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DETERMINATION OF FLAVONOIDS AND SOME CHEMICAL CONSTITUENTS OF *Convolvulus fatmensis* Ktze. AND ITS BIOLOGICAL ACTIVITY

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Convolvulus fatmensis Ktze. growing in the Egyptian desert, belongs to family Convolvulaceae. Investigation had been done for its phytochemical constituents and biological activity. The results revealed the presence of flavonoids, sterols and/or triterpenes, proteins, carbohydrates and/or glycosides. Eight flavonoid compounds were isolated and identified as Quercetin - 3-O – rutinoside (Rutin) Quercetin-3-glucoside Quercetin-7-glucoside Quercetin-3-rhamnoside kaempferol-7-L-rhamnosid kaempferol-3,7-dirhamnosid Kaempherol-3-rhamnoside, 7-rhamnoxyloside and Kaempferol-3-galactorhamnoside. The percentages of crude protein, free and total amino acids were determined using amino acid analyzer. Sterols and/or triterpenes were studied using gas chromatography (GC). Antimicrobial screening for the total extract were carried out on different bacteria (Gram+ and Gram–) and fungi strains. Antitumor activity also was investigated for the total alcoholic extract.

Keywords: Convolvulaceae, *Convolvulus fatmensis*, flavonoids, sterols, biological activity.

Family Convolvulaceae is one of plant kingdom, which includes a number of very important medicinal plants. On reviewing the Literature, it was found that it contains a vast number of species, which vary in their chemical constituents and uses. These constituents are alkaloids, carbohydrates, lipids, phenolic and resins.

Convolvulus scammonia L. is one of the most important plants of this family as it is the source of scammony which is one of the oldest remedies known for treatment of jaundice, headache, purgative, rheumatic and skin disease (Al-antaki, 1952; Ibn Sina, 1968; Egyptian pharmacoepia, 1972).

Another important plant in this family is *Ipomoea batatas* cultivated as a vegetable crop for production of sweet potato tubers, it can be considered as a main source of human food because it is very rich in vitamins B, C, D and G, its leaves contain insulin like compound so it is antidiabetic (Fawzy,1985).

In folk medicine there are many other medicinal uses of Convolvulaceae such as, tonic (*Ipomoea digitata* and *Cressa cretica*), toothache (*Convolvulus bidentatus*) (Walter and Memary, 1977), purgative (*Merremia alata*, *Argyreia capitata* and *Ipomoea pedicellaris*), laxative (*Ipomoea indet*), for headache (*Ipomoea gracilis*) (Sirivon, 1973), for rheumatoid and as skin lesion to treat dermatitis caused by the stink of jelly fish (*Ipomoea pes caprae*) (Perry and Metzeger, 1980; Hostettmann *et al.*, 1995).

Convolvulus fatmensis L. has antidiarrhoeal and antinociceptive activity (Atta and Mouneir, 2005; Atta and Elsoud, 2004) but it has not enough previous phytochemical studies.

MATERIALS AND METHODS

I- Plant Materials

The aerial parts of *Convolvulus fatmensis*. were collected from El-Arish (North Sinai) and identified by prof. N. El-Hadidi, professor of Botany, Botany Department, Faculty of Science, Cairo University and by comparison with plant description in flora of Egypt as well as herbarium specimens at Desert Research Center

II- Authentic Material

Reference material for sugars and amino acids, were purchased from E. Merck, Dermstadt, Germany.

Materials, Solvent Systems, and Reagents for Chromatography

A- Adsorbents

Pre-coated silica gel 60 G F254 plates (E-Merck) for TLC and silica gel 60, (70-230 mesh, Merck) for column chromatography, sulphonated polystyrene resin, and type Ultra Pac 8 for ion-exchange chromatography (IEC) were used.

B- Solvent systems

(a) Ethyl acetate-methanol- acetic acid water (65:15:10:10),
(b) Butanol – acetic acid – water (4:1:5),
(c) Butanol-acetic acid-water (4:1:1) and
(d) ethyl acetate –methanol-water (30:5:4) were used for developing the chromatoplates.

