



Global Advanced Research Journal of Agricultural Science (ISSN: 2315-5094) Vol. 5(2) pp. 088-093, February, 2016 Issue.
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Full Length Research Papers

Some Biological Effects of the Phenolic Content of Licorice Roots (*Glycyrrhiza glabra* L.)

Mohamed H. M. Abd El Azim^{1*}, Mohamed El-Gerby¹, Ahmed A. M. Abdelgawad², Amani M. D. El-Mesallamy¹

¹Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt.
² Medicinal and Aromatic Plants Dept., Desert Research Center, Cairo 11753, Egypt.

Accepted 14 February, 2016

The phenolic content of licorice roots (*Glycyrrhiza glabra* L.) was investigated by various chromatographic methods. The results showed, identification of 17 phenolic compounds as Protocatechuic acid, Vanillic acid, Benzoic acid, Quercetin-3-O- α -rhamnosyl(1 \rightarrow 6)- β -glucoside (Rutin), Quercetin 3-O- α -rhamnoside (Quercetrin), Naringenin 7-O-rhamnoglucoside (Naringin), (2S)-4',7-dihydroxyflavanone 4'-O- β -D-glucopyranoside (Liquiritin), Ferulic acid, *P*-coumaric acid, Cinnamic acid, Myricetin, Quercitin, Kaempferol, Apigenin, 5,7,4'-trihydroxyflavanone (Naringenin), Liquiritigenin and Flavone. Efficiency of the methanol extract of licorice roots as anticancer agent for breast, colon and liver was tested. The results showed that the IC₅₀ of the extract was 28.1 μ g / ml for anti-colon cancer and 31.3 μ g/ml for anti-breast cancer, while it was 3.42 μ g/ml for anti-hepatic cancer. The antioxidant activity was measured by DPPH radical scavenging method. This extract showed strong antioxidant activity against DPPH as compared with vitamin C. Antimicrobial activity of the methanol extract of licorice roots was studied against three bacterial and four fungal strains at concentration 0.1ml and 0.3ml (10mg/1ml). The extract showed strong inhibitory effect for most species at concentration 0.3ml.

Keywords: *Glycyrrhiza glabra*, phenolic, Antitumor, antioxidant and antimicrobial.

INTRODUCTION

The licorice (*Glycyrrhiza glabra* L., family Leguminosae) plant has a long and storied history of use in both Eastern and Western cultures pre-dating the Babylonian and Egyptian empires (Fenwickie et al., 1990; Olukoga and Donaldson 1998). There are about 30 species containing in *Glycyrrhiza* genus all over the world (Zhang et al., 2011). In Chinese traditional medicine, licorice (*Gan Cao*) remains one of the oldest and most commonly prescribed herbs

and has been used in the treatment of numerous ailments ranging from tuberculosis to peptic ulcers (Huang 1993).

Licorice has held claim for therapeutic use for fevers, liver ailments, dyspepsia, gastric ulcers, sore throats, asthma, bronchitis, Addison's disease and rheumatoid arthritis and has been used as a laxative, antitussive and expectorant (Anon 2005; Schulz et al., 1998 and Wang et al., 2000). Among its most consistent uses are as a demulcent for the digestive system, to treat coughs, to soothe sore throats, and as a flavoring agent. According to Duke (Duke 1985), the tobacco industry is the primary user of licorice derivatives

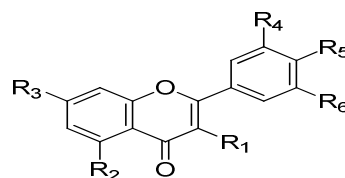
*Corresponding Author's Email: drmhm1982@gmail.com

in the United States, with the remainder equally divided among the food and pharmaceutical industries.

The roots of *Glycyrrhiza glabra* L., have anti-acid, anti-ulcer (Hikino 1985), anti-inflammatory, expectorant, diuretic (Shibata 2000), antimicrobial (Fukai et al., 2002), anxiolytic (Ambawade et al., 2002), anticonvulsant (Ambawade et al., 2001) and memory enhancing (Dhingra et al., 2004) activities. Licorice paste is the preferred form for flavoring tobacco (Carmines et al., 2005) whereas licorice powder is preferred for confectionery and pharmaceuticals. Recently, wild *Glycyrrhiza* became limited because of over-collection. With the decline of resources of wild *Glycyrrhiza*, cultivated *Glycyrrhiza* plants became an additional resource and are anticipated from the standpoint of conserving the natural environment (Dong et al., 2012).

