

An Improved Method of Analysis of Glycyrrhizic Acid in Mulethi (*Glycyrrhiza glabra* L.)

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An improved technique for the estimation of glycyrrhizic acid has been developed in *Glycyrrhiza glabra* L. The technique is based on the estimation of glycyrrhizic acid through its aglycone, the glycyrrhetic acid. The procedure reported so far give erroneous results because of the fact that the amount of non-glycyrrhizic acid components accompanying glycyrrhizic acid were not taken into consideration during the processing of samples. This method is considered to be more accurate as it also estimates the glycyrrhizic acid present during the preliminary processing of samples i.e. filtration and washings etc.

Key words: Mulethi, *Glycyrrhiza glabra*, Chemical analysis

Roots of *Glycyrrhiza glabra* L. and their aqueous extracts and formulation are extensively used in medicine (Brown and Dattner 1998). The active principle responsible for biological activity is glycyrrhizic acid, an acidic saponin. It is present in roots as calcium or potassium salts, known as glycyrrhizin along with fats, sterols, flavonoids, carbohydrates etc. A number of procedures have been reported for the extraction, isolation and estimation of glycyrrhizin and glycyrrhizic acid (Gou *et al.*, 1999, Lay *et al.*, 2000, Lee *et al.*, 1995, Tsai and Chen, 1991). Most of them are based on the solubility of glycyrrhizin in water, precipitation of glycyrrhizic acid with dilute mineral acid followed by conversion of the acid into its ammonium salts. Generally, it has been observed that the extraction is carried out either by dilute alcohol or water. None of these procedures have proved to be very accurate for quantitative determination for quality assay.

The erroneous results may be due to the loss of glycyrrhizin during the course of extraction with water or dilute alcohol. The glycyrrhizic acid is soluble in water and is lost during the initial processing, *i.e.*, washings and filtration of the samples. Earlier, an attempt has been made to estimate it through glycyrrhetic acid by using charcoal, but with little success. About three per cent glycyrrhetic acid is reported to be lost during the course of estimation. It has also been found that precipitated glycyrrhizic acid contained small amount of neutral, phenolic and solvent insoluble matter, which add to the inaccuracy of such procedures. In view of the necessity of an accurate estimation procedure for the quality assay of the samples, an improved method has been developed to hydrolyse glycyrrhizin to glycyrrhetic acid with dilute sulphuric acid.

Materials and Methods

Following steps were conducted to estimate the glycyrrhizic acid and glycyrrhetic acid from the roots of *Glycyrrhiza glabra*.

Isolation and purification of glycyrrhizic acid and extent of hydrolysis of glycyrrhizic acid to glycyrrhetic acid

The powdered root sample of *Glycyrrhiza glabra* was extracted with methanol. The glycyrrhizic acid was isolated from the water soluble part of the methanolic extract by precipitation with hydrochloric acid. The glycyrrhizic acid was purified by converting it into its ammonium salt and re-precipitated by using hydrochloric acid. More than 99 per cent glycyrrhetic acid was obtained by hydrolyzing glycyrrhizic acid, so obtained using 10 per cent sulphuric acid.

Extraction, isolation and estimation of glycyrrhizic acid by precipitation method

Ten gram of dried and powdered roots were extracted by continuous hot extraction in Soxhlet's apparatus with methanol for six hours. Methanol was then distilled off under vacuum. The flask and filter paper were washed thrice with distilled water and make up the volume up to 15 ml. The combined aqueous extract was then cooled to 4-6° C and treated with cold 1 per cent sulphuric acid. The glycyrrhizic acid was dissolved in methanol and filtered. The filtrate was transferred to a previously weighed evaporating dish. Methanol was evaporated on a gently boiling water bath and the glycyrrhizic acid left behind was dried in oven at 100-105° C till constant weight (Table 1).

Table 1. Glycyrrhizic acid analysis by precipitation method

Accessions	Glycyrrhizic acid (g)	Glycyrrhizic acid (%)
EC2708	0.5964	5.964
EC19741	0.3790	3.790
EC25371	0.5148	5.148
EC21950	0.4504	4.504
EC25967	0.4694	4.694
EC25409	0.4208	4.208

Isolation of glycyrrhetic acid, neutral, phenolic and solvent insoluble matter from the precipitated glycyrrhizic acid

The glycyrrhizic acid so obtained was hydrolysed with 10 per cent sulphuric acid. The hydrolysed products were extracted with ether. The ethereal extract was washed with 5 per cent potassium hydroxide solution leaving behind only non acidic substances in ether. The potassium hydroxide extract was acidified and extracted with ether to get glycyrrhetic acid and phenolic substances. These were then acetylated which made it possible to separate glycyrrhetic acid from phenolic substances. Insoluble organic matter was separated earlier from precipitated glycyrrhizic acid by dissolving it in methanol and filtration (Table 2).

The filtrate and washings from the precipitated glycyrrhizic acid were collected and hydrolysed with 10 per cent sulphuric acid. Glycyrrhetic acid was then separated according to the same procedure used earlier. The acid thus obtained was dried to constant weight. The corresponding amount of glycyrrhizic acid was found out and its percentage calculated (Table 3).