C- The following chromatographic reagents were prepared (Stahl, 1969)

1- Naphthoresorcinol-sulphuric acid (for carbohydrate).
2- Aniline phthalate (for carbohydrates).

2- Ninhydrin (0.2% w/v in acetone) for amino acids.

For spectrophotometric estimation of amino acids in IEC fractions, ninhydrin was used as 0.3% (w/v) solution in dimethyl sulfoxide/lethium hydroxide containing 0.4% hydrantine at pH 5.2.

III- Phytochemical Study

A- Phytochemical screening

Powdered sample of *Convolvulus fatmensis* was subjected to preliminary phytochemical screening to investigate the active constituents.

B- Identification of carbohydrate content

1- Preparation of carbohydrate extracts

Low molecular weight sugar components were extracted by boiling (100 g) from the plant powder with ethanol (90%) (Karawaya *et al.*, 1984). The residue left after evaporation of ethanol was dissolved in hot pyridine, filtered and evaporated to dryness at room temperature. The pyridine extract was dissolved in 2ml aqueous isopropanol (10%) and saved for chromatographic study.

Water-soluble polysaccharides of the plant were obtained from the residual marc (after extraction with ethanol) by successive extraction with water (Whistler, 1965), ammonium oxalate (Whistler and smart, 1965) and dilute hydrochloric acid (Kertse, 1951). Polysaccharides were precipitated from each prepared extract by the addition of ethanol 95% (1.5 volumes). Purification of the precipitate was carried out by solubility in water re-precipitation and thorough washing with ethanol. Purified precipitates were kept dry (vacuum desiccators) and their yield and physico-chemical characters were recorded.

2- Preparation of polysaccharide hydrolysates

One hundred mg of purified extracts (aqueous, amm.oxalate and dil. HCl) were hydrolysed by heating with 2ml of 0.5M H_2SO_4 (Chrums and Stephen, 1973) in sealed ampoules for 20 hours at 100°C. The hydrolysates were freed from (SO_4) by treating with $BaCO_4$. The resulting solutions were extracted with hot pyridine and treated as for ethanolic extracts and saved for chromatographic study.

3- Chromatographic investigation of carbohydrate extracts

The two carbohydrate extracts viz. ethanolic (90%) and polysaccharide hydrolysates of the plant were examined for simple sugars by thin layer chromatography (TLC) (silica gel G, solvent system a), and paper chromatography (PC) (solvent system b). Spray reagent No. (1).

C- Investigation of Amino Acids and Protein Content

To evaluate the nutritive value of the plant, a detailed study of the amino acids and protein content was carried out, this study comprised:

1- Determination of crude protein

This was carried out by microkjedahl method (British pharmacopia, 1980).

2- Qualitative investigation of amino acids

2-1 Extraction of free amino acids

Defatted powdered plant sample (10g were percolated with 50% ethanol (Awapora, 1948). The concentrated residue was dissolved in absolute ethanol, left overnight in a refrigerator, and filtered to dispose the precipitated extraneous matter. The concentrated residue was finally dissolved in 2ml 10% aqueous isopropanol and kept for chromatographic study.

2-2 Isolation and hydrolysis of protein

The defatted powdered plant sample (40g) were stirred in 10% sodium chloride solution for one hour (Ledered, 1975) and filtered. The filtrate was treated with an equal volume of trichloroacetic acid (10%). The precipitated protein was separately collected by centrifugation, successively washed with trichloroacetic acid (5%), ethanol and ether followed by drying in vacuum desiccators.

Acid hydrolysis was carried out by refluxing 10 mg of the isolated protein of each sample with 10ml 6N HCl for 20 hours (Olson *et al.*, 1978). The reaction mixture was evaporated to dryness under reduced pressure at 30°C. A part of the residue was dissolved in 10 ml water to which 10 mg activated charcoal was added, stirred, and filtered. The concentrated filtrate was dissolved in 1ml 10% aqueous isopropanol and saved for chromatography. PC and TLC were performed using ascending double development technique with solvent systems (c) and (a) the air dried chromatograms were sprayed with Ninhydrin reagent.

