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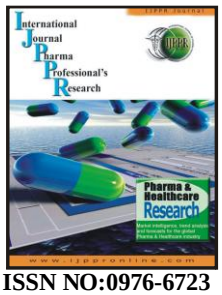

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Research Article

A COMPARISON BETWEEN DIFFERENT METHODS FOR EXTRACTION OF GLYCYRRHETINIC ACID FROM LIQUORICE STOLONS

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Abstract

The dried roots, rhizomes and stolons of the plant *Glycyrrhiza glabra* L., belongs to family Leguminosae. It is commonly known as liquorice. It has been used as medicine since ancient times. Recently, it is also widely used in both food and pharmaceutical industries.A triterpenoid saponin glycoside, glycyrrhizin (2-20%) is the major constituent of plant, which yield one molecule of glycyrrhetinic acid (aglycon) and two molecules of glucuronic acid (glycon) on hydrolysis in acidic medium due to the breakage of ester linkage between glycon and aglycon. Glycyrrhizin is fifty times sweeter than sucrose. The techniques used for the production of glycyrrhetinic acid include production from salt of glycyrrhizin, by enzymatic reaction and by hydrolysis of liquorice roots/stolons.The present investigation deals with comparison between various extractions techniques of glycyrrhetinic acid from the stolons of liquorice. Three different extraction methods namely maceration, solvent treatment and Soxhlet extraction method were compared to determine percent yield of glycyrrhetinic acid as ammonium salt. The maximum extraction of glycyrrhetinic acid was found by solvent treatment method, which utilized change of pH of extraction solvent.

It was concluded from the result that the extraction ratio of glycyrrhetinic acid can be increased by changing in pH of extraction solvent. Isolated component obtained from above three methods were investigated further for physical characterization, organoleptic properties, melting point, loss on drying, pH, UV, IR.

Keywords: - Licorice, *Glycyrrhiza glabra*, glycyrrhetinic acid, stolon, extraction methods.

Introduction

Liquorice consists of the dried peeled or unpeeled root and stolon of plant *Glycyrrhiza glabra* L., (Family: Leguminosae) (1-5). This plant is widely used in both food and pharmaceutical industries. Major constituent of licorice is a triterpenoid saponin glycoside; glycyrrhizin (2-20%), which is a mixture of potassium and calcium salts of glycyrrhizic acid and is 50 times sweeter than sucrose and safe to be used in diabetes. Glycyrrhizin loses its sweet taste and yield one molecule of glycyrrhetinic acid (aglycon) and two molecules of glucuronic acid (glycon) on hydrolysis in acidic medium due to breakage of ether bond between glycone and aglycone. Glycyrrhizin is freely soluble in hot water and alcohol, but practically insoluble in ether.

Glycyrrhetinic acid is a pentacyclic triterpenoid derivative of β-amyrin type. It is freely soluble in chloroform and acetic acid. Minor constituents are triterpenoid saponin (glabranin A & B, glycyrrhetol, glabrolide, isoglabrolide), isoflavone (forminonetin, glabrone, neoliquiritin, hispaglabridin A&B), coumarins (herniarin, umbelliferone), triterpene sterols (onocerin, β-amyrin, stigmasterol), flavonoid glycosides (isoliquiritin and liquiritin), coumarin glycosides (herniarin, umbelliferone). It has some proved clinical activities like antiulcer, anti-asthmatic, anti-diuretic, hepatoprotective, antibacterial, antioxidant, anti-spasmodic, anti-inflammatory, estrogenic (7-10). It is applied in eczema and psoriasis and also used against herpes virus and HIV (1, 3, 4, 10-13).

This research provide the information regarding comparison of extraction techniques, pre-formulation studies of glycyrrhetinic acid ammonium like physical characterization, organoleptic properties, melting point, loss on drying, pH, UV, IR.

