LIQUIRITIAE RADIX AND POSSIBLE SUBSTITUENTS – COMPARATIVE LC/MS ANALYSIS OF SPECIFIC FLAVONOIDS

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Keywords: *licorice, liquiritin, liquiritigenin, isoliquiritigenin, glabridin*

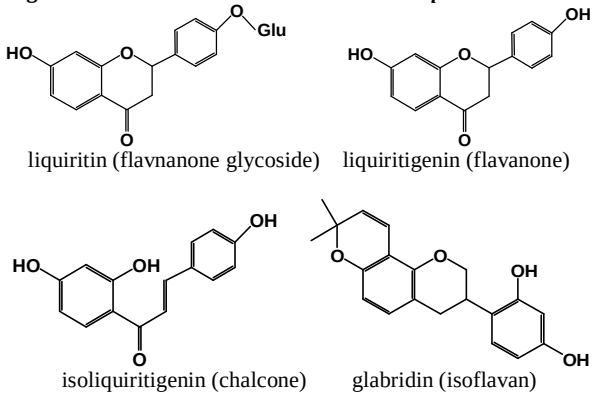
Abstract: *Licorice (Glycyrrhiza glabra. L) has been used since ancient times in order to treat gastric ulcers. The major active compounds are saponins and flavonoids. They are responsible for several other properties of the extracts: antibacterial, antiviral, depigmenting. The roots of the plant deposit these complex substances. Radix vegetal products may be easily substituted, making difficult their identification in absence of other vegetative organs such as leaves, flowers or fruits. Glycyrrhiza echinata L. is widerly spread in Romania than the consecrated medicinal species G. glabra. The aim of this study is to determine whether the roots of the two species have similar flavonoid characteristics, using Liquid Chromatography – Mass Spectrometry (LC/MS), a sensitive analysis method. The phytochemistry of licorice roots differs from that of G. echinata roots. The two are not equivalent. G. echinata roots lack the specific compounds correlated to the therapeutic activity of licorice.*

INTRODUCTION

Medicinal plants have been used since antiquity. Licorice is mentioned by Chinese documents from the 2nd century BC (1), also it is one of the most employed ingredients of ayurvedic medicine.(2) Dioscorides refers to the exploitation of licorice in ancient Greece.(3) Modern medicine has identified active compounds and has explained their mechanisms of action. The identity and the amount of active compounds in vegetal products are essential for the onset of the expected effects.

Licorice (*Glycyrrhiza glabra* L.) is a shrub encountered in Europe, Asia and Africa. *Liquiritiae radix* contains saponins and flavonoids (figure no. 1). The representative saponin is glycyrrhizin, a compound up to 50 times sweeter than sucrose.(4) Liquiritin, liquiritigenin, isoliquiritigenin and glabridin are specific flavonoids synthetized by this plant.(4,5) Licorice has several pharmacological activities, such as: antiulcer, anti-inflammatory, expectorant, antiviral, antibacterial, depigmenting, anti-proliferative, hepatoprotective, antidiabetic, antiasthma.(6-11)

Figure no. 1. Characteristic flavonoids of *Liquiritiae radix*



In Romania, *Glycyrrhiza echinata* L. is widerly spread than *G. glabra*. The two species can be easily differentiated only when fruits are present. *G. echinata* has spiny pods. *G. echinata* roots could be mistakenly presented as *Liquiritiae radix*.

G. glabra roots are light yellow and taste sweet-bitter, different from sugar. Young licorice roots have small amount of active compounds, making difficult their organoleptic identification. *G. echinata* roots are light grey and taste slightly bitter.

Recent studies on *G. echinata* extracts have shown the presence of fatty acids and volatile compounds and the antioxidant potential of the plant.(12) This species needs more investigations regarding the chemical composition and possible other therapeutic effects.

The composition of *G. echinata* roots should be studied using sensitive and specific methods of analysis.

PURPOSE

In order to establish whether to expect similar therapeutic activity to the consecrated medicinal plant, the aim of the study is the identification and quantification of specific flavonoids in the roots of the two *Glycyrrhiza* species.

