ISSN No:-2456-2165

Preliminary Pharmacognosy Evaluation of the *Yashtimadhu*: *Glycyrrhiza Glebra* (Linn.)

[1] Dr. Sukha ram*, [2]Gaurav Sharma, [3]Mita Kotecha, [4]Dr. Krutika Chaudhary, [5]Dr. Amit Choudhary
[1] P.G. Scholar, P.G. Department of Dravyaguna Vigyan
[2]Pharmacologist, P.G. Department of Dravyaguna Vigyan
[3]Professor and H.O.D., P.G. Department of Dravyaguna Vigyan
[4]Lecturer, P.G. Department of Dravyaguna Vigyan
[5] P.G. Scholar, P.G. Department of Dravyaguna Vigyan
NATIONAL INSTITUTE OF AYURVEDA, JAIPUR

Abstract:-

AIM:– Analysis of *Yashtimadhu* sample: *Glycyrrhizaglebra* (Linn.) collected on the market according to pharmacognostical, physicochemical and phytochemical parameters. MATERIALS AND METHODS: - Collection, identification and authentication of *Glycyrrhizaglebra* (Linn.) Moisture content, pH value, alcohol andwater-soluble extractive value, total ash,acid insoluble and water-soluble ash, carbohydrate, alkaloids, amino acids, proteins, saponin, phenolic compound, tanninsand Thin Layer Chromatography etc. was determined by pharmacognostical, physicochemical and phytochemical experiments of the *Yashtimadhu* sample. OBSERVATIONS AND RESULTS: - Pharmacognostical, physicochemical, phytochemical and T.L.C. of collected sample *Glycyrrhizaglebra* (Linn.). CONCLUSION: - Pharmacognostical, physicochemical and phytochemical studies provide information on the identification and validation of *Glycyrrhizaglebra* (Linn.). This study will help ensure the identification, validity, purity, safety and efficacy of the drug.

Keywords:- *Yashtimadhu, Glycyrrhizaglebra (Linn.), Carbohydrate, Tannin, T.L.C.*

I. INTRODUCTION

Glycyrrhizaglebra(Linn.), belonging to family Fabaceaeis a hardy herb or undershrub attaining a height up to 2 metres, leaves multifoliolate, imparipinnate, flowers in axillary spikes, papilionaceous, lavender to violet in colour, pods compressed, containing reniform seeds. *Glycyrrhizaglebra*(Linn.) is known as *Mulathi* in Hindi, *Jethimadha* in Gujarati, *Yashtimadhukam*in Malayalam, *Jeshthamadh* in Marathi and Liquorice in English etc.[1]The historical evidence of *Yashtimadhu*is traced from *Vedic* period, *Samhita* period and ancient *Nighantu* period to current modern texts.

*Yashtimadhu*is a very famous skin-benefiting *Ayurvedic* drug. The drug is used in*Chakshusya, Vrishya, Keshya, Kanthya, Varnya, Virajaniya, Ropaniya Karma.*[2]*Yashtimadhu* has*Madhura Rasa, Guru – Snigdha Guna, ShitaVirya, Madhura Vipaka*and *Vatapittashamaka Karma* etc., and attributedgastric ulcer, fever, skin diseases, ophthalmic diseases, hemorrhoids, consumption, hoarseness of voice and hiccoughetc. properties.Major chemical constituents of *Glycyrrhizaglebra*(Linn.) are Glycyrrhizine, prenylatedbiaurone, licoagrone, 7- acetoxy- 2- methyl- isoflavone, 7- methoxy- 2- methylisoflavone and 7- hydroxy- 2 methyl isoflavoneis also present in minor quantity.[3]

Verification of *Glycyrrhizaglebra* (Linn.) at the macroscopic and microscopic level is an hour requirement because various drugs are classified as adulterous and take place in market samples.

