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PHENOLIC CONSTITUENTS AND BIOLOGICAL ACTIVITY OF THE AERIAL PARTS OF THE EGYPTIAN LICORICE (*GLYCYRRHIZA GLABRA* L.)

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ABSTRACT

Four isoflavones (glabrisoflavone, prunitin, santal and genisetin), two flavonols (kaempferol and quercetin) and one phenolic acid (4-hydroxy phenyl acetic acid) beside β-sitosterol and β-sitosterol-O-glucoside, were isolated from the aerial parts of the Egyptian licorice (*G. glabra*). Santal, 4-hydroxy phenyl acetic acid and β-sitosterol-O-glucoside are reported here for the first time in the genus *Glycyrrhiza*. The structures of the isolated compounds were determined by IR, UV, MS, ^{1}H-NMR and ^{13}C-NMR (2D experiments), as well as comparison with reference samples. Preliminary pharmacological testing of the total alcoholic extract and some of its fractions demonstrated potent anti-inflammatory and hepato-protective activities compared to hydrocortisone and silymarin, respectively.

INTRODUCTION

Licorice is the roots and stolons of various species of *Glycyrrhiza* (Leguminosae), of which *G. glabra* is considered as the main source of licorice in the Arab area[1,2]Licorice as a herbal drug has long been employed in pharmacy as a flavoring and sweetening agent, demulcent, expectorant, antiulcerogenic, spleen tonic and detoxicant, and also to treat rheumatoid arthritis and to restore vitality[2-4]. Licorice root is a component in over 60 percent of Chinese and Japanese traditional herbal formulae, and is used in the treatment of muscle spasm of the G.I.T., stomach and peptic ulcers and eczema[5]. It is reported to improve the metabolism of fats and reverses the degeneration of liver cells by improving detoxification. This makes it useful for treating fatty livers, lowering cholesterol, and treating chronic hepatitis. It also improves immunity by increasing interferon production[5].

Glycyrrhizin which is the main active glycoside of licorice has a molecular structure which is very similar to that of cortisone, and is responsible in part for the anti-inflammatory and anti-ulcer effects of licorice[5,6].

Although the chemical constituents of *G. glabra* roots and stolons have been extensively studied in details[7-14], those of the aerial parts have not received much attention, only few reports are available[15-20]. The later included the isolation of flavonoids[15-19] as well as triterpenoids[20]. The intensive use of large amounts of the roots and stolons of licorice generates massive amounts of non-used aerial parts which can be considered as waste products. Subsequently, and in continuation of our search for biologically useful secondary metabolites from plant waste products[21,22], the aerial parts of Egyptian grown *G. glabra* has been investigated herein.

This paper describes the isolation of four isoflavones, two flavonols and one phenolic acid beside β-sitosterol and β-sitosterol-O-glucoside from the aerial parts of the Egyptian licorice. Some of the isolated compounds are reported for the first time in the genus *Glycyrrhiza*.

EXPERIMENTAL

General: Melting points were determined on Gallenkamp melting point apparatus (UK), and were uncorrected. IR (KBr) spectra were run on a Bruker FT-IR Spectrophotometer (Germany). UV spectra were measured on a Shimadzu UV-260 UV Spectrophotometer. ^{1}H- and ^{13}C-NMR, and 2D experiments (APT, COSY and HETCOR) spectra were obtained on a Varian Mercury VX 300 NMR Spectrometer, at 300 and 75 MHz, respectively. Chemical shifts are given in δ ppm with TMS as internal standard. EI-MS were measured on a Finnigan Mat SSQ 7000 (UV) spectrometer (70 eV). Silica gel 60 (Merck) was used for CC, and precoated TLC plates (Merck) were used.

Plant Material: *G. glabra* aerial parts were collected in April 2000 from the Experimental Garden of the Faculty of Pharmacy, Zagazig University, Zagazig, Egypt. The identity of the plant was kindly confirmed by Dr. N. El-Hadidi, Prof. of Taxonomy, Faculty of Science, Cairo University. A voucher specimen was deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University.

Extraction and Isolation: The dried and crushed aerial parts of *G. glabra* (4 Kg) were repeatedly extracted with 95% EtOH (3×6L) at room temperature. The residue (420 g) obtained on removal of the solvent was fractionated to give light petroleum extract **I** (30 g), chloroform extract **II** (216 g) and ethyl acetate extract **III** (10 g). The remaining aqueous extract was tested for glycyrrhizin as previously described[23], and no glycyrrhizin was detected.

The light petroleum extract **I** was saponified in the usual way, and the unsaponifiable matter was CC over silica gel to provide 160 mg of β-sitosterol.

A part of the chloroform extract **II** (85 g) was chromatographed on a column (90 × 5 cm) of silica gel. Elution was attained using chloroform to which methanol was added in a gradient mode to provide compounds **1** (80 mg), **2** (176 mg) and **3** (50 mg). Another part of the chloroform extract **II** (130 g) was treated with 10% aqueous solution of NaOH and then re-extracted with chloroform to give fraction $\mathbf{II_a}$. The remaining alkaline solution was rendered acidic with conc. HCl and extracted with chloroform to give fraction $\mathbf{II_b}$. The remaining acidic solution was then extracted with ethyl acetate to provide fraction $\mathbf{II_c}$ (14.2 g). The waxy fractions $\mathbf{II_a}$, $\mathbf{II_b}$ (60 g) was subjected to CC on silica gel to provide β-sitosterol and β-sitosterol-O-glucoside (70 mg). Fraction $\mathbf{II_c}$

(14.2 g) was chromatographed on a column (60 × 5cm) of silica gel using chloroform to which methanol was added in increasing amounts to yield compound **4** (120 mg).

The ethyl acetate extract **III** was subjected to CC using a column of silica gel (60 × 2 cm) and was eluted with chloroform then chloroform- methanol mixtures of increasing polarity to give compounds **5** (20 mg), **6** (127 mg) and **7** (50 mg).