MATERIALS AND METHODS

Plant material: three samples were studied: *G. glabra* from cultures of an authorised medicinal plant producer and distributor, *G. glabra* and *G. echinata* from the botanical garden of "Victor Babeş" University of Medicine and Pharmacy of Timişoara, institution that also holds voucher specimens code CC-GG-002 and CC-GE-002. The roots were harvested in October, they were washed, sliced and dried in the sun for 5 days, then in the drying stove, at 30ºC, to constant weight. Solutions of 1% (mg/ml) in methanol were used for the analysis. The roots were chopped in order to pass through sieve number VI. They were macerated using methanol, for 24 hours, at room temperature, then 3 times 15 minutes in the ultrasonic bath, followed by 30 minutes in the boiling water bath, in a round bottom flask with ascendant condenser.

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The hydrolysed extracts were obtained by maintaining a mixture of 1:1 total extract and HCl 6N in the water bath for 40 minutes at 80˚C.

All solutions were diluted 1/5 before analysis.

Standards: liquiritin, liquiritigenin, isoliquiritigenin, glabridin (Extrasynthese, France).

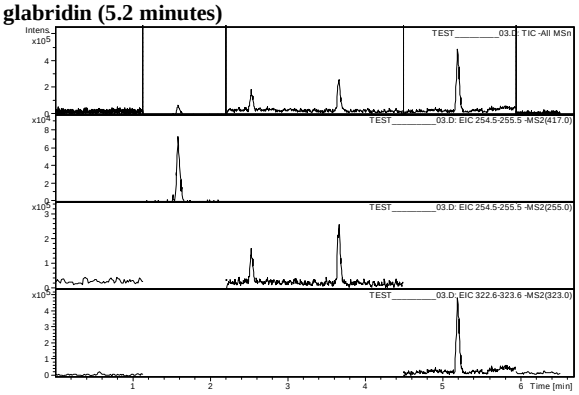
LC/MS systems: HP 1100 Series binary pump, auto sampler HP1100 Series, thermostat HP 1100 Series, Agilent Ion Trap 1100 SL mass spectrometer

Experimental conditions: analytical column Gemini NX C18 50 mm x 2.0 mm i.d., 3 µm; mobile phase: methanol and acetic acid 0.1% (V/V), gradient elution; 0.6 ml/minute, 45ºC; ESI-MS detection, negative ionisation, selected ion monitoring (SIM) or selected reaction monitoring (SRM); injected volume 2 µL.

The mass spectres and the chromatograms of the standards (figure no. 2) and of the samples were obtained.

The accuracy of the method was performed by recovery studies.

Figure no. 2. HPLC-MS chromatogram of the standards; 1 – all standards; 2 – liquiritin (1.5 minutes); 3 – liquiritigenin (2.4 minutes) and isoliquiritigenin (3.65 minutes); 4 – glabridin (5.2 minutes)



RESULTS

Figure no. 3. The percentage content of the analysed substances in the vegetal products

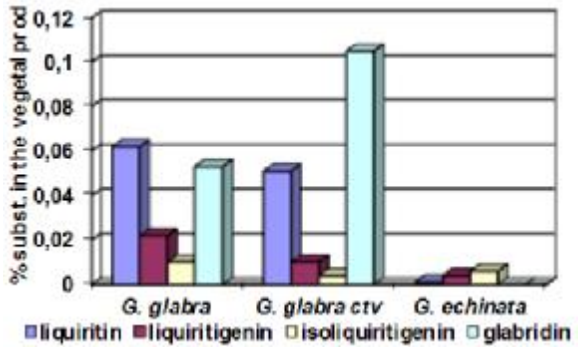


Table no. 1. The concentration of the analysed substances in the non-hydrolysed extracts

Vegetal product	liquiritin (ng/ml)	liquiritigenin (ng/ml)	isoliquiriti-genin (ng/ml)	glabridin (ng/ml)
G. glabra	6211.1	2205.1	1099.5	5375.2
***G. glabra* ctv**	5154.3	1060.6	457.4	10907.5
G. echinata	193.3	427.0	625.0	0.0

Table no. 2. The concentration of the analysed substances in the hydrolysed extracts