II. MATERIALS AND METHODS

Microscopic, physicochemical and phytochemical studies including quantitative analysis of *Glycyrrhizaglebra* (Linn.)Were performed to obtain diagnostic features for the diagnosis and regulation of solid and powdered drugs.

Collection of sample:

Glycyrrhizaglebra (Linn.)was purchased from market Jaipur, Rajasthan. The crude drug was identified and authenticated by CSIR - National Institute of Science Communication and Information Resources, New Delhi - 110012 (NISCAIR), vide reference number NISCAIR/RHMD/Consult/2019/3487-88-1 as *Glycyrrhizaglebra* (Linn.)and belong to family Fabaceae. After identifying the plant, the root of *Yashtimadhu* was powdered, labelled, packed and subjected for organoleptic and other analytic studies.

Moisture Content[4]: The moisture content is determined by placing a sample of 5 g of the drug in the oven for 105º 5 hours, and calculating the weight of the sample every 30 minutes, until the sample weight is constant, without any weight differences being recorded. This sample was allowed to cool at room temperature in a desiccator for 1 hour before measuring.

pH[5]: Immerse pre-calibrated electrode of pH meter in 5 % w/v solution of sample and note down the value of pH.

ISSN No:-2456-2165

Extractive values Aqueous/Alcoholic[6]: 5 g of air-dried powdered medicine was mixed with 100 ml of Distilled Water / Alcohol for specified strength in a closed flask for twenty-four hours. It was then continuously moved for 2 hours using a rotary shaker and allowed to stand for eighteen hours. Content filtered using filter paper. The filtrate is transferred to a low-calorie container and evaporates in a water bath. After that, the container was kept in the oven at 105º, to constant weight and weight. The percentage of Aqueous / alcohol excretion was calculated with the air-dried drug.

Ash value[7]:

Total Ash: - Accurately weighed 2 g of air-dried drug in a silica container and heated to a temperature not exceeding 450ºC until dissolved in carbon. After that, it's down and weighed. The percentage of ash is calculated based on the air-dried drug.

Acid Insoluble Ash: - Boil the whole ash with 25 ml of 2M hydrochloric acid for 5 minutes, collect the insoluble matter in a Gooch crucible or paper ashes, washed with hot, hot water, cooled on a desiccator and measured. Calculate the percentage of acid-insoluble ash with an air-dried drug.

Water – Soluble Ash: - Boiled the total ash for 5 minutes with 25 ml of water; collected the insoluble matter in a Gooch's Crucible or on an ash less filter paper, washed with hot water and ignite for 15 minutes at a temperature not exceeding 450ºC. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represented the water-soluble ash. Calculate the percentage of water-soluble ash regarding the air-dried drug.

Qualitative analysis of Photochemical (Primary and Secondary Metabolites):

Name of Test	Procedure	Observation	Result
Carbohydrate			
Molish's test	2 ml Test Solution + 2 ml Molisch's reagent & shake carefully + 1ml. of conc. H_2SO_4 Wait for one 1 minute.	A Purple colour ring at the junction of the two layers	Carbohydrate present
Benedict's test	4 ml Test solution + 1 ml Benedict's solution + ▲	Formation of green, yellow, orange, red or brown colour in order of increasing concentrations of simple sugar in the test solution, due to the formation of cuprous oxide.	Reducing sugars present
Fehling's test	Fehling A 1 ml + Fehling B 1 ml + 2 ml Test solution + ▲	Brick Red ppt.	Generally used for reducing sugars
Alkaloids			
Dragendorff's test	2 ml test Solution + 2 ml Dragendorff's reagent	Orange precipitate	Alkaloidspresent
Wagner's test	Test solution + few drops of Wagner's reagent	The reddish-brown precipitate	Alkaloidspresent
Hager's test	Test solution + Hager's reagent	Orange-yellow precipitate	Alkaloidspresent
Amino acids			
Ninhydrin test	Test solution + Ninhydrin + ▲	Characteristic deep blue or pale yellow colour	Presence of alpha-amino acids and proteins containing free amino groups.
Protein			
Biuret test	Test solution + 1 ml of 4% NaOH solution + 1 drop of 1% solution of $CuSo_4$.	Development of violet or pink colour	Presence of proteins.
Xanthoproteic test	Test sample + 2 ml of water + 0.5 ml of conc. HNO_3	Development of yellow colour	Presence of proteins.
Millon's test	Test solution + 2-3 ml of Millon's reagent were added.	White precipitate slowly turning to pink	Presence of proteins.
Saponin			
Foam test	Test solution + water+ shake	A stable, characteristic honeycomb-like froth	Presence of saponins.
Glycosides			
Borntragor's test	1 ml Benzene + 0.5 ml Dil. NH_4 Sol. + Test Solution	Formation of reddish pink colour.	Presence of anthrax Quinone glycosides
Phenolic compound			
Phenolic test	Test Solution + ▲ + 2 ml of $FeCl_3$ sol.	Formation of green and blue colour.	Presence of phenols
Steroids			