2-3 Quantitative investigation of amino acids

Free and protein amino acids were qualitatively determined using LKB 4151 plus amino acid analyser, 0.5ml volume of the obtained solution was injected in the amino acid analyser (Steven *et al.*, 1989).

D- Identification of lipid content

Preparation of the lipid sample

Three hundred gm of air dried powdered plant extensively extracted with petroleum ether (40-60): ether (1:1) using soxhlet apparatus. The extract was evaporated till dryness under reduced pressure and the residue was prepared for the following studies:

1- Separation and investigation of unsaponifiable matter fraction using Gas-Liquid chromatography (GLC): (Christie, 1982)

Five gm lipid of *Convolvulus fatmensis* in 480ml ethanol was saponified with solution of 40gm of potassium hydroxide in 100ml of distilled water and the mixture was refluxed on boiling water bath for three hours. The solution was then concentrated.

Excess of water was then added and the soap solution was extracted in a separating funnel with peroxide-free ether. The combined ethereal extracts and washings were washed with water until free from alkalinity, dried over

anhydrous sodium sulphate then filtered. The filtrate was evaporated to dryness under vacuum and the residue was subjected to Gas-Liquid chromatography investigation.

The Gas-liquid chromatography apparatus, equipped with flame ionization detector, was used in the identification of unsaponifiable matter. The operation was carried out isothermally and authentic samples were also injected under the same conditions and the relative retention times (RRT) were calculated. The results of Itoh *et al.* (1973) and Farag *et al.* (1986) were used as a guide to characterize some of the unknown compounds. The relative percentage of each unsaponifiable compound was determined using triangulation method according to Nelson *et al.* (1969).

2- Separation and identification of saponifiable fraction

After removal of the unsaponifiable fraction with ether, the soapy solution was converted into the corresponding free fatty acids by mean of 2.5% sulphuric acid and extracted with petroleum ether. The petroleum ether extract was washed several times with distilled water until free from acids and filtered over anhydrous Na_2SO_4. The petroleum ether was removed by distillation under vacuum at 40°C.

The extracted fatty acids and the standard ones were converted to the corresponding methyl esters using ethereal solution of diazomethane (Farag *et al.*, 1986). The methyl esters of the fatty acids were analyzed with a GCV Pye-Unicam series 304 gas chromatographic apparatus. Peak identification was performed. Relative proportions of individual compound were estimated as the ratio of partial areas to the total area, (Fryer *et al.*, 1960; Nelson *et al.*, 1969; Farag *et al.*, 1986 and Khalil, 1987).

E- Isolation and purification of phenolic compounds

1- Extraction

The defatted powder plant aerial part (1 kg) was extracted in a soxhlet apparatus with 80% ethanol. The ethanolic extract was dried under reduced pressure and then the precipitation of salts was carried out by dissolving the extract dropwise on excess of ethanol with continous stirring. The solution was filtered, concentrated and re-dissolved in alcohol. This process was repeated several times till no further salt precipitated.

2- Isolation and purification using chromatographic methods

The concentrated extract was dissolved in very small amount of alcohol and mixed with about 10gm of silica gel for column; the alcohol was evaporated on water bath with continous titurating till form free flowing dry powder. The powder was then introduced on the top of glass column containing silica gel for column packed by dry method using chloroform then gradual increasing of polarity with ethyl acetate and methanol. Elution was done and each eluate was concentrated separately under reduced pressure, and then subjected to TLC using system (d). Fractions were collected and then subjected to prepartative TLC using system (d), bands

corresponds to flavonoids were visualised under UV, scratched and eluted with methanol and water. Elutes were dried and purified on sephadex LH20 column.

IV- Biological Studies

A- Antimicrobial activity

1- Preparation of extract

Total extract was dissolved in a concentration of 10% alcohol.

