/kg)	Time (s) required by mice to fall			
		1 h	2 h	3 h	4 h
Control	0	138.6 ± 11.6	139.5 ± 11.4	138.6 ± 11.6	140.6 ± 12.5
Chlorpromazin	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 $p \leq 0.05$ and $p \leq 0.01$

TABLE-4
TRANQUILLIZING ACTIVITY OF *CONVOLVULUS ARVENSIS* EXTRACTS IN MICE USING TRACTION TEST

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

Successive plant extracts were used to study the effect on isolated rabbit's intestine, effect on the uterine motility of rats[25], effect on the isolated heart of rabbits[38] and effect on isolated tracheal strips of guinea pigs[39]. Human tumour cell lines U251 and MCF7 (brain tumour and breast carcinoma cell lines respectively) were used for measurement of potential cytotoxicity by SRB assay.

NMR spectral analyses were carried out on a Bruker AMX and/or Varian Inova, both 500 MHz; mass spectra were recorded using a Finigan SQ700. UV-Vis spectrophotometer. Schimadzu UV 240 was used for recording UV spectra.

Effect on liver and kidney functions

Single administration: Twenty mature albino rats of 150–180 g b.wt. were divided into 5 equal groups. The 1st group was left as control, while the 2nd and 3rd groups were administrated a single oral dose of the plant extracts (400 mg/kg), blood samples were collected from the orbital plexus of rats, 6 h 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 aspirate aminotransferase (AST) and alanine am inotransferase (ALT)[36]. Levels of urea[37] and creatinine were also estimated[35].

TABLE-5
THE EFFECT OF *CONVOLVULUS ARVENSIS* EXTRACTS ON PT AND PTT IN RATS

Organ	Group	PT (s)	PTT (s)
	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

*, †, ‡ Significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$
PT: prothrombin time
APTT: activated partial prothrombin time

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 Table-6.

TABLE-6
EFFECT OF SINGLE AND REPEATED ORAL ADMINISTRATION OF DIFFERENT EXTRACTS ON BLOOD GLUCOSE LEVEL IN NORMAL AND ALLOXAN DIABETIC RATS

Group	Dose (mg/kg b.wt.)	Blood glucose level (mg/mL)			
		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 $p \leq 0.001$

RESULTS AND DISCUSSION

The compounds, all known, were identified by R_f values, colour reactions and spectroscopic data, which were identical with those of authentic samples. The flower alcoholic extract exhibited the most activity of all extracts of *Convolvulus arvensis*, especially antimicrobial activity on bacteria and fungi (Table-1), probably due to the high concentration of flavonoids and coumarins.

Ethanolic extract of *Convolvulus arvensis* was non-toxic (LD_{50} over 5 g/kg b.wt.); this indicates that the plant is highly safe for human use. This was proved by its use as a source of food for goats[4]. The results of the pharmacological study proved that there is no antiasthmatic effect and the plant has neither antipyretic, analgesic, antiinflammatory, antiulcerogenic nor anticonvulsant activity.

The present work revealed the moderate diuretic (Table-2) and tranquillizing activity (Tables 3 and 4) of both roots and flower extracts. Both leaves and root are used as antihemorrhagic, but the results obtained show that both green parts and flower have anticoagulant (Table-5) activity at a dose of 400 mg/kg b.wt.; this may be due to the presence of coumarins. Successive extracts of both green part and roots also exert the same effect as the total alcoholic extracts.

Concerning hypoglycemic effect, it was observed that green parts had significant effect, which may be attributed to the presence of flavonoids. It was also observed that green parts extract has hypoglycemic effect (Table-6) on diabetic rats only and not on normal ones which may be attributed to the presence of polysaccharides and flavonoids[40]. Polysaccharides have been found to stimulate insulin secretion[41]. The anticoagulant activities are probably due to the presence of flavonoids[42]. The plant could also 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 decreased both liver (AST and ALT) and kidney (urea and creatinine) functions (Tables 7 and 8). It was reported that *Convolvulus arvensis* plant material is used to treat jaundice due to the presence of convolvuline resinous glycosides[6].

TABLE-7
EFFECT OF SINGLE AND REPEATED ORAL ADMINISTRATION OF DIFFERENT EXTRACTS ON LIVER FUNCTION ACTIVITY RATS

Group	Dose (mg/kg b.wt.)	Single		Repeated	
		AST μ/L	ALT μ/L	AST μ/L	ALT μ/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	37.00 ± 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 $p \leq 0.001$

TABLE-8
EFFECT OF SINGLE AND REPEATED ORAL ADMINISTRATION OF *CONVOLVULUS ARVENSIS* EXTRACTS ON KIDNEY FUNCTION TEST IN RATS

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†

†, ‡ Significant at $p \leq 0.01$ and $p \leq 0.001$

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