Compound 1 (glabrisoflavone): yellow crystals, mp 250-252°C ($CHCl_3$/MeOH). UV λ_{max}^{MeOH} nm: 266, 342 (sh); $\lambda_{max}^{MeOH/AlCl_3}$ nm: 266, 340 (sh). EI-MS, m/z (rel. int. %): 354 $[M]^+$ (10.76), 323 (13.8), 322 (14.9), 321 (43.0), 285 (13.6), 284 (78.0), 283 (100), 271 (17.1), 270 (18.7), 153 (13.5), 123 (13.1), 118 (14.1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.35 (1H, *s*, H-2); δ 1.78 (3H, *s*, CH_3-12), 3.39 (2H, *d*, J = 7.2 Hz, H-9), 5.50 (1H, *t*, J = 7.2 Hz, H-10), 3.84 (2H, *d*, J = 5.5, H-13) and 4.63 (1H, *t*, J = 5.5, OH-13); δ 7.46 (2H, *d*, J = 8.7 Hz, H-2`,6`) and δ 6.90 (2H, *d*, J = 8.7 Hz, H-3`,5`); δ 6.53 (1H, *s*, H-8). The OH groups appeared at δ 13.29 (OH-5), 10.86 (OH-7), and at 9.57 (OH-4`). ^{13}C-NMR: Table 1.

Compound 2 (prunitin): white granules, mp 240°C ($CHCl_3$/ MeOH). UV λ_{max}^{MeOH} nm: 260, 330 (sh); $\lambda_{max}^{MeOH/NaOMe}$ nm: 271, 355 (sh); $\lambda_{max}^{MeOH/AlCl_3}$ nm: 271, 310 (sh), 371 (sh); $\lambda_{max}^{MeOH/NaOAc}$ nm: 260, 330 (sh). EI-MS, m/z (rel. int. %): 284 $[M]^+$ (100), 256 (2.14), 255 (8.32), 254 (2.2), 167 (20.9), 166 (20.9), 142 (9.7), 138 (12.0), 133 (2.6), 123 (2.23), 118 (7.7). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.46 (1H, *s*, H-2); δ 6.48 (1H, *d*, J=2.1 Hz, H-6) and δ 6.73 (1H, *d*, J=2.1 Hz, H-8); δ 7.48 (2H, *d*, J=8.4 Hz, H-2`,6`) and δ 6.91 (2H, *d*, J=8.4 Hz, H-3`,5`); δ 3.95 (1H, *s*, OCH_3); δ 13.00 (1H, *s*, 5-OH); δ 9.59 (1H, *s*, 4`-OH). ^{13}C-NMR: Table 1.

Compound 3 (7-methyl orobol, santal), faint yellow crystals, mp 223°C ($CHCl_3$/MeOH). UV λ_{max}^{MeOH} nm: 262, 295 (sh), 335 (sh); $\lambda_{max}^{MeOH/AlCl_3}$ nm: 269, 295 (sh), 375 (sh); $\lambda_{max}^{MeOH/AlCl_3/HCl}$ nm: 269, 370 (sh); $\lambda_{max}^{MeOH/NaOAc}$ nm: 260, 295 (sh), 330 (sh). EI-MS, m/z (ret. int. %): 300 $[M]^+$ (100), 284 (3.20), 283 (1.56), 272 (1.47), 271 (5.20), 257 (1.05), 244 (1.76), 243 (6.14), 167 (34.57), 166 (9.2) , 141 (7.88), 138 (6.39), 134 (9.55), 127 (10.55), 123 (2.02), 118 (0.35), 105 (2.14). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.87 (3H, *s*, OCH_3); δ 8.35 (1H, *s*, H-2); δ 6.63 (1H, *s*, H-8) and δ 6.40 (1H, *s*, H-6); δ 7.01 (1H, *s*, H-2`), δ 6.79 (1H, *d*, J = 8.1 Hz, H-5`), and δ 6.81 (1H, *d*, J = 8.1, H-6`); δ 12.97 (1H, *br s*, OH-5). ^{13}C-NMR: Table 1.

Compound 4 (4-hydroxy phenyl acetic acid): colorless crystals, mp 148-150°C (benzene-acetone); IR (KBr) cm^{-1}: 2300-3500 (OH), 3056 (C-H aromatic), 2800-2950 (C-H aliphatic), 1707 (C=O, acidic), 1607, 1517 (C=C aromatic), 1447 (C-H bending of CH_2), 1214 (C-O of phenol), 824 (C-H bending representing the *p*-substitution). **EI-MS**, m/z (rel. int.%): 152$[M]^+$ (23.1), 108 (7.6), 107 (100), 78 (6.3), 77 (25.2). ^{1}HNMR (300 MHz, DMSO-d_6): δ 3.47 (2H, *s*, CH_2); δ 7.09 (2H, *d*, J=8.4, H-2,6) and δ 6.73 (2H, *d*, J=8.4, H-3,5). ^{13}C-NMR: Table 1.

Compound 5 (genisctin): colorless needles, mp 297°C ($CHCl_3$/MeOH). **UV** λ_{max}^{MeOH} nm: 261, 290 (sh), 335 (sh); $\lambda_{max}^{MeOH/NaOMe}$ nm: 275, 325 (sh), 370 (sh); $\lambda_{max}^{MeOH/AlCl_3}$ nm: 271, 325 (sh), 370 (sh); $\lambda_{max}^{MeOH/NaOAc}$ nm: 263, 335 (sh). EI-MS, m/z (rel. int.%): 270 $[M]^+$ (100), 269 (19.6), 242 (1.4), 241 (2.9), 154 (1.2), 153 (16.4), 152 (9.0), 124 (5.7), 123 (4.4), 119 (1.9), 118 (7.1), 96 (1.7), 89 (2.5). ^{1}H- NMR (300 MHz, DMSO-d_6): δ 6.23 (1H, *d*, J = 1.8 Hz, H-6) and δ 6.39 (1H, *d*, J = 1.8 Hz, H-8); δ 6.81 (2H, *d*, J=8.7 Hz, H-3`,5`), and δ 7.38 (2H, *d*, J =8.7 Hz, H-2`, 6`); δ 8.29 (1H, *s*, H-2); δ 9.52 (1H, *br s*, OH-4`); δ12.91 (1H, *s*, OH-5).