2- Microorganisms used

Bacillus subtilis, Sarcina maxima, Staphylococcus aureus and Salmonilla typhi, Echerichia coli. Candida albicans, Aspergillus niger, Aspergillus flavus, Pseudomonas auregenosa, Klebsiella pneumonia, Enterobacter sp. and *Candida albicans*.

The antimicrobial activity was carried out using the paper disc technique (Duguid *et al.*, 1978). Sterilized paper discs (Whatmann No.3) of 0.5 cm diameter were impregnated with the prepared extract and placed upon the surface of the tested organism (after dryness from the solvent) inoculated plates. After incubation at 30-32 °C for 24 hours (for bacterial organisms) and 7 days (for fungi), the plates were examined for any zone of inhibition around the disc which indicate that the organisms were affected by the tested extracts. Each treatment was replicated three times. Plates containing solvent only served as control. The diameters of the inhibition zone were determined in mm.

B- Antitumor activity (cytotoxic activity)

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

1- Tumor cells

Ehrlich tumor cell lines

2- Measurement of potential cytotoxicity by SRB assay

Potential Cytotoxicity of *Convolvulus fatmensis* extracts were tested using the method of Skehan *et al.* (1990). Cells were plated in 96-multiwell plate (10^4 cells) for 24 hr before treatment with the extracts to allow attachment of cells to the wall of the plate.

Different concentrations of the plant extract (0, 1, 2.5, 5 and 10 mg/ml) were separately added to the cell mono-layer. Triplicate was prepared for each individual dose. Mono-layer cells were incubated with the extract 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 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.

RESULT AND DISCUSSION

1- Phytochemical Study

1- Phytochemical screening

Preliminary Phytochemical screening revealed the presence of carbohydrates and/or glycosides, sterols and/or triterpenes, proteins and/or amino acids, coumarins, tannins, flavonoids, alkaloids and/or nitrogenous bases and saponins. No volatile oils or cardenolides were detected.

2- Carbohydrate contents

The polysaccharides precipitated from the different plant extracts were obtained as amorphous, greyish-white, odourless and tasteless powders. They dissolved readily in 20 parts of water at 25°C forming viscous opalescent, colloidal solutions. They gave positive tests for pectin (Browse and zerban, 1979).

TLC and PC examination of ethanolic extract and pectin hydrolyzed using solvent systems (a) and (b) and authentic markers are summarised in table (1) which revealed the presence of four sugars in the ethanolic extract and six in the pectin hydrolyzate.

Table (1). TLC and PC of carbohydrate contents of *convolvulus fatmensis*.

Authentic	Ethanolic extract	Pectin hydrolyzed
Xylose	+	+
Glucose	+	+
Fructose	+	+
Galactose	+	+
Sucrose	+	-
Arabinose	-	+
Galactouronic acid	-	+

3- Protein and amino acids content

Percentage of crude protein, as determined by British pharmacoepia (1980) was found to be 35%. The free and protein hydrolysate amino acids contents of the plant which are summarised in table (2) revealed that, the plant contains sixteen known amino acids as free and protein in different concentrations. Serine represents the highest concentration as free amino acid while Leucine is the lowest concentration, on the other hand aspartic acid represents the highest concentration as protein amino acid and Methionine is the lowest.

Table (2). Free and protein amino acids of *Convolvulus fatmensis* using amino acid analyzer.

No.	RT	Amino acid	Conc. (mg/ml)	
			Free a. a.	Protein a. a.
1	11.36	Aspartic a.	4.13	5.89
2	14.55	Therionine	2.52	1.94
3	25.99	Serine	10.81	2.33
4	18.09	Glutamic a.	6.14	5.44
5	25.19	Glycine	0.92	2.13
6	26.46	Alanine	2.74	2.09
7	30.02	Valine	4.06	2.68
8	32.44	Methionine	4.69	0.64
9	34.01	Isoleucine	1.87	1.73
10	35.21	Leucine	0.63	2.74
11	39.51	Tyrosine	2.84	1.24
12	42.25	Phenyl alanine	4.40	2.44
13	50.41	Histidine	1.03	2.16
14	54.02	Lysine	1.22	2.83
15	62.71	Argenine	4.85	2.52

RT: Retention time

4- Identification of Lipid contents

GLC analysis of saponifiable and unsaponifiable matter of *Convolvulus fatmensis* are presented in tables (3 and 4). The saponifiable matter of the plant revealed the presence of nine hydrocarbon and four sterol compounds in different concentrations. By using GLC, there are fifteen fatty acids shown, the highest concentration fatty acid was Linoliec while the lowest concentration was Lauric acid.