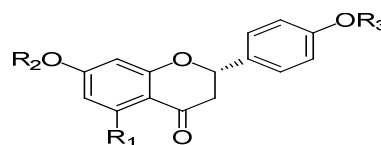
RESULTS AND DISCUSSION

Investigation of the phenolic compounds were done by fractionation of the extract, over polyamide column and elution with methanol/bidistilled water, and then subjected to rechromatography for several times led to the separation of seventeen pure phenolic compounds. The structure of these compounds was confirmed by comparison of their physical and spectral data with those of reported compounds. **(1)** Protocatechuic acid (C₇H₆O₄, 52mg), **(2)** Vanillic acid (C₈H₈O₄, 55mg), **(3)** Benzoic acid (C₇H₆O₂, 70mg), **(4)** Quercetin-3-O- α -rhamnosyl(1 \rightarrow 6)- β -glucoside (Rutin) (C₂₇H₃₀O₁₆, 84mg), **(5)** Quercetin 3-O- α -rhamnoside (Quercetrin) (C₂₁H₂₀O₁₁, 52mg), **(6)** Naringenin 7-O-rhamnoglucoside (Naringin) (C₂₆H₃₀O₁₄, 41mg), **(7)** (2S)-4',7-dihydroxyflavanone 4'-O- β -D-glucopyranoside (Liquiritin) (C₂₁H₂₂O₉, 105mg), **(8)** Ferulic acid (C₁₀H₁₀O₄, 15mg), **(9)** P-coumaric acid (C₉H₈O₃, 19mg), **(10)** Cinnamic acid (C₉H₈O₂, 17mg), **(11)** Myricetin (C₁₅H₁₀O₈, 15mg), **(12)** Quercitin (C₁₅H₁₀O₇, 90mg), **(13)** Kaempferol (C₁₅H₁₀O₆, 10mg), **(14)** Apigenin (C₁₅H₁₀O₅, 29mg), **(15)** 5,7,4'-trihydroxyflavanone (Naringenin) (C₁₅H₁₂O₄, 19mg), **(16)** Liquiritigenin (C₁₅H₁₂O₄, 35mg) and **(17)** Flavone (C₁₅H₁₀O₂, 12mg).



	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆
Flavone	H	H	H	H	H	H
Kaempferol	OH	OH	OH	H	OH	H
Quercetin	OH	OH	OH	OH	OH	H
Quercetrin	O-Glu	OH	OH	OH	OH	H
rutin	O-Glu-O-rham	OH	OH	OH	OH	H
Myricetin	OH	OH	OH	OH	OH	OH
Apigenin	H	OH	OH	H	OH	H

Glu-O-rham = Glucorhamanoside



Liquiritigenin	R ₁ = R ₂ = R ₃ = H
Liquiritin	R ₁ = R ₂ = H, R ₃ = Glucopyranoside
Naringenin	R ₁ = OH, R ₂ = R ₃ = H
Naringin	R ₁ = OH, R ₂ = rhamnoglucoside, R ₃ = H

Antitumor activity

The potential cytotoxicity activity of the methanol extract of licorice roots was tested against three human cell lines [HEPG-2 (liver carcinoma cell line), MCF-7 (breast carcinoma cell line) and HCT-116 (colon carcinoma cell line)] by SRB (Sulphorhodamine-B) assay. The results showed that the extract has strong activity against all cell lines tested. The antitumor activity of the tested extract is summarized in figure 1. The IC₅₀ values (the concentrations of thymoquinone required to produce 50% inhibition of cell growth) of the extract against each cell lines were 28.1 μ g / ml, 31.3 μ g / ml and 3.42 μ g / ml for HCT-116, MCF-7 and HEPG-2, respectively.

Antioxidant activity

The DPPH scavenging activity of the methanol extract of licorice roots is summarized in figure 2. It was observed that the scavenging activity of the extract at all concentrations (25, 50 and 100 μ l) is rather strong (32.6-72.3%) as compared with vitamin C. The remarkable antioxidant activity of methanol extract of licorice roots might be due to the higher concentration of phenolic compounds. IC₅₀ value for the methanol extract = 64 μ g/ml, while for vitamin C = 17 μ g/ml.