Compound 6 (kaempferol): bright yellow needles, mp 280°C ($CHCl_3$/MeOH). **UV** λ_{max}^{MeOH} nm: 270, 307 (sh), 371; $\lambda_{max}^{MeOH/NaOMe}$ nm: 278, 316 (sh), 417 (sh); $\lambda_{max}^{MeOH/AlCl_3}$ nm: 268, 304 (sh), 349 (sh), 422. EI-MS, m/z (rel. int.%): 286 $[M]^+$(100), 285 (39.2), 258 (14.2), 257 (12.1), 229 (13.6), 213 (7.4), 153 (5.4), 137 (3.2), 136 (6.3), 121 (18.3), 118 (0.9). ^{1}H-NMR (300 MHz, CD_3OD): δ 6.16 (1H, *d*, J=2 Hz, H-6) and δ 6.37 (1H, *d*, J=2 Hz, H-8); δ 6.89 (2H, *dd*, J=6.9, 2 Hz, H-3`,5`) and δ 8.07 (2 H, *dd*, J=6.9, 2 Hz, H-2`,6`). ^{13}C-NMR: Table 1.

Compound 7 (quercetin) dark yellow fine granules, mp 314 °C ($CHCl_3$/MeOH). **UV** λ_{max}^{MeOH} nm: 256, 270 (sh), 299 (sh), 375; $\lambda_{max}^{MeOH/NaOMe}$ nm: 245( ), 292 (sh), 329 (sh), 414; $\lambda_{max}^{MeOH/AlCl_3}$ nm: 271, 300 (sh), 327 (sh), 360 (sh), 443; $\lambda_{max}^{MeOH/AlCl_3/HCl}$ nm: 265, 300 (sh), 361(sh), 428; $\lambda_{max}^{MeOH/NaOAc}$ nm: 268, 382 (sh), 426; $\lambda_{max}^{MeOH/NaOAc}$ nm: 262, 392. EI-MS, m/z (rel. int. %): 302 $[M]^+$ (100), 301 (30.1), 286 (13.2), 285 (9.2), 274 (11.2), 273 (12.5), 257 (4.3), 153 (12.5), 137 (10.0), 134 (6.05), 124(4.1), 121 (6.2), 118 (6.0), 109 (7.1), 108 (7.0). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 6.18 (1H, *s*, H-6) and δ 6.38 (1H, *s*, H-8); δ 6.89 (1H, *d*, J = 8.4 Hz, H-5`), δ 7.61 (1H, *d*, J = 8.4 Hz, H-6`), δ 7.73 (1H, *s*, H-2`).

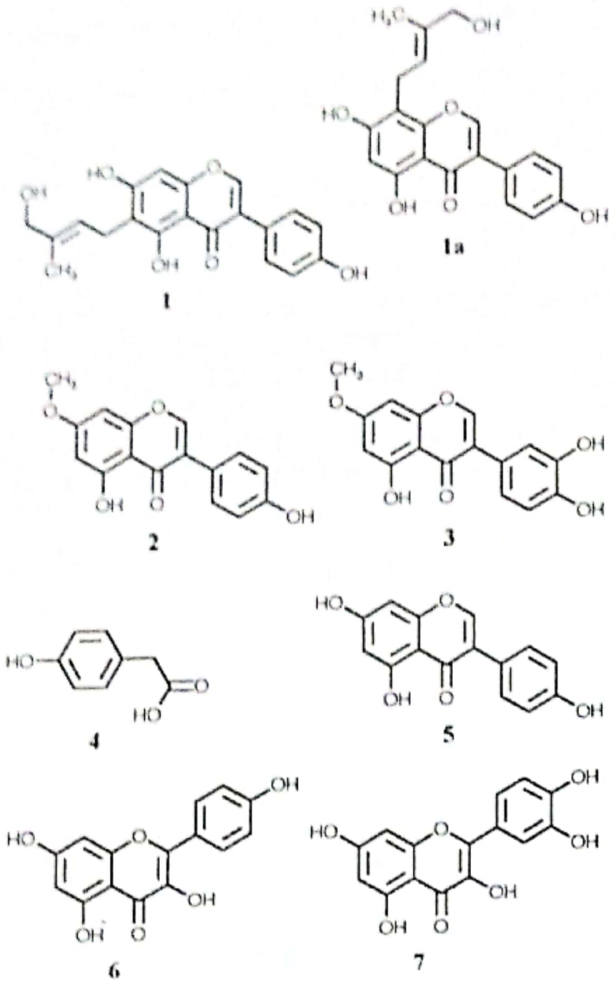
***β*-sitosterol** and ***β*-sitosterol-O-glucoside** were identified through comparison with authentic samples (co-TLC, mmp, IR and MS) as well as hydrolysis of the *β*-sitosterol-O-glucoside and preparation of the acetyl derivative of *β*-sitosterol.

Preliminary Pharmacological Testing

Materials- Adult male albino rats weighing 150-200 g were used in this study. Rats were obtained from the animal house of the National Research Center, Dokky, Cairo. Animals were kept for one week in the animal house of the Faculty of Pharmacy, Zagazig University under twelve hours day and night cycle, for accommodation. They were left free on excess food and water *ad libitium*.

Preparation of the extracts

The powdered aerial parts (50 g) of *G. glabra* were extracted with ethanol 95% at room temperature to give the total alcoholic extract. A part of the later was fractionated to light petroleum, chloroform and ethyl acetate extracts.



Anti-inflammatory activity of the different extracts

Seven groups of the albino rats (each of 6 rats, n=6) were used. The first group served as a control, and was given gum acacia mucilage (10%), the second group was given hydrocortisone (solu-cortif®-UPJON), in a dose of 200 mg/kg. The third and fourth groups received the total alcoholic extract in doses of 200 and 400 mg/kg, respectively. The remaining groups were given the light petroleum extract, chloroform extract, and ethyl acetate extract, respectively, each in a dose of 400 mg/kg. Each extract was suspended in gum acacia mucilage (10%), and was injected intraperitoneally to the rats.