Vegetal product	liquiritin (ng/ml)	liquiritigenin (ng/ml)	isoliquiriti-genin (ng/ml)	glabridin (ng/ml)
G. glabra	1188.3	21648.5	3944.5	2222.8
***G. glabra* ctv**	1836.1	41736.2	11388.3	3553.0
G. echinata	128.3	836.3	0.0	0.0

DISCUSSIONS

Both *G. glabra* samples present all the studied substances (figure no. 6, table no. 1). The vegetal product obtained from cultivated species (*G. glabra* ctv) contains higher amounts of glabridin, but less liquiritigenin and isoliquiritigenin than the other *G. glabra* sample. The glabridin content is double in the cultivated species and the liquiritigenin and isoliquiritigenin content represents only half of the values obtained by the other *G. glabra* sample. The liquiritin content can be considered similar for both samples. The first *G. glabra* sample contains mainly liquiritin (621.1 µg/g of vegetal product) and glabridin (537.52 µg/g of vegetal product) in similar quantities. The same substances are also predominant in the vegetal product obtained from cultivated licorice, but the quantitative ratio differs, the glabridin (1090.75 µg/g of vegetal product) content is double in comparison to liquiritin (514.43 µg/g of vegetal product). This vegetal product contains the highest amount of glabridin among the three analysed samples. The glabridin content of *Liquiritiae radix* was shown to vary depending on the location of the plant. Vegetal products from Italy and Northern Spain contain 0.07-0.80 % glabridin.(13)

The hydrolysed samples (table no. 2) possess higher amounts of liquiritigenin and isoliquiritigenin in comparison to the original extracts, confirming their presence as aglicons in different glycosidic structures. Isoliquiritigenin is chemically unstable in the hydrolytic conditions, it is oxidised and it becomes undetectable in the *G. echinata* hydrolysed extract. Degradation of glabridin also occurs.

Glabridin has numerous pharmacologic effects: antioxidant (4,14), antimycotic (15-17), antitumor (18), estrogenic (19), depigmenting (20), serotonin reuptake inhibitor.(21)

Liquiritin exhibits neuroprotective effects on cognitive defficits chemically induced in rats (22). Also in rats, liquiritin improves the learning and memory ability of the subjects of an Alzheimer's disease model (23). This substance is a promising agent for the treatment of vasculopathy in diabetic patients.(24) Liquiritin in combination with isoliquiritin and liquiritigenin induce apoptotic cell death in the A549 non-small cell lung cancer cells.(25)

Liquiritigenin and liquiritin hold antioxidant capacity. Dermatology is one of the branches of medicine directly interested in this effect. New topical pharmaceutical formulations target to deliver these active substances at specific depths in the skin.(26)

Pharmacological properties of isoliquiritigenin include: anti-inflammatory (27), antitumor (28), antioxidative, hepatoprotective, cardioprotective activities.(29)

G. echinata extracts contain only three of the analysed substances: liquiritin, liquiritigenin and isoliquiritigenin. Glabridin could be considered a marker of *Liquiritiae radix* and could be used to rapidly identify a possible substitution of the medicinal vegetal product with *G. echinata* roots. Even thou the other three flavonoids were identified in *G. echinata* extracts, the liquiritin content of *G. echinata* represents less than 5% comparison with that of *G. glabra*.

The small amount of some flavonoids and the absence of others make *G. echinata* a nonviable substitute for *Liquiritiae*

radix.

CONCLUSIONS

Licorice (*G. glabra*) extracts contain saponins and flavonoids and exibit numerous pharmacologycal activities.

In order to establish the degree of similarity between *G. glabra* and *G. echinata* roots, liquiritin, liquiritigenin, isoliquiritigenin and glabridin were quantified by LC/MS in 1% methanolic total extracts.

G. glabra contains all the analysed flavonoids, among them liquiritin and glabridin are present in higher concentrations.

Glabridin is absent in *G. echinata* roots and the liquiritin, liquiritigenin and isoliquiritigenin content is by far inferior to *G. glabra*.

G. echinata roots should not replace the consecrated medicinal product *Liquiritiae radix* due to the absence of glabridin and the reduced concentrations of the other analysed flavonoids.

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