MATERIALS AND METHODS

A. COLLECTION AND AUTHENTICATION

The dried stolen of liquorice (*Glycyrrhiza glabra*) was used for extraction of glycyrrhetinic acid. Liquorice was procured from Meerut market. Dried stolon was authenticated by Dr. Anjula Pandey, Principal Scientist, National Beuro of Plant Genetic Resources (NBPGR), New Delhi. The chemicals used in present study include ammonium hydroxide, orthophsophotic acid, sulphuric acid and charcoal, chloroform, ethyl acetate, n-butanol, 2-propanolol and acetic acid, acetonitrile, which were procured from SD Fine Chem, Mumbai and were of analytical grade.

B. EXTRACTION OF GLYCYRRHETINIC ACID AS ITS AMMONIUM SALT

Extraction procedure

1. Maceration method

Weighted amount of liquorice stolons were allowed to soak in 5% H_2SO_4 solution in 500 ml distilled water for 6 hours. Filter the mixture and residual cake was further mixed with 500 ml warmed, distilled water for 2 hours. Both filtrates were mixed and neutralized with sufficient amount of alkali (Strong ammonia solution). The mixture was decolorized by passing it through charcoal column. The mixture was the concentrated on rotary evaporator and crystallized with ethanol. Then the component was purified by column chromatography and analyzed for further identification.

2. Solvent treatment method

Weighted amount of liquorice stolons were allowed to soak in 5% H_2SO_4 solution in 500 ml distilled water for 6 hours. The mixture was filtered (Fraction A). Residual cake was mixed with 500 ml of alkali (Strong ammonia solution). After 2 hours mixture was filtered (Fraction B). Both fractions 'A' and 'B' were neutralized with alkali (Strong ammonia solution). The mixture was decolorized by passing it through charcoal column. The mixture was the concentrated on rotary evaporator and crystallized with ethanol. Then the component was purified by column chromatography and analyzed for further identification.

3. Soxhlet extraction method

Weighted amount of liquorice stolons were packed in Soxhlet column and refluxed with 1000 ml of acidified water for 6 hours. The extract was neutralized with alkali (Strong ammonia solution). The mixture was decolorized by passing it through charcoal column. The mixture was the concentrated on rotary evaporator and crystallized with ethanol. Then the component was purified by column chromatography and analyzed for further identification.

C. IDENTIFICATION OF COMPOUND (GLYCYRRHETINIC ACID AMMONIUM)

Chemical identification of component is essential to confirm the molecular identity. Component extracted and purified by above three methods were investigated for physical characterization, organoleptic properties, melting point, loss on drying, pH, UV, IR.

1. PHYSICAL CHARACTERIZATION

All salts were studied for physically characterization which includes determination of physical state.

2. ORGANOLEPTIC PROPERTIES

All salts were studied for color, odor and taste.

3. MELTING POINT

The measurement of the melting point is of major concern to identify the compound which also reflects the solubility characteristics, purity of component and crystalline habit (crystalline or amorphous). The melting point of glycyrrhetinic acid ammonium was determined by the capillary melting technique. Firstly, the melting point apparatus was calibrated using L-ascorbic acid AR and sodium carbonate AR. Then the small quantity of glycyrrhetinic acid ammonium was taken in a capillary tube and put in the digital melting apparatus and average melting point was determined.

4. LOSS ON DRYING

Accurately weighed 10 g of compound was placed in hot air oven, preadjusted at 105°C. Weight the sample after each 1 hour until two constant readings of weight are obtained.

5. DETERMINATION OF pH

The 1% aqueous solution of compound prepared and the pH was determined with a standardized glass electrode, precalibrated at pH 4, 7 and 9.

6.DETERMINATION OF λ_{max} AND PREPARTION OF CALIBRATION PLOT BY UV SPECTROPHOTOMETRIC ANALYSIS

It involves following steps

a. SELECTION OF MEDIA

The 0.1N HCl was used to prepare calibration curve by UV spectrophotometer.

b. PREPARATION OF STOCK SOLUTION

A stock solution of concentration 1 mg/ml was prepared in 0.1N HCl in 100 ml volumetric flask. The 0.1N HCl was used as blank/reference. Sample was scanned to determine the λ_{max} with the help of ultraviolet spectrophotometer (Shimadzu 1700S). The dilutions (1-10 µg/ml) were prepared and scanned at λ_{max} to measure absorbance. Finally calibration curve of glycyrrhetinic acid ammonium was prepared and equation of line was established.

