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DETECTION AND IDENTIFICATION OF SOME BIOACTIVE COMPOUNDS IN ROOTS OF *GLYCYRRHIZA URALENSIS* FISCH

Licorice is one of the most important medicinal plants not only in Mongolia, but also worldwide because of its medicinal properties and agricultural applications. The resources of licorice are rapidly dwindling because the plant root is frequently used in a variety of treatments. Thus, plant tissue culture research has accelerated in recent years around the world in order to increase the source of important plants like licorice. The purpose of the study was to determine biological active components of wild licorice (*Glycyrrhiza uralensis* Fisch) in Mongolia and to optimize condition for in vitro seed propagation. From the results, moisture was 5.6 %, total minerals 8.8 %, total protein 9.6 %, total lipid

0.9 %, monosaccharides 0.5 % and disaccharides were 7.2 % respectively in the sample. Also, it has included that 6.5 % glycyrrhizic acid, 0.4% flavonoids, 0.1 % lignin, 34.3 % water-soluble extracts and no alkaloids. The yield of ammonium glycyrrhizinate was 1.2g/100g sample. *Glycyrrhiza uralensis* Fisch seeds were inoculated in ½ MS medium under a 8-hours photoperiod by cool white lamp. After 4 days, the first seed were germinated, germination of the seeds completed within 28 days.

Key words: *Licorice, glycyrrhizic acid, plant root, Glycyrrhiza uralensis Fisch., bioactive compounds.*

Introduction

The *Glycyrrhiza* L. genus, also known as licorice, is a member of the Leguminosae family and composes of about 30 species [1]. It is extensively spread in the Mediterranean basin of Africa, Europe, and Asia, extending to Australia, North America. Licorice is widely used in food and herbal medicine all over the world, and it accounts for a significant portion of the global market. *Glycyrrhiza uralensis* Fisch., *Glycyrrhiza glabra* L., and *Glycyrrhiza inflata* Bat. are commercially the most important species. The roots have traditionally been used as an important medicinal herb in countries ranging from Assyria, Egypt, Greece, Roman empires to Chinese, Indian, Japanese, Turkish, and Mongolian traditional medicine. Licorice is a small perennial herb that has long been used to treat a wide range of ailments, including fever, stomach ulcers, skin diseases, respiratory disorders, epilepsy, paralysis, rheumatism, hemorrhagic diseases, and jaundice. Modern clinical and experimental studies revealed that it has a wide range of pharmacological activities, including hepatoprotective, antioxidative, antiulcer, anti-inflammatory, antiallergic, antiviral, antidiabetic, anticancer, antidepressant and expectorant, and memory-enhancing properties. Researchers have conducted extensive research on the chemical constituents of liqorice in recent years [2]. Liqorice contains protein, amino acids, polysaccharides and simple sugars, essential oils, mineral salts, pectins, resins, starches, sterols, and gums. Nearly 400 chemical compounds, including flavonoids, glycosides, triterpenoid saponins, coumarin, pterocarpan, phenolics, and alkaloids that are responsible for hepatoprotection, neuroprotection, anti-inflammatory, detoxification, and other bioactivities, have been systematically excavated for various pharmacological activities in three varieties of licorice. *Glycyrrhiza uralensis* Fisch. ex DC., is a traditional plant that has long been recognized for its numerous health benefits and medicinal uses. It is also the most common species, accounting for more than 90% of total licorice production. Glycyrrhizin (glycyrrhizic acid) the principal substance present in licorice roots, along with the flavonoid liquiritin apioside. *G. uralensis* differs from *G. glabra* and *G. inflata* in that it has a greater variation in the six main constituents of licorice than *G. glabra* and *G. inflata*. [3; 4].

Materials and methods

Plant materials

Plant materials, whole plants were collected in the region of Bayankhongor aimag. The fresh root of *Glycyrrhiza Uralensis Fisch* collected and washing with under tap water to remove any impurities and then drying at room temperature without light, the sample put in grinder transfer it to powder. Certified reference seeds for use in plant tissue culture experiments were obtained from the Institute of Botany at the Mongolian Academy of Sciences.

Extraction and Purification

Biologically active compounds such as tannins, flavonoids, alkaloids and glycyrrhizic acid were identified in plant samples using spectrophotometric and volumetric methods.

For the reflux experiment, the powdered sample (approximately 100 g) was accurately weighed into a round bottom flask (1 L) and the distilled water (500 ml) added. The sample was refluxed for 5-6 h. The water extract was discharged. The residue was treated with 500 ml of distilled water with boiling for 3 h and allowed to cool before decanting into the same flask. After cooling, the extract was concentrated to twice its volume and 9 ml of concentrated H_2SO_4 was added to obtain a thick, brown crude extract of glycyrrhizic acid.

