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# Anti-tumor, antioxidant and antimicrobial and the phenolic constituents of *Glycyrrhizaglabra*

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#### ABSTRACT

Seventeen phenolic compounds were defined from the methanol extract of licorice roots (*Glycyrrhizia glabra* L.) by chromatographic methods. Efficiency of the methanol extract of licorice roots as anticancer agent for breast, colon and liver was tested. The results showed that the IC<sub>50</sub> of the extract was  $28.1 \mu g/ml$  for anti-colon cancer and  $31.3 \mu g/ml$  for anti-breast cancer, while it was  $3.42 \mu g/ml$  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. © 2014 Trade Science Inc. - INDIA

#### **KEYWORDS**

*Glycyrrhiza glabra;* Phenolic; Antitumor; Antioxidant; Antimicrobial..

#### INTRODUCTION

The licorice (*Glycyrrhizia 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<sup>[1,2]</sup>. There are about 30 species containing in Glycyrrhiza genus all over the world<sup>[3]</sup>. 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<sup>[4]</sup>. 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<sup>[5-7]</sup>. Among its most consis-

tent uses are as a demulcent for the digestive system, to treat coughs, to soothe sore throats, and as a flavoring agent. According to Duke<sup>[8]</sup>, the tobacco industry is the primary user of licorice derivatives in the United States, with the remainder equally divided among the food and pharmaceutical industries.

The roots of *Glycyrrhiza glabra* L., have antacid, anti-ulcer<sup>[10]</sup>, anti-inflammatory, expectorant, diuretic<sup>[11]</sup>, antimicrobial<sup>[12]</sup>, anxiolytic<sup>[13]</sup>, anticonvulsant<sup>[14]</sup> and memory enhancing<sup>[15]</sup> activities. Licorice paste is the preferred form for flavoring tobacco<sup>[16]</sup> 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<sup>[17]</sup>.

#### **RESULTS AND DISCUSSION**

Investigation of the phenolic compounds was 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 ( $C_7H_6O_4$ ) 52mg), (2) Vanillic acid ( $C_8H_8O_4$ , 55mg), (3) benzoic acid ( $C_7H_6O_2$ , 70mg), (4) quercetin-3-O- $\alpha$ -rhamnosyl  $(1\rightarrow 6)$ - $\beta$ -glucoside (Rutin) (C<sub>27</sub>H<sub>30</sub>O<sub>16</sub>, 84mg), (5) Quercetin 3-O- $\alpha$ -rhamnoside (Quercetrin)  $(C_{21}H_{20}O_{11}, 52mg)$ , (6) Naringenin 7-0rhamnoglucoside (Naringin) ( $C_{26}H_{30}O_{14}$ , 41mg), (7) (2S)-4',7-dihydroxyflavanone 4'-O-β-D-

glucopyranoside (liquiritin)  $(C_{21}H_{22}O_9, 105mg)$ , (8) Ferulic acid  $(C_{10}H_{10}O_4, 15mg)$ , (9) p-coumaric acid  $(C_9H_8O_3, 19mg)$ , (10) Cinnamic acid  $(C_9H_8O_2, 17mg)$ ,11) Myricetin  $(C_{15}H_{10}O_8, 15mg)$ , (12) Quercitin  $(C_{15}H_{10}O_7, 90mg)$ , (13) Kaempferol  $(C_{15}H_{10}O_6, 10mg)$ , (14) Apigenin  $(C_{15}H_{10}O_5, 29mg)$ , (15) 5,7,4'-

trihydroxyflavanone (Naringenin) ( $C_{15}H_{12}O_4$ , 19mg), (16) (2*S*)-4',7-dihydroxyflavanone 4'-O- $\beta$ -D-glucopyranoside (Liquiritin)( $C_{15}H_{12}O_4$ , 35mg) and (17) Flavone ( $C_{15}H_{10}O_2$ , 12mg).

#### Antitumor activity

The potential cytotoxicity activity of the methanolic extract of licorice roots was tested against three human cell lines [HEPG2 (liver carcinoma cell line), MCF7 (breast carcinoma cell line) and HCT116 (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

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



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

tested extract is summarized in Figure 1. The  $IC_{50}$  values (the concentrations of thymoquinone required to produce 50% inhibition of cell growth) of the extract against each cell lines were  $28.1 \mu g/ml$ ,  $31.3 \mu g/ml$ and 3.42µg/ml for HCT116, MCF7 and HEPG2, respectively.

#### Antioxidant activity

The DPPH scavenging activity of the methanolic 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  $100\mu$ l) is rather strong (32.6-72.3%) as compared with vitamin C. The remarkable antioxidant activity of methanolic extract of licorice roots might be due to the higher concentration of phenolic compounds.  $IC_{50}$  value for the methanolic extract =  $64\mu g/ml$ , while for vitamin C =  $17\mu g/ml$ .