7. FOURIER TRANSFORM INFRARED SPECTROSCOPY

The Fourier transform infrared spectroscopy of the product was performed on FTIR (FTIR 8400S, CE, Software Irresolution). The perfectly dried glycyrrhetinic acid ammonium (1 mg) was mixed with potassium bromide KBr powder (10 mg) in a mortar pestel. Prepared mixture was then compressed into fine disc by KBr press at pressure of 15,000 Psi. Prepared disc was placed on window of IR spectrometer to determine various bonds and group present in compound.

8.DIFFERENTIAL SCANNING CALORIMETRY (DSC)

DSC was performed on DSC Q200 V24.4 Build 116 (Universal V4.5A Instruments) at IIT, Delhi.

9. NUCLEAR MAGNETIC RESONANCE (NMR)

The ^{1}H-NMR spectra were recorded on an ADVANCE II 400 (Bruker) spectrometer from Punjab Technical University. Sample was prepared using DMSO as solvent at 400MHz.

RESULT AND DISCUSSION

A. COLLECTION AND AUTHENTICATION

The dried stolon of plant *Glycyrrhiza glabra* was authenticated by the National Bureau of genetic resources (NBPGR), New Delhi with voucher no. NHCP/NBPGR/2009-30/4812.

B. EXTRACTION OF GLYCYRRHETINIC ACID AS ITS AMMONIUM SALT

Yield was varied between 5.23-6.79%.

EXTRACTION PROCEDURES	% Yield
Maceration	5.23
Solvent treatment	6.79
Soxhlet	5.68

Table 1: Percent yield by different extraction processes

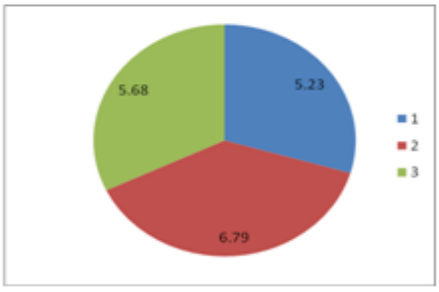


Chart 1: Pie chart representing % yield By different extraction processes

C. IDENTIFICATION OF COMPOUND (GLYCYRRHETINIC ACID AMMONIUM)

1. PHYSICAL CHARACTERIZATION

Glycyrrhetinic acid ammonium was crystalline powder in nature and results are shown in table 2.

2. ORGANOLEPTIC PROPERTIES

Color, taste and odor of glycyrrhetinic acid ammonium are shown in table 2.

3. MELTING POINT

The average melting point of glycyrrhetinic acid ammonium is shown in table 2 for prepared salts.

4. LOSS ON DRYING

Loss on drying was found to be less than 0.1% and results are shown in table 2.

5. DETERMINATION OF pH

The 1% solution of glycyrrhetinic acid ammonium exhibited a pH of 4.2, 4.1 and 4.2.

Procedures	Maceration	Solvent treatment	Soxhlet
% Yield	5.23	**6.79**	5.68
Physical form	Crystalline	Crystalline	Crystalline
Color	White	White	White
Odour	Odorless	Odorless	Odorless
Taste	Characteristic	Characteristic	Characteristic
Melting point	293±0.12°C	292±0.5°C	294±0.15°C
Loss on drying	0.1%	0.1%	0.1%
Determination of pH	4.2	4.1	4.2

Table 2: Results of above studies

6. DETERMINATION OF λ_{max} AND PREPARTION OF CALIBRATION PLOT BY UV SPECTROPHOTOMETRIC ANALYSIS

The λ_{max} of glycyrrhetinic acid ammonium was found to be 251-252 nm in 0.1N HCl.