Rats of all groups received the given injections 1.5 hours before the induction of inflammation. Inflammation was induced in the right hind paw of the rats, by injection of 0.05 ml of 1% carragenin solution, dissolved in normal saline, into the sub-planter region of the hind paw[24]. The size of the hind paw was measured before, and after 0.5, 1.5, 2, 3, 6, and 8 hours of the injection of carragenin. The control group was injected with normal saline, instead of carragenin. The size of thickness of the induced hind paw edema was measured for each rat and the mean of the induced thickness was calculated together with the standard error of the mean. The total area under the curve (AUC), representing the thickness of the edema and the time in hours (%.hr), were calculated by the trapezoidal method.

Hepato-protective activity

Thirty six rats were divided into six groups (n=6): the control, cirrhotic control, silymarin (200 mg/kg), total extract (400 mg/kg), chloroform extract (400 mg/kg), and ethyl acetate extract (400 mg/kg) groups. Drugs and extracts were suspended in 10% gum acacia mucilage and were injected IP daily for 7 days. The control and cirrhotic control received gum acacia 10% only. In the eighth day animals were made cirrhotic by IP injection of CCl_4 in a dose of 35ul/100g rat (diluted with liquid paraffin 0.3 ml/100g rat).[25] Blood samples were collected after 22 hours of the carbon tetrachloride injection; the serum was used to determine liver enzymes (ALT, AST, and alkaline phosphates), total cholesterol, and liver proteins (albumin, globulin, and albumin-globulin ratio).

RESULTS AND DISCUSSION

The ethanolic extract of the aerial parts of *Glycyrrhiza glabra* L. was subjected to various fractionation procedures, followed by classical chromatographic isolation techniques as described in the experimental section. This afforded the isolation of *β*-sitosterol, *β*-sitosterol-O-glucoside and compounds **1-7**.

Compound **1** showed UV absorptions (342 and 266 nm) and a proton singlet at δ 8.35 (H-2) indicating an isoflavone[26,27]. The MS exhibited a molecular peak at m/z 354 fitting nicely with the molecular formula $C_{20}H_{18}O_6$, beside fragments at m/z 323, 270 and 283 typical for a 3-hydroxymethyl-2-butenyl moiety at ring A[27,28]. Mass fragments at m/z 153, and 123, indicated two OH groups at ring A, and that at m/z 118, indicated one OH group at ring B[27]. The ^{1}H-NMR spectrum showed a A_2B_2 system (δ 7.46 *d*, J = 8.7 Hz ,H-2`, 6` and δ 6.90 *d*, J = 8.7 Hz, H-3`, 5`) characteristic for a substitution at 4`, beside signals assignable to 3-hydroxymethyl-2-butenyl moiety at ring A (δ 1.78 (3H, *s*, CH_3-12), δ 3.39 (2H, *d*, J = 7.2 Hz, H-9), δ 5.50 (1H, *t*, J = 7.2 Hz, H-10), δ 3.84 (2H, *d*, J = 5.5, H-13) and δ 4.63 (1H, *t*, J = 5.5, OH-13)). These results beside the obtained ^{13}C-NMR data (Table 1) indicated that compound **1** is glabrisoflavone or its isomer ganconin-C **1a**. Comparing the chemical shift of the free proton at ring A (δ 6.53, H-8) with those reported for **1a** (δ 6.37)[28], beside comparison of ^{13}C-NMR with those of **1a**[28,29] indicated that compound **1** is glabrisoflavone[30]. Confirmation was achieved through comparing the obtained data with those reported for glabrisoflavone previously isolated from the aerial parts of *G. glabra* grown in Uzbekistan[30].

The UV absorption (260 and 330 nm) and the presence of one proton singlet at δ 8.46 (H-2) in the ^{1}H-NMR spectrum of compound **2** indicated an isoflavone structure[26,27]. The MS showed a molecular ion peak $[M]^+$ at 284, corresponding to the molecular formula $C_{16}H_{12}O_5$. The significant MS fragments at m/z 118 indicated a 4`-monohydroxylation at ring B. The ^{1}H-NMR spectrum exhibited a typical AX system (a pair of doublets at δ 6.48, J=2.1 Hz, and δ 6.73, J=2.1 Hz) assignable to H-6 and H-8, respectively; a typical A_2B_2 system (a pair of doublets, at δ 7.48,

J=8.4 Hz, and at δ 6.91, J=8.4 Hz), attributed to H-2`,6`, and H-3`,5`, respectively; a singlet at δ 3.95 was assigned for the OCH_3 group, and two singlets at δ 13.00 and δ 9.59 attributed to 5-OH and 4`-OH, respectively. The $^{13}$C-NMR indicated the presence of 16 carbon atoms, which were assigned by the aid of APT and 2D experiments as shown in Table 1. The above mentioned data and comparison with reported data confirmed that **2** is prunetin (5, 4`-dihydroxy-7-methoxy isoflavone), which was previously isolated from *G. glabra* root[31] and aerial parts[19,32].

Compound **3** displayed UV absorptions at 262 and 335 nm beside a proton singlet at δ 8.35 (H-2) confirming an isoflavonoid nature[26,27]. The UV spectra indicated a free OH at C-5 (band I bathochromic shift of 40 nm with $AlCl_3$) and *O*-coupled OH groups in ring B (band I hypsochromic shift of 5nm, induced by addition of HCl to the $AlCl_3$ solution)[26,27]. The MS spectrum showed a molecular ion at m/z 300 coinciding with the molecular formula $C_{16}H_{12}O_6$ and significant fragments at m/z 167 (placing on OH and one OCH_3 in ring A) and at m/z 134 and 118 (indicating two OH groups in ring B)[27]. The ^{1}H-NMR spectrum showed a typical AX system (two singlets at δ 6.63 and δ 6.40 ;H-8 and H-6), a typical ABX system (δ 7.01, *s*, H-2`; δ 6.79, *d*, J = 8.1 Hz; H-5`, and δ 6.81, *d*, J = 8.1; H-6`) confirmed that the two OH groups found on ring B were located at C-3`, and C-4`. The presence of a three proton singlet at δ 3.87 confirmed the presence of a OCH_3 group. The ^{13}C-NMR data showed signals of 16 carbons, which are assigned on basis of APT and 2D experiments as shown in Table 1 and ^{13}C-NMR confirmed the presence of one methoxy group (δ 55.99)[29]. These data suggested that compound **3** is 5, 3`,4`-trihydroxy-7-methoxy isoflavone (7-methyl orobol or santal). Comparing the obtained data with those reported for santal[33] and similar compounds[26,29] confirmed structure **3**. This is the first time to isolate such a compound from genus *Glycyrrhiza* but it was isolated before from *Pterocarpus santalinus*[33].

