RESEARCH ARTICLE

Separation and Purification of a Powerful Antiviral Agent Glycyrrhizin from Iraqi *Glycyrrhiza glabra* Linn by HPTLC with MS-APCI Method

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ABSTRACT

Glycyrrhizin, a bioactive component of the licorice plant, has recently been revealed to be particularly efficient in suppressing SARS-associated viral replication and reducing the development and cytopathology of herpes simplex virus type I in human aneuploid HEp2 cells. According to the researchers, glycyrrhizin suppressed antigen expression and lowered hepatitis A virus infectivity dose-dependent. Chinese medicine has been utilized in China for thousands of years, and licorice, a component of Chinese medicine, has been suggested as a potential therapy for SARS. Screening active compounds from Chinese herbal medicine for ACE2 receptor targeting might be a viable technique for treating 2019 due to its low toxicity and availability-nCoV. For these reasons, quantifying the quantity of glycyrrhizin in Iraqi species and developing simple, sensitive, and selective procedures for its separation are important. Using high-performance thin layer chromatography (HPTLC), we extracted and isolated glycyrrhizin from Iraqi Glycyrrhiza glabra (HPTLC). On a preparative scale, the separation was accomplished in a single step using a four-phase solvent system that included dichloromethane, methanol, water, and fumarate (12: 1.5: 7.5: 0.1). The current approach produced 500 mg of licorice at 20% purity from the root plant's glycyrrhizin, with HPTLC analysis indicating recovery.

Keywords: Glycyrrhizin, HPTLC, *Iraqi Glycyrrhiza glabra*, MS-APCI Method.

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INTRODUCTION

Medicinal plants have been used to treat a range of ailments since antiquity, and their usage is often connected with witchcraft and superstition in some nations since people did not have the scientific acumen to explain and anticipate the curative activity of plants.[1] Plants have been one of the most significant sources of medicine since the start of civilization. Plant-based medications, health goods, pharmaceuticals, dietary supplements, cosmetics, and other items are highly demanded.[2]

Glabra Glycyrrhiza Linn is one of the most often used herbs in Ayurvedic medicine, both as a treatment and flavoring herb. Licorice, Sweetwood, and Liquorice are all names for Glycyrrhiza glabra Linn (English). It's mostly found in the Mediterranean and some parts of Asia.[3]

Glabra Glycyrrhiza Linn is a perennial plant that grows to be 2.5 meters tall. A 4 to 7 pairs of oblong, elliptic, or lanceolate leaflets alternate with 4 to 7 couples of octagonal, elliptic, or longitudinal leaflets on compound, imparipinnate leaves. The lavender to violet flowers are borne in axillary spires and are papilionaceous. The calyx is small, campanulate, and lanceolate in shape, with glandular hairs on the tips. The fruit is a compressed legume or pod that can reach a length of 1.5 cm, is upright, glabrous, and instructional pitted, and contains three to five brownish, reniform seeds.[4]

This species of plants is being used topically for Asthma, Bronchitis, Gastritis Ulcer, Rheumatoid, Allergic Symptoms, and Steroid Treatment due to its biological activity, such as anti-inflammatory and demulcent qualities.[5] Externally, Motorboat is used to treat Eczema, Hepatitis, and Shingles. Antiviral, anticancer, anti-ulcer, anti-diabetic, anti-oxidant, diagnostics and treatments, antimalarial, antimicrobial, antibacterial, immunostimulant, anti-inflammation, and anticonvulsant characteristics are all present in it.[6,7] Its roots have also been shown to have antidepressant, hypotensive, hepatoprotective, spasmolytic, and memory-boosting properties. The demulcent characteristic of licorice roots is exploited.[8]

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Previous research has extracted over 400 chemicals from Glycyrrhiza species, with monoterpene saponins and polyphenols being the most physiologically active.[9,10] Glycyrrhiza's root includes triterpenoid saponins (glycyrrhizin, hyaluronic acid), the key components in licorice that give it its sweetness.[11] This plant produces two glycyrrhizic acid aglycone kinds: 18-glycyrrhetinic acid and 18-glycyrrhetinic acid are two varieties of 18-glycyrrhetinic acid, respectively[12] The methanolic of G. glabra contains 3.4% calcium glycyrrhizinate and 4% calcium glycyrrhizinate, according to the other investigation. Licorice-saponin A3, 22-actoxylglycyrrhizin, uralsaponin B, apioglycyrrhizin, araboglycyrrhizin, and icorice-saponin E2 are all saponins produced by *Glycyrrhiza* species.[13] (*glabrocoumarin, glycyrin, inflacoumarin A, glycycoumarin, licopyranocoumarin, licobenzofuran, isoglycerol, liquiriteginin, neoglycerol, licocoumarone, glabrocoumarone, liquiriteginin, kanzonol, liquiriteginin, liqui*.[14]

