Quantification of Glycyrrhizin from Different Extract of *Glycyrrhiza Glabra*

Clariya Rapheal[1], Prasanth S.S[2]

[1]*Department of Pharmaceutical chemistry, Westfort College of pharmacy*
[2] *Department of pharmaceutical analysis, Alshifa college of pharmacy*

Abstract: **Identification and quantification of phytoconstituents in *Glycyrrhiza glabra* was carried out by HPTLC. Whereas the dried root of Glycyrrhiza *glabra* was extracted successively with solvent like hexane, chloroform and methanol respectively. The phytochemical screen of each extract was performed and compares the result. The Total phenolic content of each extract were carried out using Gallic acid as reference. In that the methanolic extract had a higher amount of total phenolic content.. The anti oxidant activity of each extract were carried out by DPPH assay using ascorbic acid as reference standard. This assay revealed that methanolic extract had a lower IC $_{50}$ value .The quantification of glycyrrhizin in each extract was carried out by HPTLC using silicagel F_{254}nm plate which was developed in optimized solvent system of Chloroform:Methnol:water (6:3.5:0.5v/v/v).HPTLC revealed that methanolic extract of *Glycyrrhiza glabra* was having higher amount of Glycyrrhizin content.**

***Key Word*: - *Glycyrrhiza glabra*, Total phenolic content, DPPH assay, Glycyrrhizin, IC $_{50}$ value**

I. INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities *Glycyrrhiza glabra* is a peeled or unpeeled root and stolon of Glycyrrhiza glabra Lbelongs to the family: Leguminosae. . The saponin, glycoside, triterpenoid, potassium salt, calcium salt and glycyrrhizic acid are the major active constituent in *Glycyrrhiza glabra* and also consist of ammonium salt of glycyrrhizicacid is called Glycyrrhizin[1].

Glycyrrhizin, a triterpenoid compound, accounts for the sweet taste of licorice root[.[2]. Both glycyrrhizin and glycyrrhetic acid can exist in the 18α and 18β stereoisomers. As a tribasic acid, glycyrrhizin can form a variety of salts and occurs naturally in licorice root as the calcium and potassium salts.[3]

Traditionally the plant has been recommended as prophylaxis for gastric and duodenal ulcers and dyspepsia as an anti-inflammatory agent during allergenic reactions. In folk medicine, it is used as a laxative, emmenagogue, contraceptive, galactagogue, anti-asthmatic drug and antiviral agent [4]. Glycyrrhizin also proved effective in the treatment of chronic hepatitis and liver cirrhosis. For relieving pain, discomfort and other symptoms caused by acrid matter in the stomach, Glycyrrhiza glabra is considered as one of the best remedies. It seems to remove the irritating effects of acids in a better way than alkalies. It is used by practitioners of the indigenous systems as a tonic, as a demulcent in catarrh of the genitor-urinary passages [5-6] The phytoconstituents are present in the *Glycyrrhiza glabra* produces different pharmacological action hence these constituents are isolated and quantified for drug development.The anti oxidant activity is one of the activity for the herbal extract because the plant extract have the ability to absorb free radical.[7].The present study focused to comparative study of the different extracts of Glycyrrhiza glabra.

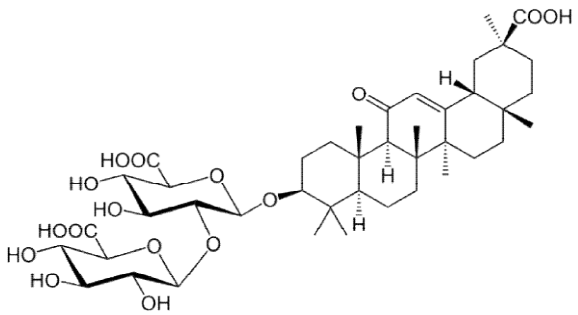

Fig1. Glycyrrhizin

II. EXPERIMENTAL REQUIREMENT

2.1 Material

The root of Glycyrrhiza glabra was collected from the local market Trivandrum.The collection was done during the month of October-November.Then the root was thoroughly washed with water to remove the dirt if any present. Identification of plant part was done in Calicut University and Dept of Botany was certified that the given specimen belong to *Glycyrrhiza glabra* of family Leguminosae