To purify this crude extract, transfer round bottom flask and was added acetone and refluxed for 3 h. Then 10 ml of 25% ammonia solution was added to this acetone extract with a stirrer to give a light-yellow precipitate.

Instrumentation and chromatographic condition

HPLC and FT-IR methods were used of quantification analysis glycyrrhizic acid and flavonoids.

The chromatographic determinations were carried out on a Shimadzu SPD-M20A equipped with a 150mm×4.6mm, 5μm particle C-18 reversed-phase column. The column was eluted with mobile phase following compositions: CH_3COOH – Methanol – 0.2M CH_3COONH_4 (1:66:33, vol.%); flow velocity, 1 mL min-1. The column effluent was monitored at 250 nm with DAD detector. Peak of glycyrrhizic acid in sample were identified by comparison with retention time of standard compounds.

Spectrum Fourier-transformer infrared spectrometer (Bruker, France), equipped with Transmission Mode is used. All IR spectra are recorded from an accumulation of 32 scans, and 0.2 cm/s of optical path difference (OPD) speed in the range of 4000–400 cm−1 with a resolution of 4 cm−1. The interferences of H_2O and CO_2 are subtracted when scanning. The crystal was then characterized by FTIR spectroscopy and by comparison with literature data and authentic sample and determined its structure to be gallic acid.

Results and discussion

Bioactive components in licorice

The sample of this research collected from Bogd sum, Bayankhongor province on July, 2017. The chemical composition was determined by quantifying protein, fats, moisture, ash, monosaccharides, and disaccharides. As a result, moisture was 5.6 %, total minerals 8.8 %, total protein 9.6 %, total lipid 0.9 %, monosaccharides 0.5 % and disaccharides were 7.2 % respectively in the sample. The bioactive components of *Glycyrrhiza uralensis* Fisch are shown in Table 1.

Table 1

The bioactive components of *Glycyrrhiza uralensis* Fisch

Sample	Contents, %				
	Tannin	Water-soluble components	Alkaloids	Flavonoids	Glycyrrhizic acid
Licorice	0.1	34.3	–	0.4	6.5
Reference	–	37.18	–	2.9	7.8

The tannins were determined by oxidoreductive titration, water-soluble extract by extraction and total flavonoid by spectrophotometric method, respectively [5]. As shown at Table 1, the sample was contained 6.5 % glycyrrhizic acid, 0.4 % flavonoids, 0.1 % lignin, 34.3 % water-soluble extracts and no alkaloids. The content of glycyrrhizic acid in the sample was 65 mg/g (Table 1) showed higher content than the results defined by Zhu, S. et al, 2009 [6].

Flavonoid and phenolics in the sample were determinated by TLC method (Figure 1). We used following four solvent systems: ethyl acetate:acetic acid (36:12:5), n-hexane:ethyl acetate:formic acid (31:14:5), toluene:acetone:formic acid (38:10:5) and petroleum ether:ethyl acetate:formic acid (30:15:5).

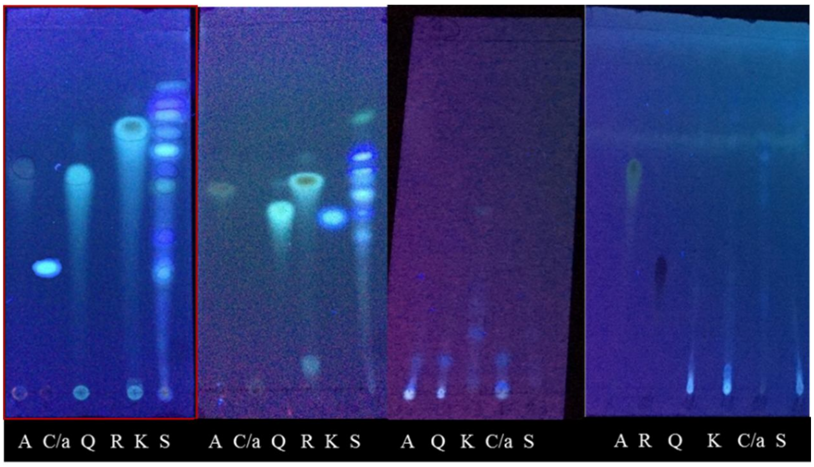

Figure 1. TLC of the flavonoids and phenolics in the licorice:

A – apigenin, C/a – caffeic acid, Q – quercetin, R – rutin, K – camperol, S – sample

As shown TLC results, the most suitable solvent system was toluene:acetone:formic acid (38:10:5), because some spots were not separated each other well or some of them weren't moved from spotting line for other solvent systems.