Antimicrobial Screening

Antibacterial activity

Data in figure 3 evaluate that the maximum inhibitory responses are indicated after the treatment of *E. coli*. and



	R ₁	R ₂		R ₁	R ₂
Benzoic acid	H	H	Cinamic acid	H	H
Protocatechuic acid	OH	OH	4-C umaric acid	H	OH
Vanillic Acid	OCH ₃	OH	Ferulic Acid	OCH ₃	OH

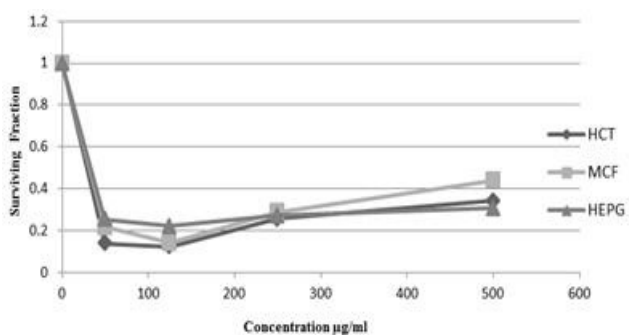


Figure 1: % of survival fraction of carcinoma cell lines (colon, breast and liver) against concentration

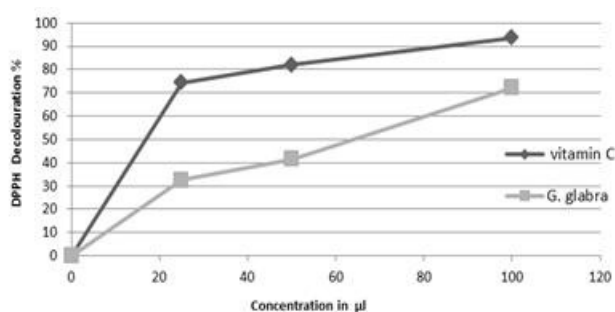


Figure 2: Antioxidant activity of the methanol extract of licorice roots compared to vitamin C

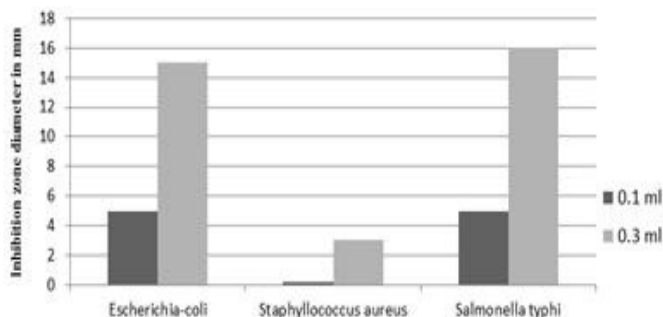


Figure 3: Antibacterial activity of the methanol extract of licorice roots

Salmonella typhi with highest concentration of the extract (0.3ml), while the moderating inhibitory response after the treatment of *E. coli.* and *Salmonella typhi* with normal concentration of the extract (0.1ml).On the other hand, *Staphylococcus aureus* had the highest resistance species to the extract at concentration 0.3 ml and no inhibitory effect at concentration 0.1ml.

Antifungal activity

The extract showed strongly inhibitory activity against *Trichoderma sp.*at 0.3ml concentration and moderated inhibitory activity for *Fusarium* and *penicillium sp.*, while at 0.1ml concentration all fungal strains showed no inhibitory activity as shown in figure 4.

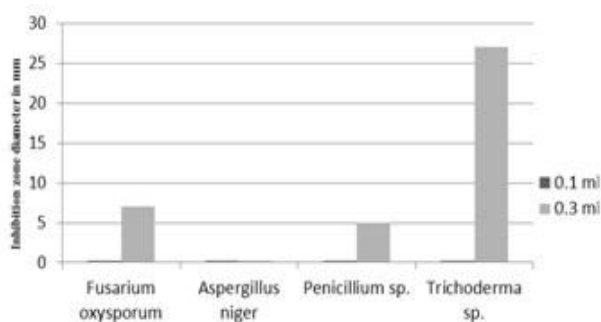


Figure 4: Antifungal activity of the methanol extract of licorice roots

Experimental

Plant Material

Licorice roots (*Glycyrrhiza glabra* L.) 2kg were provided from Lotus Company (Sekem Group, Egypt). The taxonomic identification of plant material was confirmed by Botany Department, Faculty of Science, Zagazig University (Egypt).