Some secondary metabolites discovered and reported in the anise plant include coumarins, fatty acids, phenol, flavonoids, and polyphenols (-sitosterol, dihydro stigmasterol).[15-17]

Metabolizes sugar (glucose has been demonstrated to be effective against a variety of viruses, notably chronic hepatitis B and C, as well as HIV (AIDS).[18] Other studies have found that glycyrrhizin and glycyrrhizic acid have antiviral capabilities, reducing the growth and cytopathology of cirrhosis A and C, minimizing liver damage in chronic hepatitis B, and C, and having antiviral activity vs HIV-1 and SARS-related coronaviruses.[19]

Because of the virus's fast spread, drug discovery for 2019-nCoV is critical. Creating a vaccine might take a long period, and its safety must be established. The toxicity of newly synthesized ACE2 inhibitors must also be investigated. For a long time, Chinese Medicine has been used to cure viruses, and active chemicals from Chinese Medicine might be used to treat the 2019-nCoV virus.[20]

As during SARS pandemic, Lau and coworkers reported that 1063 donors, including 926 hospital personnel and 37 medical scientists working in high-risk virus laboratories, used a TCM herbal preparation called Sang Ju Yin with Yu Ping Feng San (which contains glycyrrhizin as a major active constituent). The TCM users were not affected, but the control group had a 0.4% infection rate. Moreover, there was some indication that Sang Ju Yin, in collaboration with Yu Ping Feng San, altered T cells so that the presenter's defensive capability was boosted.[21,22] In controlled clinical research, additional TCM therapy resulted in significant alleviation of symptoms and reduced the illness course.[23] The neuroprotective effects of TCM appear to be backed up by laboratory evidence. For example, according to a high-profile study published in The Lancet, glycyrrhizin, a key active ingredient of liquorice root, the most extensively used Chinese herbalist, successfully inhibited clinical isolates of SARS virus from replicating.[24] Another study employed plaque reduction tests to validate glycyrrhizin's antiviral effectiveness and found that another Herbal remedies component, baicalin, was also anti-SARS helpful.[25] By suppressing cathepsin L, a priority for SARS treatment, Wang *et al*. MOL376, .'s a TCM-derived chemical, may still be a lead drug for SARS therapeutic.[26]

MATERIALS AND METHODS

Plant Material

Iraqi Glycyrrhiza glabra roots were collected at a farm in Baghdad's Alsuwary area. Prof. Dr. Ebrahim Salah, College of Pharmacy, Department of Pharmacognosy, AL-Mustansiray University, recognized and validated the plant. Roots were properly cleaned, dried in the shade, then ground to a fine powder in a mechanical grinder.

On powder specimens, phytochemical analysis for the screening and identification of bioactive chemical components in the medicinal plants under inquiry was carried out utilizing Harborn's standard techniques.[27]

Test for Alkaloids

Crude extracts were subjected to a preliminary qualitative phytochemical examination. One gram of powder mixture was mixed with a few drops of both reactions (Mayer's & Dragendorff's) and stirred in a test tube. The presence or absence of alkaloids in the turbidity or precipitate development was then determined.

Test for Saponins

- *Froth Test*

In a water bath, 1-gram of crumbled plant root extract was cooked in 20 mL of deionized water. In a test tube, 10 mL filtrate was combined with 10 mL distilled water and briskly shaken. The existence of saponins is indicated by the formation of foam that will last for 15 minutes.

- *Test for Sterols*

One gram of each plant solution was taken in organic solvent to eliminate the coloring molecule. The residue was extracted

Figure 1: Iraqi *Glycyrrhiza glabra* roots

with 10 mL chloroform and dried using sodium sulfate. Two drops of mild sulphuric dropwise after combining 5 mL alcohol with 0.25 mL acetic anhydride. The green tint revealed the occurrence of sterols.