The Rf value was calculated and compared with results of Medic-Saric, et al, 2004 [7]. We used same solvent systems like them. All Rf value was same as reported by Medic-Saric, et al. Thus the sample of *Glycyrrhiza uralensis* Fisch was contained caffeic acid, quercetin, camperol and flavanone.

Isolation and characteristics of glycyrrhizic acid from the sample

Ammonium glycyrrhizinate was precipitated by ammonium from water extract of licorice root. The ammonium salt was light-yellow crystal. The yield of the crude salt was 3.9 g. Then the salt was recrystallized by using following solvent system: ethanol/glacial acetic acid, 5:1(v/v). After recrystallization, the yield of ammonium glycyrrhizinate crystal was 1.2 g. The melting point of the crystal was determined 208-211°C, by melting point apparatus, Digital, SMP10.

The FTIR spectra of ammonium glycyrrhizinate are shown in Figure 2. In the spectra of the glycyrrhizinate, the stretching vibrations of the –OH group was observed at 3429 cm^{-1} , and the C–H stretching modes in alkanes appeared at 2971 cm^{-1} . The bands in the range of 1703–1373 cm^{-1} are ascribed to the characteristic absorptions of the glycyrrhizinate, which presented the stretching vibrations of –C-O (1042 cm^{-1}), –C=O (1715 cm^{-1}). From the result, the stretching vibrations of main functional group in the glycyrrhizinate was same as previous reported by Visht, S., 2012.

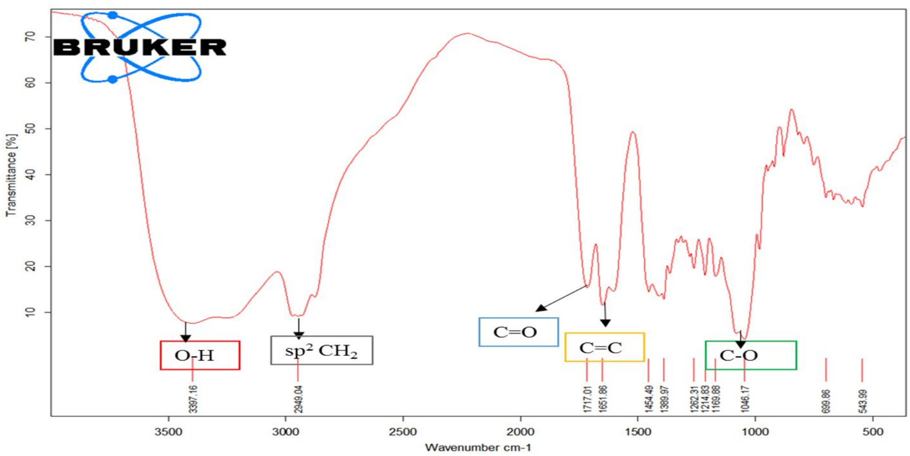

Figure 2. FT-IR spectrogram of the isolated glycyrrhizinate

The isolated glycyrrhizinate was determined by Shimadzu SPD-M20A HPLC. The chromatographic detailed condition was written in materials and methods section.