Compound **4** produced a violet color when treated with $FeCl_3$ indicating a phenolic structure. Its acidic nature was deduced as it gives effervescence with sodium carbonate and by the IR absorption at 1708 cm^{-1} (C=O, acidic)[34]. The MS showed a molecular peak at m/z 152 coinciding with the molecular formula $C_8H_8O_3$, and a characteristic fragment at m/z 107 $[M-COOH]^+$. The ^{1}H-NMR showed a typical A_2B_2 (δ 7.09, *d*, J=8.4, H-2,6 and δ 6.73, *d*, J=8.4, H-3,5), and a two protons singlet at δ 3.47 assignable to the benzylic CH_2 group[34]. The ^{13}C-NMR showed 6 signals (Table 1) representing 8 carbons, one of them is a carbonyl carbon (δ 176.16), and another one of a benzylic CH_2 (δ 41.09)[34]. Comparing the data obtained for compound **4** with those reported for 4-hydroxy phenyl acetic acid, previously isolated from *Althaea officinalis*[35] confirmed its structure. This is the first report for the isolation of this acid form the genus *Glycyrrhiza*, and may be considered as a chemotaxonomic marker for the studied species.

Compound **5** was proved to be an isoflavone by its UV absorptions at 261, 290(sh) and 335 nm and the presence of a singlet at δ 8.29 (H-2) in the ^{1}H-NMR spectrum[26,27]. The MS indicated the presence of two OH groups at ring A (m/z at 153, and 124), and one OH group at ring B (m/z at 118)[27]. The ^{1}H-NMR exhibited the presence of the 10 protons assigned as presented in the experimental section. Comparing the obtained data with those reported for genistein[27] confirmed its structure as 5,7,4`-trihydroxy isoflavone previously isolated from *G. glabra* root and aerial parts[14,18,19].

Compounds **6** and **7** were identified as kaempferol and quercetin by comparing their obtained data (UV, MS and ^{1}H-NMR, as detailed in the Experimental section) with those reported for kaempferol[26,27,36] and quercetin[26,27,36], respectively. Confirmation was achieved by comparison with authentic samples of kampferol and quercetin, respectively. Kaempferol and quercetin were reported previously in *G. uralensis*[37,38].

β-Sitosterol and β-sitosterol-O-glucoside were identified as mentioned in the experimental section. Although, β-Sitosterol was reported in *Glycyrrhiza* species[39] before, this is the first report for β-sitosterol-O-glucoside in this genus.

Results of the anti-inflammatory testing of the total extract (Table 2, and Figures 1 and 3) exhibited a dose-dependant reduction in the size of the rat hind paw edema. It produced anti-inflammatory effect in a dose of 400 mg/kg, which was greater than that produced by hydrocortisone after 1.5, and 2 hours of carragenin administration. These results (Fig. 3) showed that the total extract has an anti-inflammatory activity which was rapid in onset and shorter in duration of action when compared to hydrocortisone.

Results obtained by the different fractions of the total extract (Table 3 and Figures 2, 4 and 5) showed that only hydrocortisone, chloroform extract, and ethyl acetate extract induced significant reduction of the hind paw edema of rats. The effect of chloroform extract and the hydrocortisone were nearly similar in both the onset and the duration of action (Figure 5). However, the effect of ethyl acetate extract was less than that of chloroform and hydrocortisone, but this effect was significant compared to the control values (Figure 2). The actual significant effect of chloroform and ethyl acetate extracts started after 2 hours of the administration of carragenin and lasted for more than 6 hours.

The anti-inflammatory effect of light petroleum extract was very low compared to that of hydrocortisone. From these findings, the tested fractions can be arranged according to their relative anti-inflammatory potencies to hydrocortisone, as follows: hydrocortisone > chloroform > ethyl acetate > light petroleum.

The effect of the total extract in a dose of 400 mg/kg was greater in onset than that showed by the smaller dose 200 mg/kg, and hydrocortisone. This effect may be due to some sort of synergistic interaction of some compounds (β-sitosterol, stigmasterol, other sterols and triterpens and some flavonoids) that exerted in combination together in the

total extract. This effect actually decreased after their separation into different fractions.

Results of the hepato-protective activity (Table 4A,B) showed that the total extract of the aerial parts of *G. glabra* significantly reduced the carbon tetrachloride-induced cirrhosis, thus recording 29.59%, 38.31%, 74.89%, and 49.09%, reduction in blood levels of ALT, AST, alkaline phosphatase, and the total cholesterol, respectively. The chloroform and ethyl acetate fractions of the aerial parts of *G. glabra* significantly inhibited the capability of carbon tetrachloride to induce acute cirrhosis. Both decreased the effect of the carbon tetrachloride on ALT, AST, alkaline phosphatase, and total cholesterol by 29.99%, 44.91%, 74.94%, and 70.13 in case of the chloroform fraction, and 63.92%, 60.97%, 84.45%, and 70.75% in case of the ethyl acetate fraction, respectively (Table 4). The hepato-protective effects of the total extract, chloroform extract, and ethyl acetate extract fractions, expressed as their ability to inhibit the carbon tetrachloride-induced elevation in liver enzyme levels, may be arranged as follow; silymarin >total extract >chloroform extract > ethyl acetate extract.

The hepato-protective activity of the total extract, chloroform extract, and ethyl acetate extract can be explained on the basis of the presence of flavonoids, which were reported to have anti-oxidant and scavenging activities[40]. The levels of the liver proteins (albumin, globulin, and albumin-globulin ratio) were not affected.