Physical tests

Ultra-violet spectrophotometric analysis

Chromatographically, pure materials dissolved in analytically pure methanol were subjected to UV spectrophotometric investigation in 4 ml capacity quartz cells Zeiss spectrometer PMQ-II. In case of flavonoids, AlCl_3 , AlCl_3/HCl , fused $\text{NaOAc}/\text{H}_3\text{BO}_3$ and NaOMe reagents were separately added to methanol solution of the investigated material and UV measurements were then carried out (Harborne 1982).

NMR spectroscopic

^1H and ^{13}C chemical shifts (δ) were measured in ppm, relative to $\text{DMSO}-d_6$ and converted to TMS scale. Joel EC a 500 MHz NMR Spectrometer at 500 MHz, (Institute Fur Chemie, Humboldt Universitätzu Berlin, Germany). ^1H chemical shifts were measured in ppm, relative to TMS and ^{13}C NMR chemical shifts to $\text{DMSO}-d_6$ and converted to TMS scale by adding 39.5. Typical conditions: spectral width = 8 KHz for ^1H and 30 KHz for ^{13}C , 64 K data points and a flip angle of 45°C .

Mass spectroscopic

The isolation pure compounds were subjected (FAB –MS). The isolated pure compounds were subjected, in most cases to Fast Atom Bombardment (positive and

negative) mass spectroscopic analysis (FAB-MS) on MM 7070 E spectrometer (VG analytical). Some other compounds were subjected to electron spray ionization mass spectroscopic analysis (ESI-MS) a Varian Mat1 12-ET Spectrometer. All measurements were carried out at Institute Fur Chemie, Humboldt Universitätzu Berlin, Germany.

Extraction and isolation

The dried roots of *G. glabra* (2.0kg) were exhaustively extracted under reflux over a water bath with 10 liters of a methanol / bidistilled water (3:1) mixture for 3hours. The solvent was removed under reduced pressure at about 45°C . The residual finally yielded 60g of a sticky dark brown material. Fractionation of the extract, (30g dissolved in 100 ml aqueous methanol 3:1) over Sephadex LH-20 (200g) column (150 X 4.5cm) and elution with methanol/bidistilled water mixtures of decreasing polarities for gradient elution led to the desorption of six individual fractions (I-VI) which were dried, individually.

Fraction I: TDPC of the material of this fraction showed the presence of free reducing sugars identified by means of Co PC.

Fraction II:Compound (1): $^1\text{H-NMR}$ ($\text{DMSO}-d_6$): δ 6.85(*d*, $J=8\text{Hz}$, H-5), 7.45(*dd*, $J=8\& 2\text{Hz}$, H-6) and 7.5(*d*, $J=8\text{Hz}$, H-2). $^{13}\text{C-NMR}$ ($\text{DMSO}-d_6$): δ 122.8(C-1), 115.6(C-2), 150.7(C-3), 145.5(C-4), 117.4 (C-5), 123.6(C-6) and 168.2(C=O). **Compound (2):** $^{13}\text{C-NMR}$ ($\text{DMSO}-d_6$): δ 126.41(C-1), 119.51(C-2), 151.59(C-3), 151.92(C-4), 115.49(C-5), 127.66(C-6), 57.99(O-Me), 170.98(C=O).

Compound (3): $^1\text{H-NMR}$ (CDCl_3): δ 12.9 (s, H_{Carboxylic}), 8.20(*d*, H-2 and H-6), 7.83(*m*, H-3 and H-5) and 7.4(*m*, H-4).

Fraction III:Compound (4): $^1\text{H-NMR}$ ($\text{DMSO}-d_6$): δ (ppm) Quercetin moiety: δ 6.18(*d*, $J=2.5\text{Hz}$, H-6), 6.37(*d*, $J=2.5\text{ Hz}$, H-8), 7.55(*d*, $J=2.5\text{ Hz}$, H-2'), 6.85(*d*, $J=8\text{Hz}$, H-5'), 7.56(*dd*, $J=2.5$ and 8Hz , H-6'). Glucose moiety: δ 5.32(*d*, $J=8\text{ Hz}$, H-1"), 3-3.75(*m*, Hz, H-2" and 6"), 4.35 (broad s, $\Delta\nu_{1/2}= 4$). Rhamnosemoiety: δ 3-3.75(*m*, H-1",

H-2''' and H-5'''), 0.97(*d*, $J=6\text{Hz}$, CH₃-rhamnose). ¹³C-NMR (DMSO-d₆): Quercetin moiety: δ 156.5(C-2), 133.3(C-3), 177.4(C-4), 161.3(C-5), 98.8(C-6), 164.1(C-7), 93.6(C-8), 156.7(C-9), 104.0(C-10), 121.6(C-1'), 115.3(C-2'), 144.8(C-3'), 148.5(C-4'), 116.3(C-5'), 121.2(C-6'). Glucose moiety: δ 101.2(C-1''), 74.1(C-2''), 76.5(C-3''), 70.0(C-4''), 75.9(C-5''), 67.5(C-6''). Rhamnose moiety: δ 100.8(C-1'''), 70.4(C-2'''), 70.6(C-3'''), 71.9(C-4'''), 68.3(C-5'''), 17.85(CH₃-rhamnose).