*2.2 Chemicals***.**

Hexane, Chloroform , Methanol (Merck Life Sciences Pvt. Ltd) Conc. H_2SO_4, Conc. HCl, NaOH, $FeCl_3$ Folin-Ciocalteu reagent (Sisco reagent Pvt. Ltd) , DPPH reagent(Alfa Aesar Pvt. Ltd)

Silica Gel Column grade (Merck Life Sciences Pvt. Ltd) Glycyrrhizin (Sigma Aldrich).S

2.3 Instrument

Electronic balance(Shimadzu,), Soxhlet apparatus (Borosil) UV VISIBLE spectrometer (Shimadzu,) Rotavapor Buchi,

(Switzerland) TLC documentation system (Aetron) HPTLC CAMAG HPTLC system, Switzerland.

III. METHODS

Freshly collected root were drying for about 5-6 days. After that, the root was dried in a hot air oven at 40℃ for about 6-7 days for the complete removal moisture content, thereby further microbial attack. The dried bark was powdered using mixer grinder.

About 30g of dried powder of Glycyrrhiza glabra was loaded in a thimble of soxhlet apparatus and that connected to a round bottom flask containing hexane, chloroform, and methanol, successively. Each extraction carried out for 10 hours.. Collect each solvent extracts and dried using Rotavapor. The Rotavapor used was BUCHI Rotavapor R-210. Were used as starting vegetal material for all experiments like phytochemical analysis, TPC, DPPH, and Quantification.

3.1.Total Phenolic Content

The Folin-ciocalteu test was chosen to measure TPC of Glycyrrhiza glabra extracts. The quantitative estimation of total phenolics in extracts was done by colorimetric method taking Gallic acid as standard in the concentration range from 10-60ppm. Extract solution (1ml) was taken in a test tube and 5ml Folin- Ciocalteu reagent was added into it and the content was mixed thoroughly. After 5min 4ml of 20%.sodium carbonate (Na2CO3) was added. This mixture was allowed to stand for half an hour at room temperature in the dark before the absorbance was measured at 760nm spectrometrically[8-14]

3.2 Antioxidant Assay

DPPH Reagent: take 3.96mg of DPPH reagent in 100ml of methanol in a standard flask

Preparation of std. Ascorbic acid: Take 5 mg of ascorbic acid powder in 5ml of methanol to form a concentration of 1mg/ml. Take 2µg/ml,4µg/ml…..20µg/ml from the stock solution and make up to 1 ml with methanol. To each of these add 2ml of DPPH Reagent solution, incubated for half an hour and read the absorbance at 517nm[15-16]].

Preparation of sample: Take 2g of each extract and dissolved in 2 ml of corresponding solvents to form 1mg/ml concentration of each extract. Take 50µg/ml,100µg/ml…….300µg/ml from the stock solutions and makeup to 1ml with methanol. To each of the different concentrations of extracts add 2ml DPPH reagent solution, incubated for half an hour in dark. Read the absorbance at 517nm. Methanol is taken as blank and control as an equal amount of methanol and DPPH solu**tion.**

3.3 *Quantification of Glycyrrhizin*

5µl of 1mg/ml of standard Glycyrrhizin in ethanol and 10 µl of 100mg/ml of extracts dissolved in corresponding solvents are tracked in precoated HPTLC F254plate. The chromatograms of samples were developed in ascending technique in twin trough chamber by using Chloroform: methanol: water in the ratio 6: 3.5: 0.5v/v/v. After the chromatogram development, the spots were observed at 254nm and scanned using densitometry scanning.

IV. RESULTS

. The extractive yield for Hexane extract (3.07%), chloroform extract (1.83%) and methanolic extract (4.67%)..

Almost all phytochemicals were present in methanolic extract when compared with other extracts.