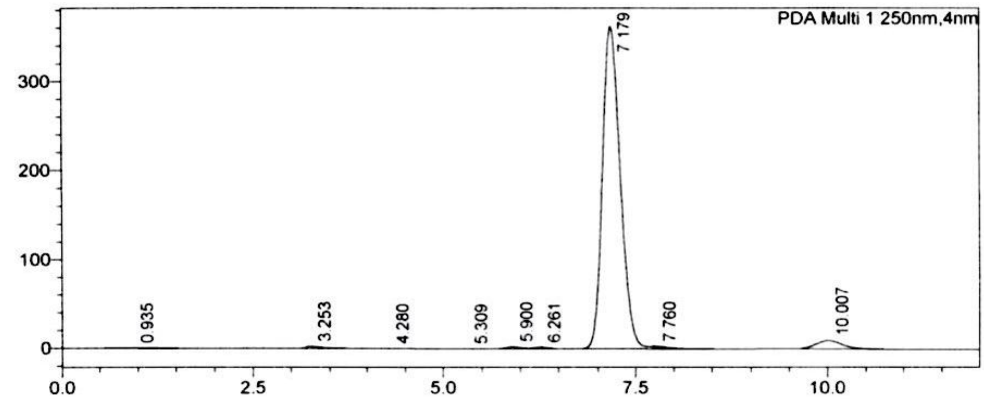

Figure 3. Chromatogram of standard glycyrrhizic acid

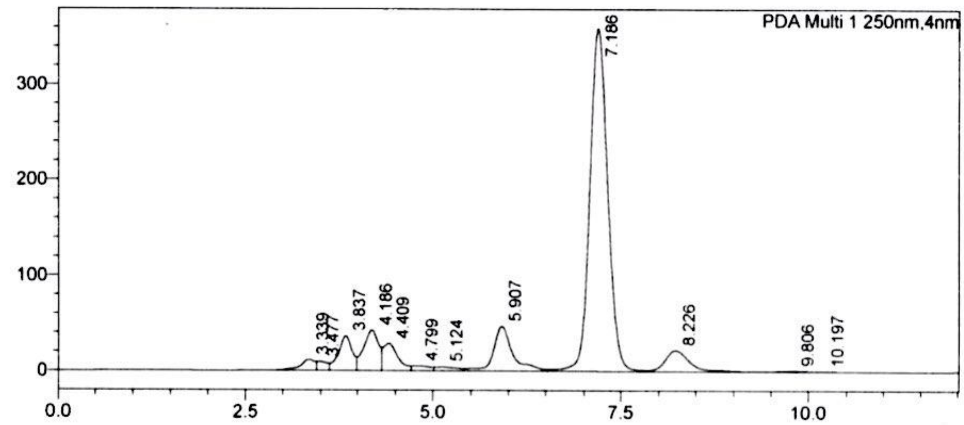

Figure 4. Chromatogram of the isolated glycyrrhizic acid

The contents of glycyrrhizic acid in Licorice samples were successfully determined. Fig. 3 showed the chromatogram of the glycyrrhizic acid standard sample solution. Fig. 4 showed chromatogram of the extract of the licorice root. The retention time of the standard was 7.179 min. The retention time of glycyrrhizic acid in the root was 7.186 min. Also this result same as the chromatographic result of Yu-hong, Y., et al, 2007 [8]. The yield of glycyrrhizic acid in wild licorice was 65.45 %.

Conclusion

The Ural licorice root was found to contain 5.7 % moisture, 8.8 % minerals, 0.9 % oil, 9.6 % protein, 0.5 % monosaccharides, and 7.2 % disaccharides in Bogd province of Bayankhongor aimag. From 100 g of Ural sugarcane roots, 1.2 g of pure glycyrrhizic acid ammonium salt was extracted. The IR spectroscopic analysis result and determination of melting point confirmed the ammonium salts of glycyrrhizic acid obtained. In addition, the purity of glycyrrhizic acid determined by HPLC was 65.44 %.

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ОБНАРУЖЕНИЕ И ИДЕНТИФИКАЦИЯ НЕКОТОРЫХ БИОЛОГИЧЕСКИ АКТИВНЫХ СОЕДИНЕНИЙ В КОРНЯХ *GLYCYRRHIZA URALENSIS* FISCH

Солодка является одним из самых важных лекарственных растений не только в Монголии, но и во всем мире из-за ее лечебных свойств и применения в сельском хозяйстве. Ресурсы солодки быстро истощаются, потому что корень растения часто используется в различных процедурах. Таким образом, в последние годы во всем мире ускорились исследования культуры растительных тканей, чтобы увеличить популяцию таких важных растений, как солодка. Целью исследования было определение биологически активных компонентов дикой солодки (*Glycyrrhiza uralensis* Fisch) в Монголии и оптимизация условий для размножения семян in vitro. Из результатов следует, что влажность составляла 5,6 %, общее количество минералов 8,8 %, общий белок 9,6 %, общий липид 0,9 %, моносахариды 0,5 % и дисахариды составляли 7,2 % соответственно в образце. Кроме того, он включал в себя 6,5 % глицирризиновой кислоты, 0,4 % флавоноидов, 0,1 % лигнина, 34,3 % водорастворимых экстрактов и никаких алкалоидов. Выход глицирризината аммония составил 1,2 г/100 г образца. Семена *Glycyrrhiza uralensis* Fisch инокулировали в среде ½ МС при 8-часовом фотопериоде при холодной белой лампе. Через 4 дня первые семена были проращены, прорастание семян завершилось в течение 28 дней.

Ключевые слова: *солодка, глицирризиновая кислота, корень растения, Glycyrrhiza uralensis Fisch., биологически активные соединения.*