S.No.	Concentration (µg/ml)	Absorbance
1	10	0.012
2	20	0.025
3	30	0.035
4	40	0.049
5	50	0.062
6	60	0.071
7	70	0.083
8	80	0.098
9	90	0.11
10	100	0.118

Table 3: Readings of calibration graph of glycyrrhetinic acid

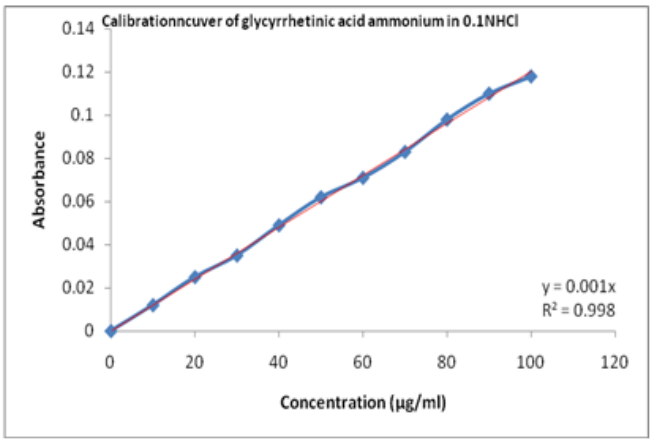


Figure 1: UV calibration graph of glycyrrhetinic acid ammonium in 0.1N HCl

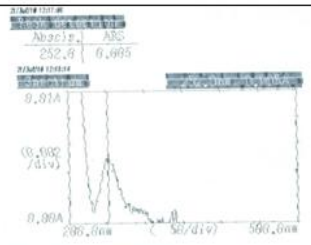
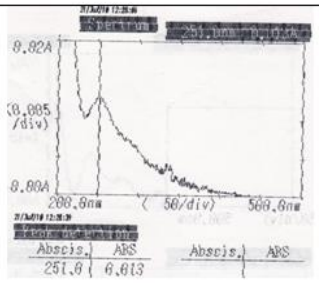
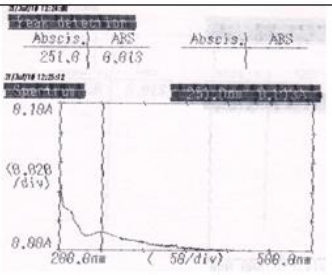
S.No.	Method	Graph	λ_{max} nm
1	Maceration		252
2	Solvent treatment		251
3	Soxhlet		251

Figure 2: UV Scan for λ_{max} of glycyrrhetinic acid ammonium in 0.1N HCl

7. INFRARED SPECTROSCOPY

The Fourier transform infrared spectroscopy of the product was obtained at a frequency of 400.1299 MHz which showed a considerable difference in bands about frequency 617, 837, 980, 1110, 1384, 1623, 2071, 2117, 2372, 3413 nm.

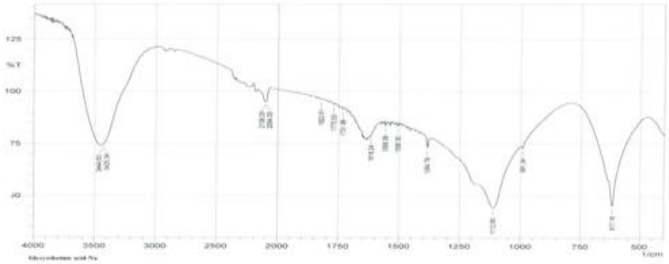


Figure 3: FTIR spectra of glycyrrhetinic acid ammonium obtained by maceration method

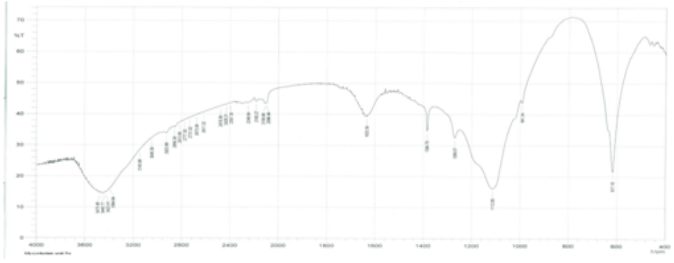


Figure 4: FTIR spectra of glycyrrhetinic acid ammonium obtained by solvent treatment method