#### **Antimicrobial screening**

#### Antibacterial activity

Data in Figure 3 evaluate that the maximum inhibitory responses are indicated after the treatment of E. coli. and 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, Staphyllococcusaureus 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.







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#### EXPERIMENTAL

#### **Plant material**

Licorice roots (*Glycyrrhizaglabra* 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<sub>3</sub>, AlCl<sub>3</sub>/HCl, fused NaOAc/H<sub>3</sub>BO<sub>3</sub> and NaOMe reagents were separately added to methanolic solution of the investigated material and UV measurements were then carried out<sup>[9]</sup>.

#### Nuclear magnetic resonance spectroscopic analysis

NMR spectra were measured on Jeol ECA 500 MHz NMR Spectrometer at National Research Center, Dokki, Cairo, Egypt. 1H chemical shifts (d) were measured in ppm, relative to dmso-d6 and converted to TMS scale.

#### Mass spectrometric analysis

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 analy tical). 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 Universitat zu Berlin, Germany.

#### **Extraction and isolation**

500 gm from dried clove buds exhaustively extracted under reflux over a water bath with 5 liters of a methanol / bidistilled water (3: 1) mixture for 3 hours. The solvent was removed under reduced pressure at about  $45 \,^{\circ}$ C. The residual finally yielded 30 gm of a sticky dark brown material. Fractionation of the extract, (30 gm dissolved in 100 ml aqueous methanol 3:1) over Sephadex LH-20 (200 gm) column (150 X 4.5 cm) and elution with methanol/bidistilled water mixtures of decreasing polarities for gradient elution led to the desorption of sex individual fractions (I-VI) which were dried, individually, in vacuum, and then subjected to rechromatography for several times to obtain a pure phenolic compounds. The structure of these compounds was confirmed by comparison of their physical and spectral data.

#### **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 sex 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 CoPC.

Fraction II: Compound (1): <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>): δ 6.85(*d*, *J*=8Hz, H-5), 7.45(*dd*, *J*=8& 2Hz, H-6) and 7.5(*d*, *J*=8Hz, H-2). <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>): 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): <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>): 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): <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ 12.9 (*s*, H<sub>Carboxylic</sub>), 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**): <sup>1</sup>H-NMR (DMSOd<sub>6</sub>): δ (ppm) Quercetin moiety: δ 6.18(*d*, *J*=2.5Hz, H-6), 6.37(*d*, *J*=2.5 Hz, H-8), 7.55(*d*, *J*=2.5 Hz, H-2'), 6.85(*d*, *J*=8Hz, H-5'), 7.56(*dd*, *J*=2.5 and 8Hz, H-6'). Glucose moiety: δ 5.32(*d*, *J*=8 Hz, H-1"), 3-3.75(m, Hz, H-2" and 6"), 4.35 (broad *s*,  $\Delta v_{1/2} = 4$ ). Rhamnose moiety: δ 3-3.75(*m*, H-1"', H-2"' and H-5'''), 0.97(*d*, *J*=6Hz, CH<sub>3</sub>-rhamnose). <sup>13</sup>C- NMR

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(DMSO-d<sub>6</sub>): Quercetin moiety:  $\delta$  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:  $\delta$  100.8(C-1""), 70.4(C-2"), 70.6(C-3"), 71.9(C-4"), 68.3(C-5"), 17.85(CH<sub>3</sub>-rhamnose). Compound (5): UV (MeOH):  $\lambda_{max} = 259, 297$ nm, 348nm.Ms (m/z): 449.1[M<sup>+</sup> + 1, 22.8%]. <sup>1</sup>H-NMR (DMSO-d<sub>z</sub>): Quercetin moiety: δ 6.17(d, J=2.5 Hz, H-6), 6.36(d, J=2.5Hz, H-8), 7.256(d, J=2.5, H-2'), 6.82(d, J=8 Hz, H-5'), 7.251(dd, J=2.5 and 8Hz, H-6'). Rhamnose moiety:  $\delta$  $5.20(\Delta v_{1/2} = 4 \text{ Hz}, \text{H-1"}), 3.1-3.9(m, \text{ overlapped with})$ water proton resonances, H-2" and H-6"). <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>): Quercetin moiety:  $\delta$  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:  $\delta$ 102.2(C-1"), 70.8(C-2"), 71.1(C-3"), 71.6(C-4"), 70.5 (C-5"), 18.01(CH<sub>2</sub>). Compound (6): UV (MeOH)  $\lambda_{max}$  =317, 279 nm. <sup>1</sup>H-NMR (CD<sub>3</sub>OD):  $\delta$ 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)  $\lambda_{max} = 313, 276 \text{ nm.} {}^{1}\text{H-}$ NMR (CD<sub>3</sub>OD):  $\delta$  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).<sup>13</sup>C-NMR (CD<sub>3</sub>OD): δ193.0 (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): <sup>1</sup>H-NMR (DMSOd<sub>6</sub>):  $\delta$  3.81(*s*, CH<sub>3</sub>), 6.69 (*d*, H- $\alpha$ ), 6.18(*d*, H-5), 7.10 (*d*, H-6)7.30 (*s*, H-2), 7.52(*d*, H- $\beta$ ). Compound (9): <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>):  $\delta$  6.2 (*d*, *J*=15Hz, H- $\alpha$ ),