ISSN No:-2456-2165

Salkowaski reaction	Test Solution + 2 ml of chloroform + 2 ml of conc. H_2SO_4& shake for few minutes	Development of red colour	Presence of steroids.
Tannins			
$FeCl_3$test	Test Solution + 5 % solution of $FeCl_3$ in 90 % alcohol	The appearance of dark green or deep blue colour	Presence of tannins.
Lead acetate test	Test Solution + 10 percent w/v solution of basic lead acetate in distilled water	Development of precipitate	Presence of tannins.
Pot. Dichromate test	Test Solution + Potassium dichromate Solution	The appearance of a dark colour	Presence of tannins.

Thin Layer Chromatography (TLC): -

Chromatography plates: - T.L.C. plate coated with 0.25 mm layer of silica gel 60 F_{254}**Activation of pre-coated Silica gel 60 F_{254}: -**Plates were dried in a hot oven at 105^0 C for one and half hour.

Preparation of mobile solution: - n – Butanol : Water : Glacial acetic acid (7:2:1).

Preparation of test solution: - 4 g powdered drugs were extracted with 100 ml of ethanol (90%) in a Soxhlet's apparatus consecutively three times. The extract was filtered and concentrated in 10 ml.

Sample application: - Samples were used with the help of a capillary 1 (one) cm above the base of the T.L.C. plate. It was then immersed in a mobile solution. T.L.C. the plate is removed from the mobile solution immediately after the area has reached 1 (one) cm under the T.L.C head plate.

Visualization: - Anisaldehydesulphuric acid spray.

R_f Value: -The distance traveled and recorded for each location from where it was installed and the R_f value calculated by dividing the distance traveled by the distance traveled in front of the mobile section.

Observations and results:

The different pharmacognosy parameters were studied and evaluated to standardize the drug. The results of pharmacognosy parameters i.e. microscopic study, physicochemical parameters, phytochemical analysis and T.L.C. have been cited below.

Macroscopic study of *Glycyrrhizaglebra* (Linn.)

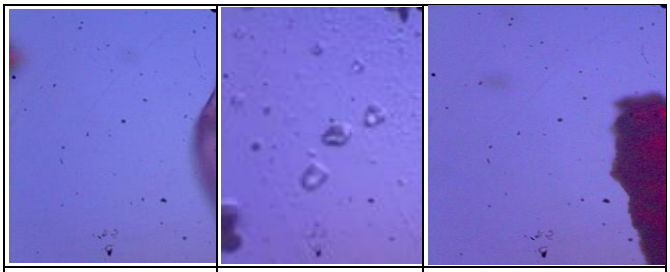
Fig. No. 1. *Glycyrrhizaglebra* (Linn.) Roots and Root Powder

Table No. 1. Macroscopic examination of *Glycyrrhizaglebra* (Linn.)