Table (3). GLC of hydrocarbons and sterols of *Convolvulus fatmensis*.

No.	Name	RT	Conc. %
1	Pentadecane	9.283	0.215
2	Octadecane	9.960	0.187
3	Eicosane	10.750	0.310
4	Heneicosane	11.617	1.747
5	Docosane	12.667	0.720
6	Hexaconsane	13.083	0.745
7	Octacosane	13.850	4.208
8	Triacontane	14.767	0.111
9	Dotriacontane	15.900	3.338
10	Cholesterol	16.767	0.940
11	Campesterol	17.800	3.910
12	Stigmasterol	18.517	5.346
13	β-Sitosterol	19.200	6.117

RT: Retention time

Table (4). GLC of fatty acids of *Convolvulus fatmensis*.

No.	Name	RT	Conc. %
1	Caprylic acid	6.067	0.197
2	Capric acid	7.400	0.033
3	Lauric acid	8.600	0.025
4	Tridecylic acid	9.250	0.161
5	Myristic acid	11.233	1.617
6	Pentadecylic acid	12.617	1.632
7	Palmetic acid	13.717	2.813
8	Margaric acid	16.033	1.594
9	Stearic acid	16.617	10.044
10	Oleic acid	18.983	10.148
11	Linoleic acid	20.050	20.324
12	Linolenic acid	22.483	5.087
13	Arachidic acid	24.833	4.597
14	Heneicosanoic acid	27.017	2.564
15	Behenic acid	28.133	1.881

RT: Retention time

5- Identification of phenolic compounds

The compounds isolated from *Convolvulus fatmensis* were eight compounds identified by R_f, UV and NMR. Their data were listed. They are Quercetin -3-O – rutninoside (Rutin) Quercetin-3-glucoside Quercetin-7-glucoside Quercetin-3-rhamnoside kaempferol-7-L-rhamnosid kaempferol-3,7-dirhamnosid Kaempherol-3-rhamnoside, 7-rhamnoxyloside and Kaempferol-3-galactorhamnoside.

Quercetin -3-O – rutninoside (Rutin): yellow crystals, R_f = 0.5 (system d), m.p 190°C UV λmax (nm), MeOH: 256, 265 (sh.), 290, 355; $ALCL_3$: 274, 302 (sh.), 330 (sh.), 432; $ALCL_3$ /HCL: 270, 298, 359, 399; NaOAc: 272, 324, 398; $NaOAc/H_3BO_3$: 263, 292(sh.), 368; NaOMe: 272, 310, 410.

^1H NMR (DMSO $-d_6$): δ 8.10 (1H, d, J= 2.5 Hz, H2`); δ 7.86 (1H, dd, J=8.5, 2.5Hz, H-6`); δ 6.89 (1H, d, J=8.5Hz, H-5`); δ 6.65 (1H, d, J=2.5Hz, H-8); δ 6.5 (1H, d, J=2Hz H6); δ5.13(1H, d, J=7.50Hz H1``glucose); δ 4.55 (1H, d, J=2.5Hz, H1```rhamnose); δ 3.47-3.87 (m, Sugar protons); δ 1.23 (3H, d, J=6 CH_3) .