Organic CHEMISTRY Au Indian Journal 6.72(*d*, *J*=8Hz, H-3 and H-5), 7.32 (*d*, *J*=8 Hz, H-2 and H-6), 7.52(*d*, *J*=15Hz, H-β). Compound (**10**): <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>): 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):  $\lambda_{max} =$ 265, 376 nm.Ms (m/z): 317.0 [M<sup>-</sup> - H, 100%].<sup>1</sup>H-NMR (DMSO-d<sub>z</sub>):  $\delta$  6.18(d, J=2.5 Hz, H-6), 6.34(d, J=2.5Hz, H-8), 7.24(s, H-2'&H-6'). Compound (12): UV (MeOH):  $\lambda_{max} = 255$ , 268, 370nm.Ms (m/z):  $300.8[M^{-} - H, 100\%]$ .<sup>1</sup>H-NMR (DMSO-d<sub>6</sub>):  $\delta$ 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').<sup>13</sup>C-NMR (DMSO-d<sub>c</sub>):  $\delta$  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):  $\lambda_{max} = 268.Ms (m/z): 285.1[M^{-} - H, 100\%].$ <sup>1</sup>H- NMR (DMSO-d<sub>6</sub>):  $\delta$  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'). <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>):  $\delta$ 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):  $\lambda_{max} =$ 266, 335nm.Ms (m/z): 270.2[M<sup>+</sup>, 100%].<sup>1</sup>H-NMR  $(DMSO-d_{a}): \delta 6.18(d, J=2.5Hz, H-6), 6.47(d, d)$ J=2.5Hz, H-8), 6.77(s, H-3), 6.92(d, J=8Hz, H-3' and H-5'), 7.93(d, J=8Hz, H-2' and H-6').<sup>13</sup>C-NMR (DMSO-d<sub>2</sub>): 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 nm. <sup>1</sup>H-NMR (CD<sub>3</sub>OD): 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 nm. <sup>1</sup>H-NMR (CD<sub>3</sub>OD):  $\delta$  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).<sup>13</sup>C-NMR (CD<sub>2</sub>OD): δ 193.4(C-4), 166.6(C-7), 165.4(C-9), 158.8(C-4'), 133.8(C-1'),

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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):  $\lambda_{max} = 251.2, 294$ nm.Ms (m/z): 224.2[M<sup>+</sup> + 2, 2.3%].<sup>1</sup>H-NMR (DMSO-d<sub>6</sub>):  $\delta$  6.79 (*s*, H-3), 4.36-8.06 (several multiplets assignable to the remaining protons). <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>):  $\delta$  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 methanolic extract of licorice roots against the liver carcinoma cell line (HEPG2), colon carcinoma cell line (HCT116) and breast carcinoma cell line (MCF7) was tested by SRB (Sulphorhodamine-B) assay using the method of<sup>[18]</sup>. 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.*<sup>[19]</sup> in Faculty of Agriculture Research Park – Cairo University (FARP). This assay was realized essentially by the method described by Joyeux *et al.*<sup>[19]</sup> and modified by Viturro*et al.*<sup>[21]</sup>.

#### 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), *Staphyllococcus aureus* (LC405) and *Salmonella typhi* (RS57) and fungi species (Laboratory collection strains) were *Fusarium oxysporum*,*Aspergillus niger*, *Penicillium sp. and Trichoderma sp*. The methanolic extract was dissolved in Dimethylforamide (DMF) for antimicrobial investigation at the final concentration of (10 mg/1 ml).

#### Antibacterial activity

In vitro antimicrobial assay of the methanolic ex-

tract was carried out according to pour plate technique at two concentrations 0.1 ml and 0.3 ml (10 mg/1 ml). 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<sup>[22]</sup>.

#### Antifungal activity

CzepakDox 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 methanolic extract were transferred into the well. Dimethyl foramide (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 methanolic extract of licorice represent a potential source of plant drugs. So, we can deduce that the methanolic extract of licorice appeared to be promising choice to be considered as antioxidant and antitumor medicines.

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