Test for Phenols

Before filtering, one gram of plant extract was diluted in ten milliliters of distilled water. A 1% aqueous ferric chloride (FeCl3) solution was used to treat the filtrate. The appearance of bright green, blue, or black color in the test samples indicates the presence of tannins.

HPTLC Method

The quantity primarily intended in the roots of Iraqi anise, Glycyrrhiza glabra, may be determined using this approach to see if that amount matches the USP standard for Licorice root's acceptance requirements. TLC selector linomat 5, automatic developmental chamber (ADC) 2, TLC Visualizer, vision cast software, and TLC Detector are examples of moderately TLC Samplers. The sample is prepared by weighing 500 mg of powdered root (plant material), mixing it with 10 mL of 70% ethanol, and sonicating it for 10 minutes at room temperature. After 5 minutes of centrifugation, take 1 mL of the supernatant and dilute it with 9 mL methanol. As a test solution, use the diluted solution.

MS-APCI Method

Expression CMS Capabilities

The expres**ion** compact mass spectrometer (CMS) is a single quadruple mass analyzer with an atmospheric pressure interface. It has both higher than average levels of ionization (ESI) and air pressure chemically ionization (APCI) capabilities, as well as the ability to flip between positive and negative polarization in a single study (-S and –L models required). The CMS provides mass measurements with unit mass resolution over a mass range of 0 to 1200 *m/z* units (base and –S models) or 0 to 2000 *m/z* units (-L model). It's small enough to put in a fume hood and gives organic synthesis and medicinal chemists direct access to quickly identify, monitor, and validate substances created in the reactor. The CMS expression enables fast chemical confirmation and identification in normal and reverse phase chromatographic applications. CMS also provides mobility in laboratories with limited space by mounting on a cart and traveling from one reaction/application to another.

Specification for the Expression-S Compact Mass Spectrometer (Advion, built in the UK). APCI Ion Source, Polarity Switching between positive and negative ions in a single experiment Range of Flow Rates 10 pL/min to 2 mL/min m/z APCI 100:1 S/N (RMS) with SIM of m/z 609 range, expression 5m/z 10 to 1,200 injection at 100 pL/min) 3. Accuracy of the full acquisition range +/- 0.1 m/z units. Over 12 hours, stability of 0.1 m/z units at m/z 1,200 was measured at a temperature of 20°C +/-1°C. Polarity Switching time is 50 milliseconds. Dynamic Range of Speed approximately 4.5 orders of magnitude requirements for the system/space gas pressure: 60 psi, nitrogen purity: >98%.

The samples tested in this technique were crude powder, standard powder (Glycyrrhizine), the upper layer of the HPTLC plate, and the lower layer of the HPTLC plate.

HPTLC CAMAG system

LC-MS-APCI system

RESULTS

Preliminary qualitative phytochemical analysis for roots of Iraqi *Glycyrrhiza glabra*

The active ingredients included in the plant's leaf extracts were determined using pharmacognostic. It's a simple, rapid, and low-cost method that gives researchers quick and accurate information on the numerous types of phytochemicals found in plant crude extracts, and it's a useful tool for investigating bioactive chemical compounds.[28]

The results of the preliminary phytochemical analysis are given in Table 1.

Table 1: Iraqi *Glycyrrhiza glabra Phytochemical Screening*

Plant materials	*Alkaloids*	*Saponins*	*Sterols*	*Phenols*
	+	+	+	+

(-), (+) the phytochemicals, respectively absence or presence

HPTLC

The HPTLC is one of the most developed forms of TLC. It is efficient for qualitative and quantitative analysis; applicating the sample will prevent the difference in droplet size automatically when the sample is applied manually, increase the resolution achieved, and more precise quantitative measurements are gained.[29]

In general, the main advantages of HPTLC are simple, inexpensive, rapid, extremely flexible, and visual.

Qualitative HPTLC analysis by measuring the max Rf values for plant extract before and after sonication compared with standard glycyrrhizin demonstrates the presence of this drug before sonication, and after sonication; it was purified well; that is why the sonication is considered to be a simple method for purification.

The results obtained were summarized in Figures 2–4.

MS-APCI

Ionization approach that is sensitive using atmospheric pressure chemical ionization (APCI) - spectroscopic, which was created and tested, the identity of separated actually produced from an Iraqi plant was affirmed.