Compound (5): UV (MeOH): $\lambda_{\text{max}} = 259, 297\text{nm}$; 348nm.Ms (m/z): 449.1[M⁺ + 1, 22.8%]. ¹H-NMR (DMSO-d₆): Quercetin moiety: δ 6.17(*d*, $J=2.5\text{ Hz}$, H-6), 6.36(*d*, $J=2.5\text{Hz}$, H-8), 7.256(*d*, $J=2.5$, H-2'), 6.82(*d*, $J=8\text{ Hz}$, H-5'), 7.251(*dd*, $J=2.5$ and 8Hz, H-6'). Rhamnose moiety: δ 5.20($\Delta\nu_{1/2} = 4\text{ Hz}$, H-1''), 3.1-3.9(*m*, overlapped with water proton resonances, H-2'' and H-6''). ¹³C-NMR (DMSO-d₆): Quercetin moiety: δ 156.9(C-2), 134.6(C-3), 178.2(C-4), 161.7(C-5), 99.19(C-6), 164.7(C-7), 94.15(C-8), 157.8(C-9), 104.5(C-10), 121.2(C-1'), 115.9(C-2'), 145.7(C-3'), 148.9(C-4'), 116.1(C-5'), 121.6(C-6'). Rhamnose moiety: δ 102.2(C-1''), 70.8(C-2''), 71.1(C-3''), 71.6(C-4''), 70.5(C-5''), 18.01(CH₃).

Compound (6): UV (MeOH) $\lambda_{\text{max}} = 317, 279\text{ nm}$. ¹H-NMR (CD₃OD): δ 7.37(*d*, H-2' and H-6'), 6.85(*d*, H-3' and H-5'), 6.23(*d*, H-6), 5.48(*d*, H-8), 5.41(*dd*, H-2), 3.8(*dd*, H-3a), 2.69(*dd*, H-3b), 5.12(*d*, H-1''), 3.1-3.65(*m*, H-2'' and 6''), 3.1-3.65(*m*, H-1''', H-2''' and H-5'''). **Compound (7):** UV (MeOH) $\lambda_{\text{max}} = 313, 276\text{ nm}$. ¹H-NMR (CD₃OD): δ 7.74(*d*, $J=8.8$, H-5), 7.44(*d*, $J=8.4$, H-2' and H-6'), 7.15(*d*, $J=8.4$, H-3' and H-5'), 6.51(*dd*, $J=8.8$ and 2.0, H-6), 6.37(*d*, $J=2.0$, H-8), 5.45(*dd*, $J=12.8$ and 2.8, H-2), 4.95(*d*, $J=7.2$, H-1''), 3.91(*dd*, $J=12.0$ and 1.6, H-6''a), 3.71(*dd*, $J=12.0$ and 5.6, H-6''b), 3.04(*dd*, $J=16.8$ and 12.8, H-3a), 2.73(*dd*, $J=16.8$ and 2.8, H-3b). ¹³C-NMR (CD₃OD): δ 193.0(C-4), 166.6(C-7), 165.2(C-9), 159.0(C-4'), 134.3(C-1'), 129.7(C-5), 128.7(C-2' and C-6'), 117.7(C-3' and C-5'), 114.9(C-10), 111.7(C-6), 103.7(C-8), 102.1(C-1''), 80.6(C-2), 78.1(C-5''), 77.9(C-3''), 74.8(C-2''), 71.3(C-4''), 62.5(C-6''), 45.0(C-3).