Results of the pharmacological experiments recommend further comprehensive pharmacological studies to determine the mechanism of actions and the safety of the studied extracts. This would lead to the production of economic pharmaceutical preparations with potent anti-inflammatory and hepato-protective effects from the aerial parts of licorice produced as waste products during the preparation of licorice roots and stolons.

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Table 1: ^{13}C NMR data of compounds **1-4** and 6

Position	(1)	(2)	(3)	(3a)*	(4)	(6)
1	--	--	--	--	126.79 (s)	--
2	154.26 (d)	154.28 (d)	153.67 (d)	153.77	131.31 (d)	162.48 (s)
3	122.55 (s)	122.40 (s)	121.26 (s)	122.10	157.39 (d)	137.10 (s)
4	180.34 (s)	180.32 (s)	180.13 (s)	180.55	116.22 (s)	177.35 (s)
4a	105.32 (s)	105.31 (s)	104.14 (s)	104.58	--	104.54 (s)
5	161.67 (s)	161.65 (s)	158.75 (s)	159.66	157.39 (d)	160.52 (s)
6	97.91 (d)	97.93 (d)	110.72 (s)	98.58	131.31 (d)	99.48 (d)
7	157.36 (s)	165.11 (s)	161.89 (s)	161.69	--	165.55 (s)
8	92.28 (d)	92.29 (d)	92.77 (d)	105.79	--	94.27 (d)
8a	165.09 (s)	157.39 (s)	155.26 (s)	154.93	--	158.20 (s)
1'	121.36 (s)	120.95 (s)	122.03 (s)	121.39	--	123.73 (s)
2'	116.42 (d)	130.07 (d)	130.06 (d)	130.06	--	130.67 (d)
3'	144.82 (s)	157.39 (d)	114.94 (d)	115.10	--	116.30 (d)
4'	145.50 (s)	114.98 (s)	157.26 (s)	157.39	--	148.05 (s)
5'	115.29 (d)	157.39 (d)	114.94 (d)	115.10	--	116.30 (d)
6'	119.86 (d)	130.07 (d)	130.06 (d)	130.06	--	130.67 (d)
9	--	--	20.39 (t)	20.51	--	--
10	--	--	120.80 (d)	121.16	--	--
11	--	--	135.16 (s)	135.53	--	--
12	--	--	13.51 (q)	13.51	--	--
13	--	--	66.16 (t)	66.34	--	--
OCH_3	55.99 (q)	55.99 (q)	--	--	--	--
CH_2	--	--	--	--	41.09 (t)	--
COOH	--	--	--	--	176.16 (s)	--

Assignment and multiplicities were determined by the aid of chemical shifts, and 2 D NMR experiments (APT, COSY, and HETCOR) * from reference 32

Table 2: The anti-inflammatory activity of hydrocortisone (200mg/kg), and the total extract of *G. glabra* (200, & 400 mg/kg)

Treatment	AUC (%.hr)			
	Mean ±S.E.	% Effect	% Change	Relative Potency (RP)
Solvent (control)	285.800 ±41.82	100.000	0.00	--
Hydrocortisone	222.540 ±26.03	77.866	-22.13	1.000
Total Extract (200mg/kg)	281.530 ±30.57	98.506	-1.49	0.790
Total Extract (400mg/kg)	201.260 * ±22.65	70.420	-29.58	1.106

RP: Relative potency to hydrocortisone.
* Significantly different from control group at P<0.05

Table 3: The anti-inflammatory activity of hydrocortisone (200 mg/kg), and the fractions of the total extract of *G. glabra* (400 mg/kg, each)

Treatment	AUC (%.hr)			
	Mean ±S.E.	% Effect	% Change	Relative Potency (RP)
Solvent (control)	176.780 ±32.73	100.000	0.00	--
Hydrocortisone	54.360 * ±11.38	30.750	-69.25	1.000
Light Petroleum Extract	136.570 ±14.05	77.254	-22.75	0.398
Chloroform Extract	57.960 * ±9.41	32.787	-67.21	0.938
Ethyl acetate Extract	105.960 * ±16.71	59.939	-40.06	0.513

RP: Relative potency to hydrocortisone.
* Significantly different from control group at P<0.05

Table 4: Effect of silymarin, total extract, chloroform extract, and ethyl acetate extract of *G. glabra L.* on the liver enzymes (ALT, AST, alkaline phosphatase and total cholesterol) of CCl_4 induced acute cirrhosis in rats.

A:

Group	ALT				AST			
	Mean ±S.E.	% Effect	% Change	Relative Potency (RP)	Mean ±S.E.	% Effect	% Change	Relative Potency (RP)
Normal Control	59.286 ±4.341	-	-	-	83.429 ±11.592	-	-	-
CCl_4 Control	126.714 ±10.058	100.000	0.00	-	209.714 ±14.025	100.000	0.00	-
Silymarin	31.000* ±6.638	24.464	-75.54	1.000	53.833* ±7.391	25.670	-74.33	1.000
Total Extract	37.500* ±7.584	29.594	-70.41	0.827	80.333* ±21.557	38.306	-61.69	0.670
Chloroform Extract	38.000* ±10.507	29.989	-70.01	0.816	94.200* ±12.261	44.918	-55.08	0.571
Ethyl Acetate Extract	81.000* ±17.776	63.923	-36.08	0.383	127.857* ±28.697	60.967	-39.03	0.421

Table 4: B

Group	Alkaline Phosphatase				Total Cholesterol			
	Mean ±S.E.	% Effect	% Change	Relative Potency (RP)	Mean ±S.E.	% Effect	% Change	Relative Potency (RP)
Normal Control	32.357 ±1.515	-	-	-	82.571 ±4.995	-	-	-
CCl_4 Control	39.500 ±0.4082	100.000	0.00	-	151.429 ±11.004	100.000	0.00	-
Silymarin	28.417* ±0.455	71.941	-28.06	1.000	81.000* ±5.391	53.491	-46.51	1.000
Total Extract	29.583* ±0.952	74.895	-25.11	0.961	74.333* ±7.0459	49.088	-50.91	1.090
Chloroform Extract	29.600* ±0.748	74.937	-25.06	0.960	106.200* ±12.540	70.132	-29.87	0.763
Ethyl Acetate Extract	33.357* ±1.8475	84.448	-15.55	0.852	107.143* ±9.7576	70.755	-29.25	0.756