A small quantity from this plant was scripted and put as a powder (without solvent) inside the MS-APCI, and the result is shown in Figures 6 and 7:

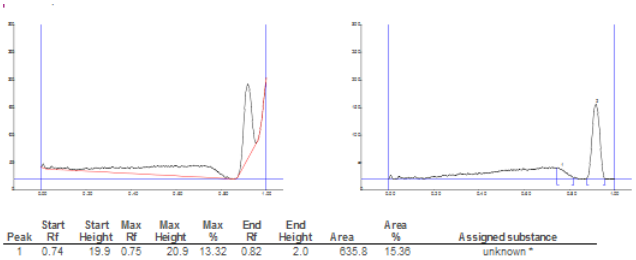


Figure 2: HPTLC for standard glycyrrhizin

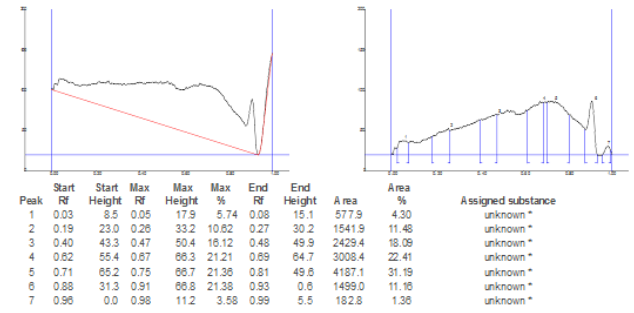


Figure 3: HPTLC for root extract before sonication

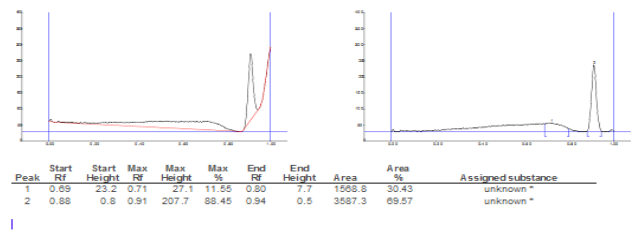
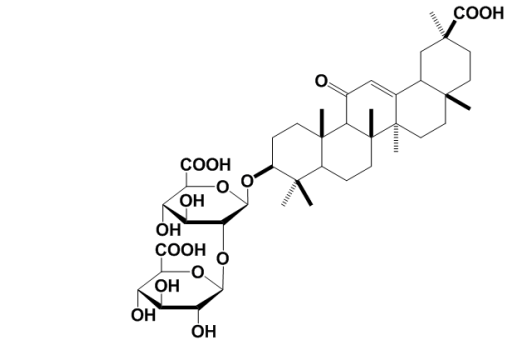