Tests	Hexane	Chloroform	Methanolic
Tannins	-	-	+
Saponins	-	-	+
Flavanoids	-	-	+
Quinine	+	+	++
Terpenoid	+	+	++
Glycoside	-	-	-
Cardiac glycoside	-	-	-
Triterpenoid	-	-	-
Phenols	-	-	++
Steroids	+	+	+
Carbohydrate	-	+	+
Protein	-	-	-
Alkaloid	-	-	++

4.1 Total Phenolic Content

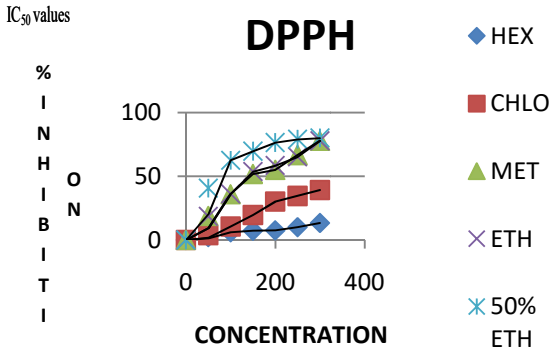

Fig .2 calibration curve

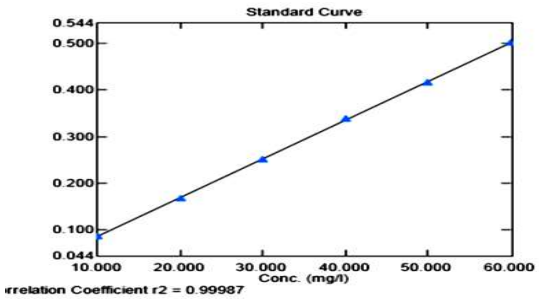

Fig .3 DPPH assay

The TPC of the extracts was expressed as µg of gallic acid equivalent The phenolic content of plants is strongly associated with their antioxidant capacity. The total phenolic content of various solvent extracts of *Glycyrrhiza glabra* was determined using Folin-Ciocalteu reagent. The TPC in root extracts was quantified using Gallic acid as standard.

Table.1. Phytochemical analysis of extracts

SL no.	EXTRACTS	AMOUNT (µg)
1	Hexane	3.667±0.198
2	Chloroform	6.467±0.390
3	Methanol	11.888±0.294

4.2 Antioxidant Assay

IC_{50} values of each extract of the root of *Glycyrrhiza glabra* were compared with a standard IC_{50} value of ascorbic acid

8.0113±0.9043. The IC $_{50}$ value of hexane, chloroform and methanol extract were 1275.734±0.6948, 340.150±1.404 and 114.28±1.389 respectively

4.3 Quantification - HPTLC Method

From HPTLC, it was reported that standard Glycyrrhizin spot were obtained with R_f value 0.14. The sport was detected in 254nm and 366nm. Better spot development was obtained with a mobile phase chloroform:methanol:water (6: 3.5: 0.5)for Glycyrrhizin.