Isolation of glycyrrhetic acid from the hydrolysis product of root extract and estimation of glycyrrhizic acid

The methanolic extract of the roots was dissolved in 10 ml. water and filtered. The filtrate and the flask were washed thrice with distilled water (15 ml). The filtrate containing glycyrrhizin was then hydrolysed with 10 per cent sulphuric acid. Glycyrrhetic acid along with phenolic

and neutral substance was extracted with ether from the hydrolysis products. It was then acetylated with acetic anhydride-toluene and pure glycyrrhetic acid was separated from the phenolic and neutral substances. Corresponding amount of glycyrrhizic acid was then calculated from the amount of glycyrrhetic acid thus obtained and the percentage of glycyrrhizic acid was determined (Table 4).

The water soluble part of the methanolic extract of the root was hydrolysed with 10 per cent sulphuric acid and the hydrolysis product extracted with ether and acetylated. Glycyrrhetic acid was then separated from neutral and phenolic substances in one stage. The amount of glycyrrhizic acid corresponding to glycyrrhetic acid was found out and the percentage of glycyrrhizic acid was calculated from the formula:

$$\text{Per cent glycyrrhizic acid} = \frac{AB}{C} \times \frac{100}{D}$$

Where, A is the molecular weight of glycyrrhizic acid; B, the weight of glycyrrhetic acid obtained; C, the molecular weight of glycyrrhetic acid, and D, the weight of powdered roots.

Results and Discussion

The above data showed that the yield of glycyrrhetic acid was over 99 per cent. The percentage of glycyrrhizic acid in various samples by precipitation method is given in Table 1, whereas, Table 2 contains the data which bring about interesting observations and results. The precipitated glycyrrhizic acid on hydrolysis yields glycyrrhetic acid, neutral and phenolic substances in addition to a small

Table 2. Determination of Glycyrrhizic acid, neutral, phenolic and solvent insoluble matter in precipitated glycyrrhizic acid

Accessions	Glycyrrhizic acid obtained (g)	Glycyrrhizic acid (%)	Glycyrrhetic acid from glycyrrhizic acid	Phenolic and neutral matter in glycyrrhizic (g)	Solvent insoluble matter in glycyrrhizic acid (g)	Glycyrrhizic acid corresponding to glycyrrhetic (g)	Glycyrrhizic acid in precipitated glycyrrhizic acid (g)(%)
EC 2708	0.5964	5.964	0.3166	3.28	3.86	0.5537	92.84
EC 19741	0.3790	3.790	0.2033	2.64	3.52	0.3555	93.80
EC 25371	0.5148	5.148	0.2802	2.21	2.60	0.4900	95.19
EC 21950	0.4504	4.504	0.2398	3.09	3.77	0.4194	93.12
EC 25967	0.4694	4.694	0.2470	3.62	4.36	0.4319	92.02
EC 25409	0.4208	4.208	0.2234	3.33	3.80	0.3907	92.85

Table 3. Determination of glycyrrhizic acid from precipitated glycyrrhizic acid during preliminary processing

Accession Nos.	Glycyrrhizic acid in precipitated glycyrrhizic acid	Glycyrrhetic acid from the filtrate	Glycyrrhizic acid corresponding to glycyrrhetic acid (g)	Total Glycyrrhizic acid (g)	Glycyrrhizic acid in the filtrate (%)
EC 2708	0.5537	0.0630	0.1101	0.6638	16.58
EC 19741	0.3555	0.618	0.1080	0.4635	23.30
EC 25371	0.4900	0.628	0.1098	0.5998	18.13
EC 21950	0.4194	0.0609	0.1065	0.5259	20.25
EC 25967	0.4319	0.0619	0.1080	0.5399	20.00

Table 4. Estimation of glycyrrhizic acid through glycyrrhetic acid

Accessions	Glycyrrhizic acid (g)	Glycyrrhizic acid corresponding to glycyrrhetic acid (g)	Glycyrrhizic acid (g)
EC2708	0.3790	0.6628	6.628
EC19741	0.2633	0.4605	4.605
EC25371	0.3422	0.5984	5.984
EC21950	0.2978	0.5208	5.208
EC25967	0.3091	0.5406	5.406
EC25409	0.2822	0.4935	4.935

amount of solvent insoluble organic matter obtained on dissolving glycyrrhizic acid in methanol. It is evident from the data that 92.02 to 95.19 per cent of pure glycyrrhizic acid was recovered.

The solubility of glycyrrhizic acid in water and the extent to which it lost during filtration and washing is presented in Table 3. The amount of glycyrrhetic acid obtained from the filtrate and washings correspond to the amount of glycyrrhizic acid to the extent from 16.58 to 23.30 per cent which was lost during the course of filtration and washing. Table 4 shows the data related to estimation of glycyrrhizic acid through glycyrrhetic acid.

The amount of glycyrrhizic acid thus obtained is almost equal to the sum of the glycyrrhizic acid present

in the precipitated glycyrrhizic acid and in the filtrate and washings thus providing more accurate and reproducible results. This technique provides more accuracy and reproducibility. It is of immense value in the quality assay of mulethi samples where an even minor difference in the glycyrrhizic acid content plays very important role.

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