RP: relative potency to silymarin.
* Significantly different from control cirrhotic rats at P<0.05

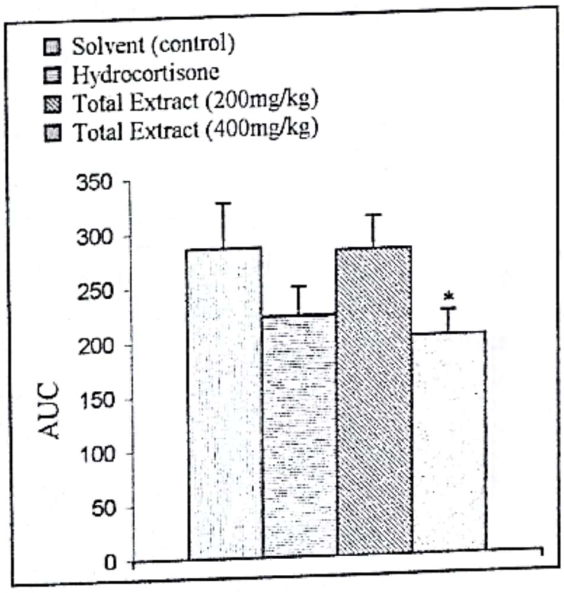

Figure 1: The anti-inflammatory activity of hydrocortisone (200mg/kg), the total extract of *G. glabra* (200, & 400 mg/kg)
* Significantly different from control group at P<0.05

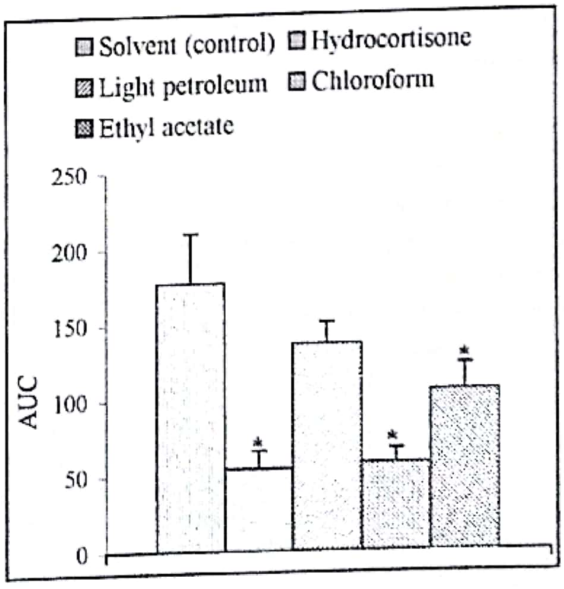

Figure 2: The anti-inflammatory activity of hydrocortisone (200 mg/kg), light petroleum, chloroform, and ethyl acetate fractions of the total extract of *G. glabra* aerial parts (400 mg/kg, each)
• Significantly different from control group at P<0.05

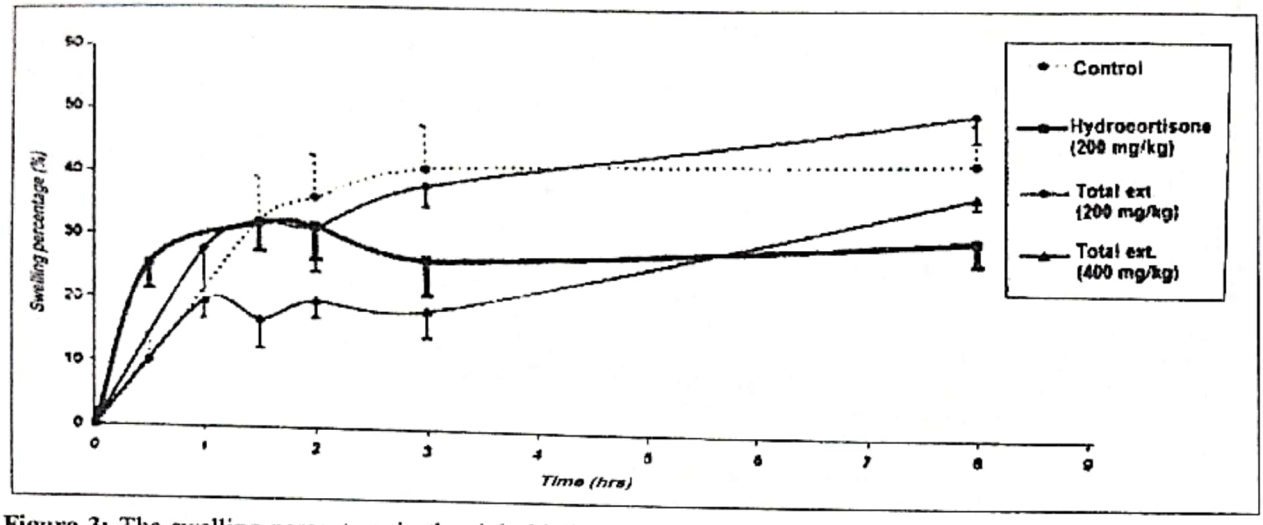

Figure 3: The swelling percentage in the right hind paw of rats, after the injection of carrageenin solution to rates treated with hydrocortisone (200 mg/kg) and the total extract of *G. glabra* in doses of 200 and 400 mg/kg.