Fraction IV: Compound (8): ¹H-NMR (DMSO-d₆): δ 3.81(*s*, CH₃), 6.69(*d*, H- α), 6.18(*d*, H-5), 7.10(*d*, H-6) 7.30(*s*, H-2), 7.52(*d*, H- β). **Compound (9):** ¹H-NMR (DMSO-d₆): δ 6.2(*d*, $J=15\text{Hz}$, H- α), 6.72(*d*, $J=8\text{Hz}$, H-3 and H-5), 7.32(*d*, $J=8\text{ Hz}$, H-2 and H-6), 7.52(*d*, $J=15\text{Hz}$, H- β). **Compound (10):** ¹H-NMR (DMSO-d₆): δ 6.46(*d*, H- α), 7.40(*t*, H-3 and H-5), 7.42(*t*, H-4), 7.557(*d*, H-2 and H-6), 7.806(*d*, H- β).

Fraction V: Compound (11): UV (MeOH): $\lambda_{\text{max}} = 265, 376\text{ nm}$.Ms (m/z): 317.0 [M⁻ - H, 100%]. ¹H-NMR (DMSO-d₆): δ 6.18(*d*, $J=2.5\text{ Hz}$, H-6), 6.34(*d*, $J=2.5\text{Hz}$, H-8), 7.24(*s*, H-2'&H-6'). **Compound (12):** UV (MeOH): $\lambda_{\text{max}} = 255, 268, 370\text{nm}$.Ms (m/z): 300.8[M⁻ - H, 100%]. ¹H-NMR (DMSO-d₆): δ 6.19(*d*, $J = 2.5$, H-6), 6.4(*d*, $J = 2.5$, H-8), 7.64(*d*, $J = 2.5$, H-2'), 6.88(*d*, $J = 8.5$, H-5'), 7.53(*dd*, $J = 2.5\&8.5$, H-6'). ¹³C-NMR (DMSO-d₆): δ 147.0(C-2), 135.8(C-3), 176.2(C-4), 160.5(C-5), 99.2(C-6), 164.0(C-7), 93.7(C-8), 156.4(C-9), 103.5(C-10), 122.2(C-1'), 115.3(C-2'), 145.1(C-3'), 148.0(C-4'), 115.6(C-5') and 120.2(C-6'). **Compound (13):**

UV (MeOH): $\lambda_{\text{max}} = 268\text{.Ms}$ (m/z): 285.1[M⁻ - H, 100%]. ¹H-NMR (DMSO-d₆): δ 6.4(*d*, $J=2.5$, H-8), 6.18(*d*, $J=2.5$, H-6), 8.14(*d*, $J=8$, H-2' and H-6'), 6.89(*d*, $J=8$, H-3' and H-5'). ¹³C-NMR (DMSO-d₆): δ 146.8(C-2), 135.4(C-3), 175.9(C-4), 161.0(C-5), 98.6(C-6), 164.2(C-7), 93.8(C-8), 156.4(C-9), 103.7(C-10), 121.9(C-1'), 129.9(C-2' and C-6'), 115.8(C-3' and C-5') and 159.5(C-4').

Fraction VI: Compound (14): UV (MeOH): $\lambda_{\text{max}} = 266, 335\text{nm}$.Ms (m/z): 270.2[M⁺, 100%]. ¹H-NMR (DMSO-d₆): δ 6.18(*d*, $J=2.5\text{Hz}$, H-6), 6.47(*d*, $J=2.5\text{Hz}$, H-8), 6.77(*s*, H-3), 6.92(*d*, $J=8\text{Hz}$, H-3' and H-5'), 7.93(*d*, $J=8\text{Hz}$, H-2' and H-6'). ¹³C-NMR (DMSO-d₆): δ 163.8(C-2), 102.8(C-3), 181.5(C-4), 161.3(C-5), 98.7(C-6), 163.6(C-7), 93.9(C-8), 157.2(C-9), 103.6(C-10), 121.1(C-1'), 128.3(C-2' and C-6'), 115.8(C-3' and C-5') and 161.4(C-4').

Compound (15): UV (MeOH) $\lambda = 307.54\text{ nm}$. ¹H-NMR (CD₃OD): δ 7.39(*d*, H-2' and H-6'), 6.78(*d*, H-3' and H-5'), 5.98(*dd*, H-6), 5.47(*d*, H-8), 5.41(*dd*, H-2), 3.7(*dd*, H-3a), 2.72(*dd*, H-3b).