^{13}C NMR (Methanol-D_6): δ ppm 174.3 (C-4) , 164.5 (C-7), 161.2 (C-5) , 156.6 and 156.4 (C-2 and C-9 respectively), 148.5 (C-4') , 144.8 (C-3'), 133.3 (C-3) , 121.6. (C-6') , 121.2 (C-1') , 116.1(C-2'), 115.2 (C-5') , 103.8 (C- 10), 98.8 and 93.7 (C-6 and C-8 respectively), 100.7(C-1''), 76.5(C-3``), 75.9 (C-5''), 74.1(C-2''), 71.5.(C-4``), 62.8(C-6``), 101.3 (C-1```), 71.9 (C-4```), 70.6 (C-2```), 70.4 (C-3```), 70 (C-5```) and 17.6 (C-6```).

Quercetin-3-glucoside: yellow crystals; its mp. 228-230 °C, R_f =0.80(system d). UV λ max (nm) MeOH: 265, 350 $AlCl_3$ 265, 300(sh), 440 $AlCl_3$/ HCl: 265, 350, 420 NaOAc: 270, 300, 380 NaOAc/H3BO3: 270, 310, 373 NaOMe: 275, 330, 430.
^{1}H-NMR (DMSO- d_6): δ 7.62 (1H, d, J = 8 .5Hz, H2`), δ 7.49 (1H, dd, J = 8.5,2.5 Hz ,H6`), δ 6.85 (1H, d, J=8.5Hz, H5`), δ 6.37 (1H, d, J = 2.5 Hz, H8), δ 6.14 (1H, d, J= 2.5 Hz, H6), δ 5.7 (1H, d, J=7 Hz, H 1`` glucose) and δ 3.5-4 (m, sugar protons).

Quercetin-7-glucoside: yellow crystals; its mp.226-228°C, R_f=0.78(system d). UV λ max (nm) MeOH: 265,350,370; $AlCl_3$: 265,300 (sh), 450; $AlCl_3$/ HCl: 265, 350,420; NaOAc: 270,300,380,420 NaOAc/H3BO_3: 270,310,380 NaOMe: 275, 330, 450.
^{1}H-NMR (DMSO- d_6): δ 7.52 (1H, d, J = 8.5 Hz, H2`), δ 7.49 (1H, dd, J = 8.5,2.5 Hz ,H-6`), δ 6.8 (2H, d, J = 8.5 Hz, H5`), δ 6.37 (1H, d, J = 2.5 Hz, H8), δ6.14 (1H, d, J = 2.5 Hz, H6), δ 5.0 (1 H, d, J=7 Hz, H 1`` glucose) and δ 3.5-4 (m, sugar protons).

Quercetin-3-rhamnoside: yellow crystals; mp. 224-226°C, R_f=0.79(system d). UV λ max (nm) MeOH: 260,358 $AlCl_3$: 272,300 (sh), 440; $AlCl_3$/ HCl: 272, 300 (sh),420 NaOAc: 275,300 (sh),395 NaOAc/H3BO_3: 260,325 (sh),375; NaOMe: 272, 320 (sh), 430.
^{1}H-NMR (DMSO- d_6): δ 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, H5`), δ 6.5 (1H, d, J = 2.5 Hz, H8), δ 6.2 (1H, d, J = 2.5 Hz, H6), δ 5.4 (1H, d, J=2 Hz, H 1`` rhamnose), δ 3.5-4 (m, sugar protons) and δ 1.2(3H, d, J=6Hz, CH3 rhamnose).

kaempferol-7-L-rhamnosid: yellow crystals; mp. 242-243°C, R_f=0.36 (system d). UV λ max (nm), MeOH: 262, 366 $AlCl_3$: 266, 353, 424; $AlCl_3$/ HCl: 265, 422; NaOAc: 262, 366, 420; NaOAc/H_3BO_3: 263, 366, 420; NaOMe: 273, 294, 440.
^{1}H-NMR (DMSO- d_6): δ 8.1 (2H, d, J = 8.5 Hz, H2` and H6`), δ 6.97 (2H, d, J = 8.5 Hz, H3` and H5`), δ 6.41 (1H, d, J = 2.5 Hz, H8), δ 6.9 (1H, d, J = 2.5 Hz, H6), δ 5.6 (1H, d, J=2.5 Hz, H1`` rhamnose), δ 3.5 (m, sugar protons) and δ 1.1 (3H, d, J=6 Hz, CH_3 rhamnose).