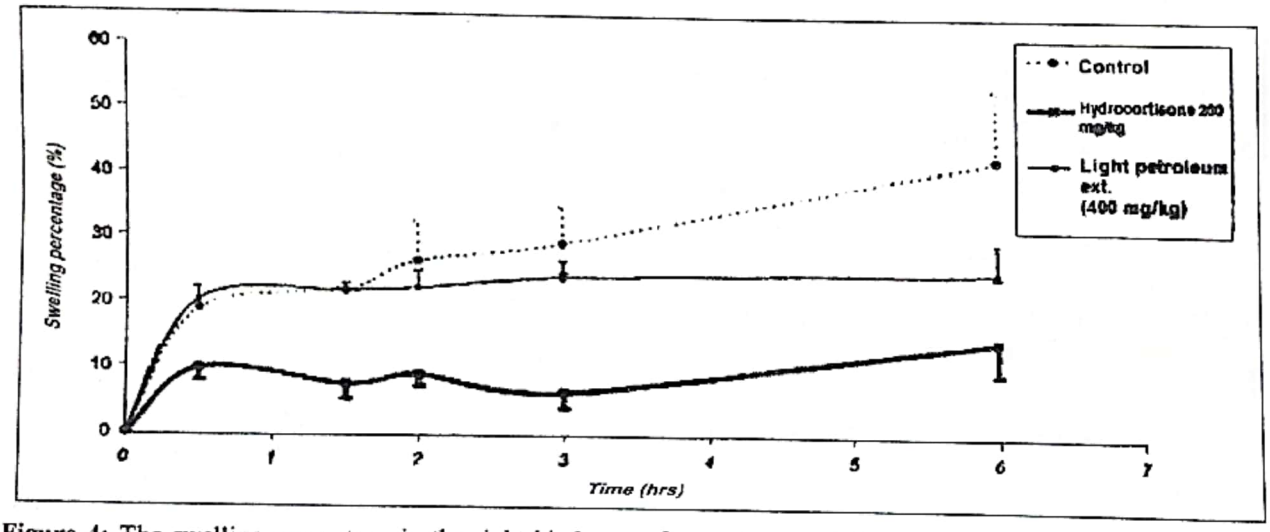

Figure 4: The swelling percentage in the right hind paw of rats, after the injection of carrageenin solution to rates treated with hydrocortisone (200 mg/kg) and the light petroleum fraction (400 mg/kg)

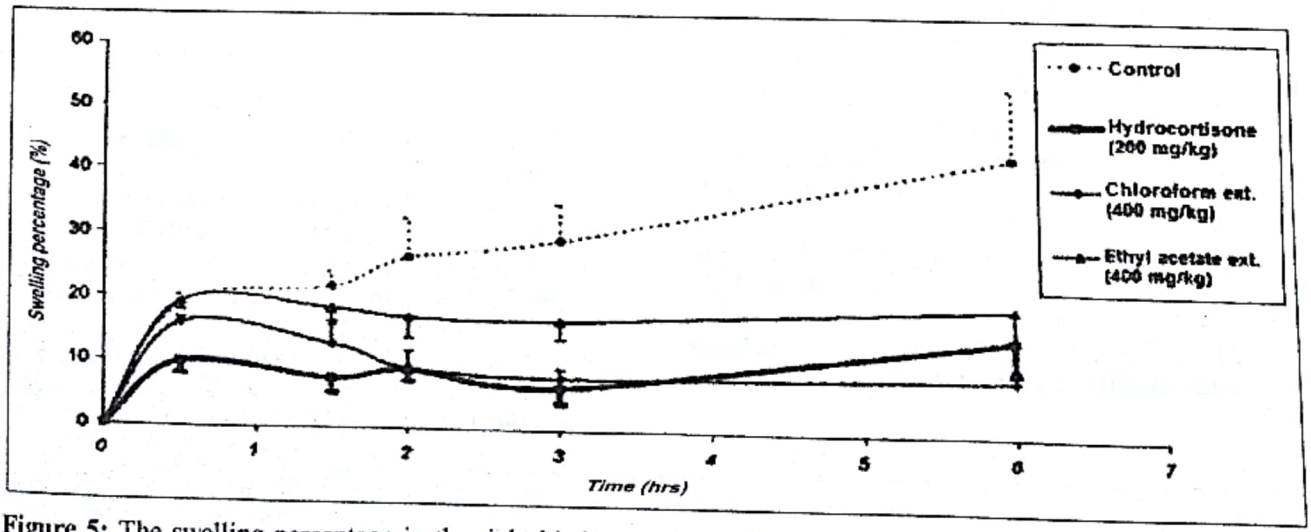

Figure 5: The swelling percentage in the right hind paw of rats, after the injection of carrageenin solution to rates treated with hydrocortisone (200 mg/kg), chloroform, and the ethyl acetate (400 mg/kg.)

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مركبات فينولية ودراسة لبعض التأثيرات الحيوية للأجزاء الخضرية لنبات عرق السوس (جليسيرهيزا جلابرا) المصري

ماهر الدمياطي – إحسان عبد العزيز – عزه الشافعي – ماجد السيد

قسم العقاقير – كلية الصيدلة – جامعة الزقازيق – الزقازيق – مصر

فـــي هذا البحث تم فصل أربعة مركبات أيزوفلافونية (سنتال، برونيتين، جلابرأيزوفلافون، جينيسيتين) و مركبان من الفلافونـــولات (كامفـــيرول، كويريســـتين) و مركـــب فينولـــي (٤–هيدروكسي فينيل حمض الخليك) و ذلك بالإضافة إلي بيتاسيتوستيرول و بيتاسيتوستيرول جلوكوزيد من الأجزاء الخضرية لنبات عرق السوس (جليسيرهيزا جلابرا) المنزرع في مصـــر. هذا وقد تم فصل المركبات سنتال و٤–هيدروكسي فينيل حمض الخليك و بيتاسيتوستيرول جلوكوزيد للمرة الأولى مـــن جـــنس جليسيرهيزا وتم التعرف علي هذه المركبات عن طريق دراسة خواصها الفيزيائية والكيميائية والطيفية (طيف الأشعة تحت الحمراء، طيف الأشعة فوق البنفسجية، طيف الكتلة، و الطنين النووي المغناطيسي للهيدروجين يد ^{1} والكربون ك^{13} ذات البعد الواحد، و ذات البعدين).

ومن الناحية الفارماكولوجية تم إختبار المستخلص الكحولي للجزء الخضري لهذا النبات و كذا بعض مستخلصاته الأخري لمعرفة أثره المضاد للإلتهابات و كذلك أثره الواقي من الإلتهابات الكبدية وذلك بالمقارنة مع مادتي الهيدروكورتيزون و السليمارين على التوالي، و قد ثبت أثر واضح لتلك المستخلصات علي هذه الإلتهابات.