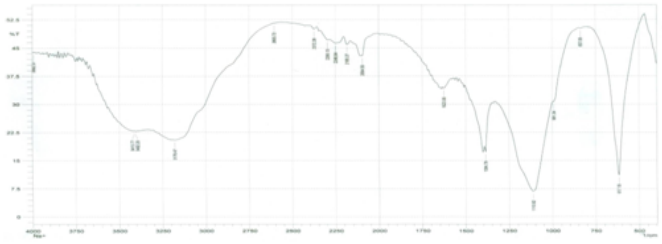


Figure 5: FTIR spectra of glycyrrhetinic acid ammonium obtained by Soxhlet extraction method

8. DIFFERENTIAL SCANNING CALORIMETRY (DSC)

DSC was performed on DSC Q200 V24.4 Build 116 (Universal V4.5A Instruments) at IIT, Delhi..

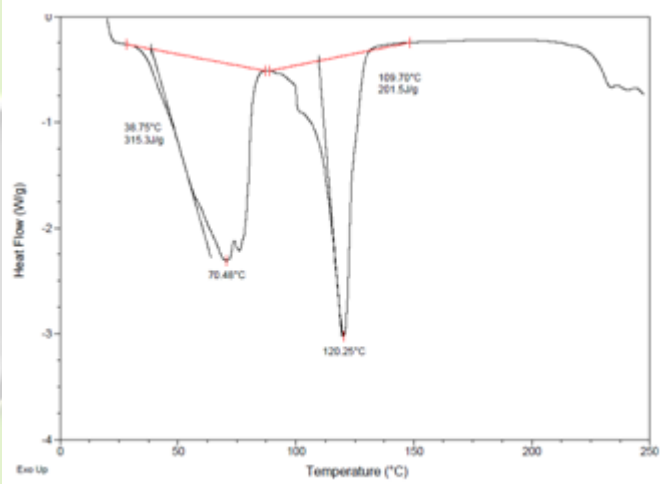


Figure 6: DSC curve of glycyrrhetinic acid ammonium

9. NUCLEAR MAGNETIC RESONANCE (NMR)

The ^{1}H-NMR spectra were recorded on an ADVANCE II 400 (Bruker) spectrometer at Punjab Technical University. Sample was prepared using DMSO as solvent at 400MHz.

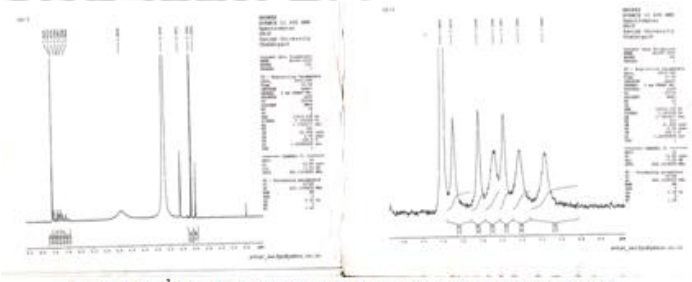


Figure 7: ^{1}H-NMR spectra of glycyrrhetinic acid ammonium

DISCUSSION

In this study, three methods for the extraction of glycyrrhetinic acid from licorice are compared. The percentage yield of glycyrrhetinic acid ammonium extracted by maceration, solvent treatment and Soxhlet

extraction method are 5.23%, 6.79% and 5.68 respectively, which indicated that the extraction of glycyrrhetinic acid can be increased by changing pH of extraction solvent. Glycyrrhetinic acid ammonium was crystalline powder in nature, white, odorless and characteristic in taste. The melting point was found to be almost identically equivalent to range 293±1ºC. Loss on drying was 0.1%. The λ_{max} of the glycyrrhetinic acid when scanned between 200-400 nm was found to be 251-252 nm in 0.1N HCl and the calibration curve obtained was found to be almost linear indicating follow up of Beer Lambert's law. The Fourier transform infrared spectroscopy of product, obtained by all the three methods indicate presence of C-C, C-O, C=O, C=C, C-H, O-H groups.

Conclusion

Solvent treatment method showed high yield which indicate pH affects the extraction of phytoconstituent.

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