kaempferol-3,7-dirhamnosid: yellow crystals; its mp. 233-234 °C, R_f=0.57 (system d). UV λ max (nm), MeOH: 268, 346 $AlCl_3$: 276, 355, 400; $AlCl_3$/ HCl: 276, 352, 400; NaOAc: 268, 346; NaOAc/H_3BO_3: 268, 346; NaOMe: 268, 380.
^{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 (1H, d, J=2.5 Hz, H1`` rhamnose), δ 5.3 (1H, d, J=2.5 Hz, H 1``` rhamnose), δ 3.4 (m, 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).

Kaempherol-3-rhamnoside, 7-rhamnoxyloside: yellow crystals; its mp.180-182 °C, R_f=0.77 (system d). UV λ max (nm), MeOH: 264, 342 $AlCl_3$: 270, 342, 400; $AlCl_3$/ HCl: 270, 342, 400 NaOAc: 270, 342, 340, NaOAc/H_3BO_3: 260, 350 NaOMe: 262, 390.
^{1}H-NMR (DMSO- d_6): δ 7.79 (2H, d, J= 8.5 Hz, H2` and H6`), δ 6.95 (2H, d, J = 8.5 Hz, H3` and H5`), δ 6.9 (1H, d, J= 2.5 Hz, H6), δ 6.7 (1H, d, J= 2.5 Hz, H8), δ 5.5 (1H, d, J=2.5 Hz, H1``rhamnose), δ 5.3 (1H, d, J=2.5 Hz, H1```rhamnose), δ 4.25 (1H,d,J=8.5Hz, H1````xylose), δ 3.4(m, sugar protons), δ 1.2 (3 H, d, J=6 Hz, CH_3 rhamnose), δ 0.8 (3 H, d, J=6 Hz, CH_3 rhamnose). Fab mass m/z 733 and in addition to m/z 579, 433 and 287.
^{13}C NMR (Methanol-D_6): δ ppm 177.7 (C-4) , 161.6 (C-4`), 161.5 ( C-7) , 161.2(C-5),157.4(C-2), 156.1 (C-9),134.4 ( C-3) , 130.4 (C-2`,6`), 119.4 ( C-1`) , 115.6. (C-3`,5`) ,99.6 (C-6) ,98.3(C-1``), 94.2 (C-8) , 69.9 (C-3``,4``), 69.7(C-2``), 69.2(C-5``), 17.4(C-6``), 100.8(C-1```), 71.7(C-4```), 70.1(C-3```), 69.7 (C-2```,5```), 17.8(C-6```), 106.2(C-1````), 76.1 (C-3```), 73.7(C-2````), 67.1(C-4````) and 65.4(C-5````).

Kaempferol-3-galactorhamnoside: yellow crystals; its mp.240-242°C, R_f=0.40 (system d). UV λ max (nm), MeOH: 264, 350 $AlCl_3$: 270, 342, 410 $AlCl_3$/ HCl: 270, 342, 410; NaOAc: 270, 342, 380; NaOAc/H_3BO_3: 260, 375; NaOMe: 262,330, 390.
^{1}H-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= 1.5 Hz, H8), δ 5.6 (1H, d, J= 1.5 Hz, H6), δ 5.4 (1H, d, anomeric proton), δ 5.2 (1H, 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 (Methanol-D_6): δ ppm 174.3 (C-4) , 160.4 (C-7), 159.6 (C-5) , 153.3 and 153.4 (C-2 and C-9 respectively), 157.4 (C-4') , 132.6 (C-3), 130.3 (C-6`,C-2`) , 121.2. (C-1`) , 114.8 ( C-3`) , 114.7(C-5`), 103.4 (C-10) , 98.8 (C-6), 98.8 and 93.7 (C-6 and C-8 respectively), 102.5(C-1''), 75.5(C-5``), 73.2 (C-3``), 71.2(C-2''), 68.5(C-4``), 59.8(C-6``), 101.3 (C-1```), 71.9 (C-4```), 70.6 (C-2```), 70.4 (C-3```), 70 (C-5```) and 17.6 (C-6```).