Compound (16): UV (MeOH) $\lambda = 313, 276\text{ nm}$. ¹H-NMR (CD₃OD): δ 7.72(*d*, $J=8.8$, H-5), 7.31(*d*, $J=8.4$, H-2' and H-6'), 6.81(*d*, $J=8.4$, H-3' and H-5'), 6.47(*dd*, $J=8.8$ and 2.0, H-6), 6.34(*d*, $J=2.0$, H-8), 5.35(*dd*, $J=13.2$ and 2.8, H-2), 3.04(*dd*, $J=16.8$ and 13.2, H-3a), 2.67(*dd*, $J=16.8$ and 2.8, H-3b). ¹³C-NMR (CD₃OD): δ 193.4(C-4), 166.6(C-7), 165.4(C-9), 158.8(C-4'), 133.8(C-1'), 129.7(C-5), 128.9(C-2', 6'), 116.2(C-3',5'), 114.8(C-10), 111.6(C-6), 103.7(C-8), 81.0(C-2), 44.9(C-3). **Compound (17):** UV (MeOH): $\lambda_{\text{max}} = 251.2, 294\text{nm}$.Ms (m/z): 224.2[M⁺ + 2, 2.3%]. ¹H-NMR (DMSO-d₆): δ 6.79(*s*, H-3), 4.36-8.06 (several multiplets assignable to the remaining protons). ¹³C-NMR (DMSO-d₆): δ 163.2(C-2), 107.38(C-3), 177.74(C-4), 126.31(C-5), 125.7(C-6), 134.46(C-7), 118.74(C-8), 156.2(C-9), 123.86(C-10), 126.65(C-2' and C-6'), 129.41(C-3' and C-5'), 131.6(C-4').

SRB assay of cytotoxic activity

Human tumor cell lines were obtained frozen in liquid nitrogen (-180 °C) from the American Type Culture Collection. The tumor cell lines were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing. Measurement of potential cytotoxicity activity of methanol extract of licorice roots against the liver carcinoma cell line (HEPG-2), colon carcinoma cell line (HCT-116) and breast carcinoma cell line (MCF-7) was tested by SRB (Sulphorhodamine-B) assay using the method of (Skehan et al., 1990). This experiment was conducted in the National Cancer Institute, Cairo, Egypt.

DPPH assay

The free radical scavenging effect of plant extracts was assessed by the decolouration solution of DPPH radical according to (Letelier et al., 2008), in Faculty of Agriculture Research Park – Cairo University (FARP). This assay was

realized essentially by the method described by (Joyeux et al, 1995) and modified by (Viturro et al., 1999).

Antimicrobial activity

Strains were obtained from the bacteria stock present at the Research Laboratory of bacteriology, Faculty of Science, Zagazig University. Gram-positive and Gram-negative bacteria species tested were *E. coli* (KQ103), *Staphylococcus aureus* (LC405) and *Salmonella typhi* (RS57) and fungi species (Laboratory collection strains) were *Fusarium oxysporum*, *Aspergillus niger*, *Penicillium sp.* and *Trichoderma sp.* The methanol extract was dissolved in Dimethyl formamide (DMF) for antimicrobial investigation at the final concentration of (10 mg / 1 ml).

Antibacterial activity

In vitro antimicrobial assay of the methanol extract was carried out according to pour plate technique at two concentrations 0.1ml and 0.3ml (10mg/1ml). Culturing and incubated of different bacteria species were carried out at 37 °C for 24 hours. After the elapse of incubation periods, the diameter of inhibition zones was measured (Vaghasiya et al., 2004).

Antifungal activity

Czepak Dox media used for cultivation of fungal species. The medium was seeded with different fungal species. After solidification of media on plates, make pores in agar with cup-borer (15mm) diameter. Two concentrations 0.1ml and 0.3ml (10mg/1ml) of the methanol extract were transferred into the well. Dimethyl formamide (DMF) was used only as a control. The plates were incubated for 7 days at 30°C. The inhibition zone formed by the extract against the particular test fungal strain determined as the antifungal activities of the extract.

CONCLUSIONS

The overall results of this study indicate that the methanol extract of licorice represent a potential source of plant drugs. So; we can deduce that the methanol extract of licorice appeared to be promising choice to be considered as antioxidant and antitumor medicines.

REFERENCES

Ambawade SD, Kasture VS, Kasture SB (2002): Anticonvulsant Activity of Roots and Rhizomes of *Glycyrrhiza glabra*. *Indian J. Pharmacol*, 34, 251-255.
 Ambawade SD, Kasture VS, Kasture SB (2001): Anxiolytic Activity of *Glycyrrhiza glabra* Linn. *J Nat Rem*, 2, 130-134.
 Anon (2005): *Glycyrrhiza glabra*, *Alternative Medicine Review*, Pp. 10, 230-237.