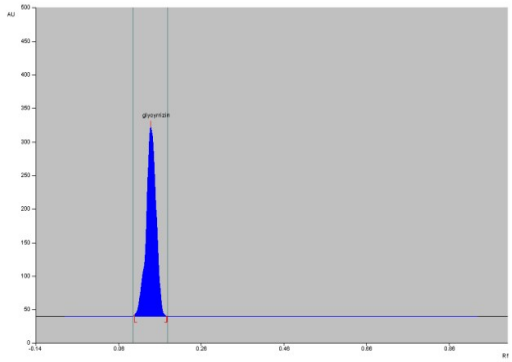

Fig 4. HPTLC Chromatogram of Glycyrrhizin

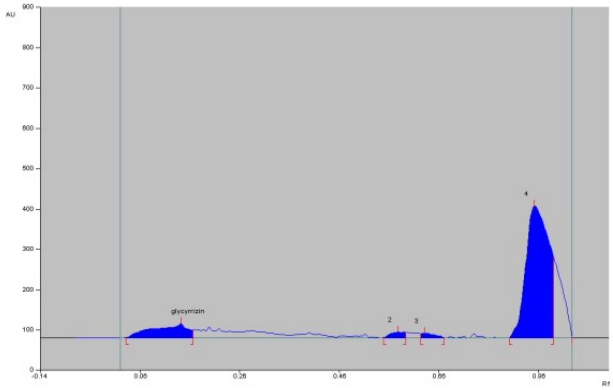

Fig.5. HPTLC Chromatogram of Hexane

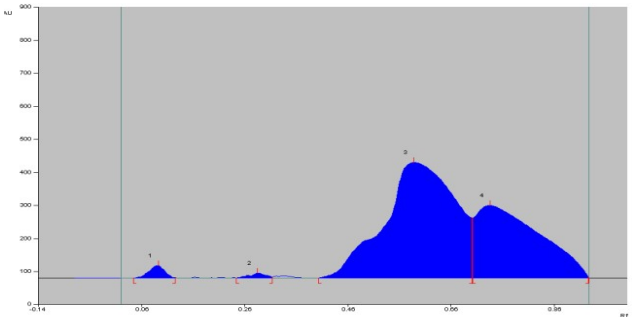

Fig.6.HPTLC Chromatogram of Chloroform

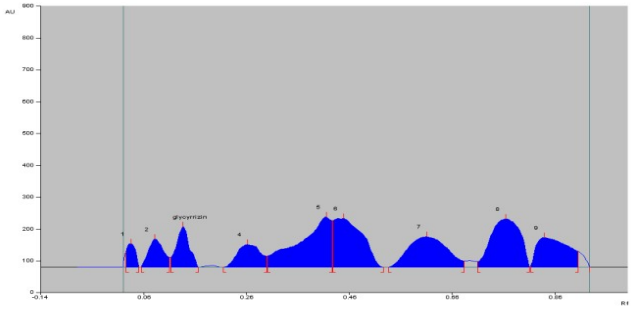

Fig.7. HPTLC Chromatogram of methanol

Sl No	EXTRACTS	AMOUNT PER 100000µg/ml EXTRACT
1	Hexane extract	1.6705µg
2	Chloroform extract	0
3	Methanolic extract	2.1667 µg

Table.3. Amount of Glycyrrhizhin

V. CONCLUSION

Herbal drugs and herbal products are used worldwide for a variety of therapeutic purposes. It is important to ensure the quality of herbal drugs and herbal products. Glycyrrhiza glabra is an herbaceous perennial plant and has been extensively studied for its different biological activities. One of the main active compound Glycyrrhizin which has hypoglycemic activity; hence particularly it has the potency to cure diabetes. From our study and with a previous literature survey we can conclude that the root of Glycyrrhiza glabra is rich in phytochemicals.

The present study deals with the extraction of the plant with different solvent and a comparative antioxidant study of these extracts. This study also confirms the presence of various chemical constituents in Glycyrrhiza glabra, the main one being the Glycyrrhizin. It is isolated from the extract. Further chromatographic fingerprinting confirms the quality, quantity, and purity of crude drugs. This ensures the availability of active chemical compounds. With support from traditional knowledge and subsequent scientific techniques, it appears to be a promising herbal drug for new drug discoveries. Nevertheless, there is more scope for the bioactivity study of

this plant. Therefore the isolation, characterization, and quantification of active constituent in extracts of Glycyrrhiza glabra by sophisticated techniques are necessary for novel drug development.

REFERENCE

[1] Visht, S., Kulkarni, G., 2012 A Comparison between different methods for extraction of glycyrrhetic acid from liquorice stolons. Int. j. Pharm. Prof.Res. 3, 622-626.

[2] Kataria R, Hemraj, Singh G, Gupth A, Jalhan S, Jindal A, Pharmacological activities on Glycyrrhiza glabra. Asian journal of pharmaceutical and clinical research 2013; Vol. 6 suppl 1: 5-7

[3] Isbrucker R A, Burdock G A. Risk and safety assessment on the consumption of Licorice root, its extract, and powder as a food ingredient with emphasis on the pharmacology and toxicology of glycyrrhizin. Regulatory Toxicology and Pharmacology; 2006, 46(3): 167–192.