II- Biological Studies

1- Antimicrobial activity

Total extract of *Convolvulus fatmensis* was applied on some microorganisms bacteria (Gram + and Gram -) and fungi using disk method

which revealed that; the plant have significant antimicrobial activity compared with control against bacteria and moderate activity against fungi as shown in tables (5 and 6).

It was found that *Klebsiella pneumonia* is the most bacteria affected by the plant extract; its inhibition zone was (32 mm) followed by *Sarcina maxima* (17 mm) while *Pseudomonas auregenosa* was not affected by the extract.

The *Candida albicans* and *Aspergillus flavus* were not affected by the plant extract, i. e. the plant is more active against bacteria than fungi.

Table (5). Effect of *Convolvulus fatmensis* extract on some bacteria (Gram + and Gram -).

Microorganism	*Bacil sub.*	*Sarc sp.*	*Staph aur.*	*Psud aurog.*	*Kleb pnem.*	*E. coli*	*Ent. Sp.*	*Salm. Typhi*
Inhibition zone	10	17	11	-	32	9	7	9

Where: *Bacil sub: Bacillus subtilis, Sarc sp.: Sarcina maxima, Staph aur :Staphylococcus aureus, Psud aurog: Pseudomonas auregenosa, Kleb pnem: Klebsiella pneumonia E. coli :Echerichia coli, Ent. Sp:Enterobacter sp.* and *Salm. Typhi :Salmonilla typhi*

Table (6). Effect of total extract of *Convolvulus fatmensis* on some fungi.

Microorganism	*Candida albicans*	*Aspergillus niger*	*Aspergillus flavus*	*Tri. Viridae*
Inhibition zone	-	12	-	8

2- Antitumour activity

The alcoholic extract of *Convolvulus fatmensis* was found to be active against Ehrlich ascites carcinoma in vitro at different doses 25, 50 and 100 mg. The percent of inhibited cells viability were 50, 70 and 85, respectively, i.e the most active dose was 100 mg/ml This activity may be due to the presence of flavonoid compounds which have antioxidant and anticarcenogenic activities (Yang *et al.*, 2001b; Jhon Finly, 2005).

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تقدير المواد الفينولية وبعض المكونات الكيميائية لنبات المداد وتأثيره البيولوجى

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ينتمى نبات المداد الى العائلة اللبلابية وينمو فى الصحارى المصرية وقد تمت دراسة محتواه من المواد الكيميائية وتأثير المستخلص الكحولى من الناحية البيولوجية حيث أثبتت النتائج احتواء النبات علي مواد فينولية وستيرولات وتربينات وبروتينات ومواد سكرية وجليكوسيدات. وقد تم فصل وتنقية وتعريف ثمانية مركبات فينولية من المستخلص الكحولي وهى: روتين, كوارستين-٣- جلوكوسيد, كوارستين-٧- جلوكوسيد, كوارستين-٣-رامنوسيد, كامبفيرول-ثلاثي جلوكوسيد, كامبفيرول-٣،٧ ثنائي رامنوسيد, كامبفيرول-٣- جالاكتورامنوسيد و كامبفيرول-٧- رامنوسيد. كما تم تقدير نسبة كل من البروتين, المحتوي الكلي من الأحماض الأمينية باستخدام جهاز تحليل الأحماض الأمينية وكذلك تم دراسة الإستيرولات والتربينات الثلاثية باستخدام جهاز كروماتوجرافيا الغاز. تناولت الدراسة البيولوجية مدى تأثير المستخلص الكحولى على بعض الكائنات الدقيقة الممرضة (بكتيريا وفطريات) كذلك تمت دراسة تأثير النبات علي بعض أنواع الخلايا السرطانية.