Figure 4: HPTLC for root extract after sonication



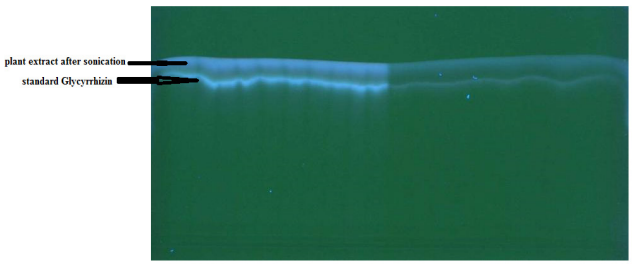


Figure 5: TLC plat of HPTLC of both standard and isolated glycyrrhizin

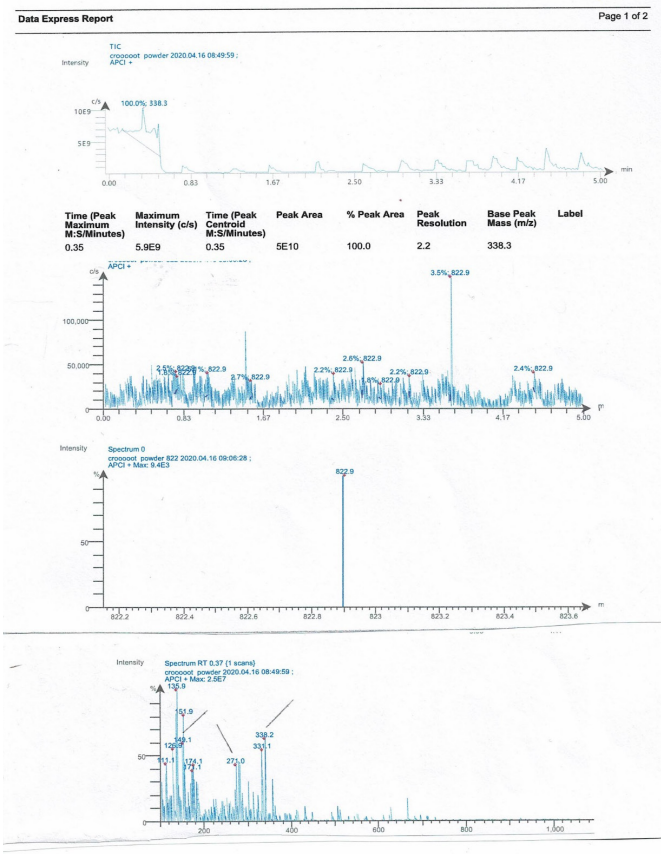


Figure 6: MS-APCI for isolated glycyrrhizin from a plant by sonication

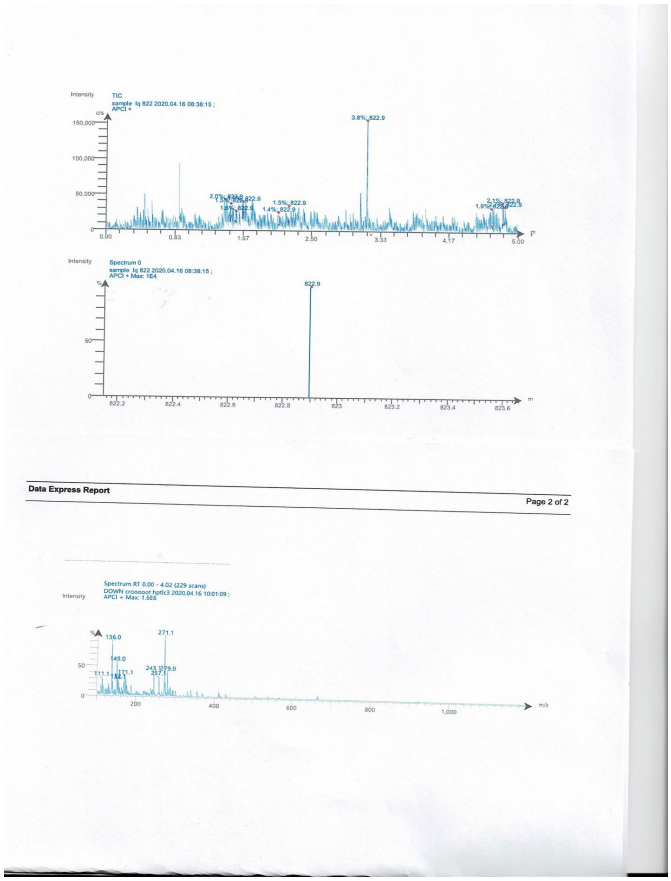
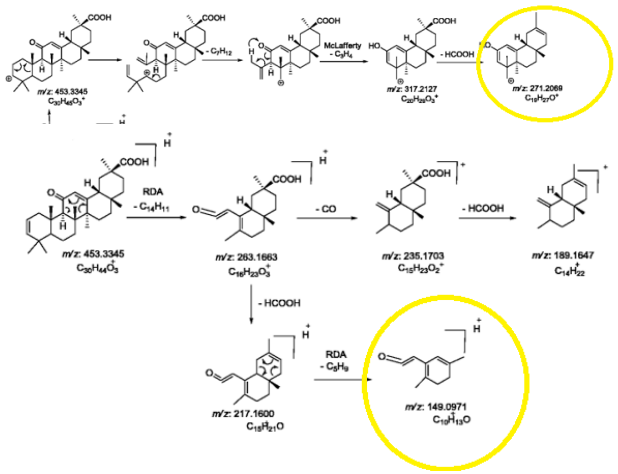


Figure 7: MS-APCI for standard glycyrrhizin

Chemical Structure for Glycyrrhizin

Symbol	
Formula	C42H62O16
Exact Mass	822.4037859360001
Average Mass	822.93208



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