[4] Yamamura Y, Kawakami J, Santa T. Pharmacokinetic profile of glycyrrhizin in healthy volunteers by a new high- performance liquid chromatographic method. J Pharm Sci. 1992; 81:1042-1046.

[5] Isbrucker R A, Burdock G A. Risk and safety assessment on the consumption of Licorice root, its extract, and powder as a food ingredient with emphasis on the pharmacology and toxicology of glycyrrhizin. Regulatory Toxicology and Pharmacology; 2006, 46(3): 167–192.

[6] Kaur R, Kaur H, Dhindas AJ, Glycyrrhiza glabra:a phytopharmacological review. IJPSR, 2013;4 (7): 2470-2477.

[7] Rossi,A,, Serraino,L., Dugo,P., Paola, R.D.,Mondello,L.G 5. V. L. Singleton and J. A. Rossi, "Colorimetry of total phenolics with phosphomolybdic and phosphotungstic acid reagents," American Journal of Enology and Viticulture, 1965:vol. 16: 144–147.

[8] V. L. Singleton and J. A. Rossi, "Colorimetry of total phenolics with phosphomolybdic and phosphotungstic acid reagents," American Journal of Enology and Viticulture, 1965:vol. 16: 144–147.

[9] Y. C. Fiamegos, C. G. Nanos, J. Vervoort, and C. D. Stalikas, "Analytical procedure for the in-vial derivatization–extraction of phenolic acids and flavonoids in methanolic and aqueous plant extracts followed by gas

[10] Chromatography with mass-selective detection," Journal of Chromatography A, vol. 1041:11–18.

[11] Saeed, N.; Khan, M. R.; Shabbir, M. Antioxidant activity, total phenolic and total flavonoid contents of the whole plant extracts Torilis leptophylla L. BMC Complement Altern Med. 2012, 12, 1-12.

[12] Ghatak A, Nair S, Vajpayee A, Chaturvedi P, Samant S, Soley K, Kudale S, et al. Evaluation of antioxidant activity, total phenol content, total flavanoid and LC-MS characterization of Saraca asoca (Roxb) De.Wilde. IJAR. (2015); 3(5):318-327.

[13] Velvizhi S, Annapurna S. Estimation of total phenolic content and free radical scavenging potential of Glycyrrhiza glabra root extract. AJPCR.(2018);11(4):213-235.

[14] M. G. L. Hertog, E. J. M. Feskens, P. C. H. Hollman, M. B. Katan, and D. Kromhout, Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study The Lancet, 1993; vol. 342: 1007–1011.

[15] M. V. Eberhardt, C. Y. Lee, and R. H. Liu, "Antioxidant activity of fresh apples," Nature, 2000:vol. 405 (.6789):.903–904.

[16] Kinoshita T, Tamura Y, and Mizutani K. The isolation and structure elucidation of minor isoflavonoids from licorice of Glycyrrhiza glabra origin. Chem Pharm Bull 2005; 53: 847– 849.

[17] Asl MN and Hosseinzadeh H. Review of pharmacological effects of Glycyrrhiza sp. and its bioactive compounds. Phytother Res 2008; 22: 709- 724.

[18] Usmanghani K. Researches on Materia Medica. Department of Pharmacognosy. Faculty of Pharmacy, University of Karachi, 1997: 29-35.

[19] Davis EA and Morris DJ. Medicinal uses of licorice through the millennia: the good and plenty of it. Molecular and Cellular Endocrinology 1991; 78: 1-6.

[20] Glycyrrhiza glabra. Monograph. Alternative Medicine Review 2005; 10: 230-237.

[21] Adel M, Alousi LA, and Salem HA. Licorice: A possible anti-inflammatory and anti-ulcer drug. AAPS Pharm Sci Tech 2005; 6: 74-82.