Carmine EL, Lemus R, Gaworski CL (2005): Toxicological Evaluation Of Licorice Extract As A Cigarette Ingredient. *Food and Chemical Toxicology*, 43, 1303-1322.
 Dhingra D, Parle M, Kulkarni SK (2004): Memory Enhancing Activity of *Glycyrrhiza glabra* In Mice, *J Ethnopharmacol*, 91, 361-365.
 Dong YY, Gao WY, Zhang JZ, Zuo BM, Huang LQ (2012): Quantification Of Four Active Ingredients And Fingerprint Analysis Of Licorice (*Glycyrrhiza uralensis fisch*) After Spaceflight By HPLC–DAD. *Res. Chem. Inter Med*, 38(8), 1719-1731.
 Duke JA (1985): *CRC Handbook Of Medicinal Herbs*. CRC Press, Inc, Boca Raton, FL, Pp. 215-216.
 Fenwick G, Lutowski J, Nieman C (1990): Licorice, *Glycyrrhiza glabra* L. Composition, Uses and Analysis, *Food Chem*, 38 (2), 119-143.
 Fukai T, Marumo A, Kaitou K, Kanda T, Terada S, Nomura T (2002): Antimicrobial Activity Of Licorice Flavonoids Against Methicillin-Resistant *Staphylococcus Aureus*, *Fitoterapia*, 73, 536-539.
 Hikino H (1985): Recent Research On Oriental Medicinal Plants, In: Wagner H, Hikino H, Farnsworth NR, Editors. *Economic And Medicinal Plant Research*, London' Academic Press, Pp. 53.
 Huang KC (1993): *The Pharmacology Of Chinese Herbs*, CRC Press, Inc, Boca Raton, FL, Pp. 275-278.
 Joyeux M, Lobstein A, Anton R, Mortier F (1995): Comparative Antilipoper Oxidant, Antinecrotic And Scavenging Properties Of Terpenes And Biflavones From Ginkgo And Some Flavonoids, *Planta Med*, 61(2), 126-129.
 Letelier ME, Molina-Berrios A, Cortés-Troncoso J, Jara-Sandoval J, Holst M, Palma K, Montoya M, Miranda D, González-Lira V (2008): DPPH And Oxygen Free Radicals As Pro-Oxidant Of Biomolecules, *Toxicology In Vitro*, 22, 279-286.
 Olukoga A, Donaldson D (1998): Historical Perspectives On Health, The History Of Licorice: The Plant, Its Extract, Cultivation, Commercialization and Etymology, *Journal Of The Royal Society Of Health*, 118, 300-304.
 Schulz V, Hnnsel R, Tyler VE (1998): *Rational Phytotherapy, A Physicians' Guide To Herbal Medicine*. Springer-Verlag, Berlin, Germany, Pp. 160-187.
 Shibata SA (2000): Drug Over The Millennia: Pharmacognosy, Chemistry And Pharmacology Of Licorice. *Yakugakuzasshi*, 2000, 120, 849-862.
 Harborne JB (1982): "The Flavonoids: Advances In Research", Chapman And Hall, London.
 Skehan P, Storeng R, Scudiero D, Monks A, McMahon J (1990): New Colorimetric Cytotoxicity Assay For Anticancer Drug Screening. *J Natl Cancer Inst*, 82, 1107-1112.
 Vaghasiya YK, Nair R, Soni M, Balujs S, Chanda S (2004): Synthesis, Structural Determination And Antibacterial Activity Of Compounds derived from vanillin and 4-aminoantipyrine. *J Serb Chem Soc*, 69(12), 991-998.
 Viturro C, Molina A, Schmeda-Hirschmann G (1999): Free Radical Scavengers From *Mutisiafriesiana* (Asteraceae) And *Sanicula graveolens* (Apiaceae). *Phytother Res*, 13(5), 422-424.
 Wang ZY, Athar M, Bickers DR (2000): Licorice In Foods And Herbal Drugs: Chemistry, Pharmacology, Toxicology And Uses. In: Mazza G, Oomah BD (Eds.), *Herbs, Botanicals And Teas*. Technomic Publishing Co. Inc, Lancaster, PA, Pp. 321-353.
 Zhang JZ, Gao WY, Gao Y, Liu DL, Huang LQ (2011): Analysis Of Influences Of Spaceflight On Chemical Constituents In Licorice By HPLC–ESI-MS/MS, *Acta Physiol Plant*, 33, 2511-20