S. No.	Observed	*Glycyrrhizaglebra* (Linn.)
1.	Colour	Dark brown
2.	Odour	Faint and Characteristics
3.	Taste	Sweet

Powder microscopic study of *Glycyrrhizaglebra* (Linn.)

In powder microscopy, a structure like Trichomes, Tracheid, Fiber, Calcium oxalate crystals and Starch grains were seen.



Trichomes **Starch Grain** **Tracheid**

Fig. No. 2. Powder microscopic characteristic of*Glycyrrhizaglebra* (Linn.)

ISSN No:-2456-2165

Physicochemical analysis:

In this study, moisture content, pH, extractive value (alcohol and water-soluble extractive value) and ash values (total ash, acid insoluble ash and water-soluble ash) were determined.

Table No. 2. Physiochemical analysis of *Glycyrrhizaglebra* (Linn.)powder

S. No.	Physiochemical Standards	Results % w/w	API standard value
1.	Moisture content	7.96%	NMT 12%
2.	pH value	6.0	--
3.	Water soluble extractive value	20.45%	NLT 20%
4.	Alcohal soluble extractive value	10.02%	NLT 10%
5.	Total ash	8.43 %	NMT 10%
6.	Acid insoluble ash	1.31 %	NMT 2.5%
7.	Water-insoluble ash	6.78 %	Not mention

Phytochemical analysis: The chemical phytochemicals of plant nutrients have protective or disease-causing properties. The plant cell produces two types of metabolites- basic metabolites that are directly involved in the growth and metabolism (carbohydrates, lipids and proteins etc.) and secondary metabolites that are not involved in metabolic function (alkaloids, phenols and sterols etc.) but acts as a protective chemical. The first phytochemical study of the aqueous and alcohol extract of *Glycyrrhizaglebra* (Linn.) was performed to reveal the presence of carbohydrates, alkaloids, amino acids, saponin, glycosides, steroids and tannins.

Table No. 3. Phytochemical analysis of *Glycyrrhizaglebra* (Linn.)powder

Name of test	*Glycyrrhizaglebra* (Linn.)	
	Aq.	Al.
(+ve) = Positive and (-ve) = Negative		
Carbohydrate test		
Molish test	+ve	+ve
Benedict test	+ve	+ve
Fehling test	+ve	+ve
Alkaloids test		
Dragendorff test	-ve	+ve
Wagner's test	-ve	-ve
Hager's test	-ve	-ve
Amino acids		
Ninhydrin test	+ve	+ve
Proteins		
Biuret test	+ve	+ve
Xanthoproteic test	+ve	+ve
Millon's test	+ve	+ve
Saponin		
Foam test	+ve	-ve
Glycosides		
Borntragor's test	-ve	+ve
Phenolic compound		
Phenolic test	+ve	+ve
Steroids		
Salkowaski reaction	-ve	+ve
Tannins		
$FeCl_3$ test	+ve	+ve
Lead acetate test	-ve	-ve
Pot. Dichromate test	-ve	+ve

Table No. 4. Thin Layer Chromatography of

Sample	*Acacia catechu* (Willd.)
R_f value	0.24, 0.42, 0.52, 0.62, 0.74, 0.86, 0.96

***Glycyrrhizaglebra* (Linn.)**

Fig.No. 3.Thin Layer Chromatography of *Glycyrrhizaglebra* (Linn.)

III. DISCUSSION

Glycyrrhizaglebra (Linn.)is a sweet taste, faint and characteristics odour,dark browncolour. Powder microscopic study of powder of *Glycyrrhizaglebra* (Linn.)revealed Trichomes, Starch grain and Tracheid grainsafter observation under the microscope. Loss of drying is a water-holding property of the test substance. Moisture content and pH value were found to be 7.96% and 6.0. Extractive value is directly relative to the strength or potency of the drug which estimates in different solvents. Water-soluble extractive value and alcoholic extractive value in the sample were found at 20.45% and 10.02%. Ash value is the indicator of the presence of inorganic and earthy matter in the plant.The higher ash value is suggestive of thermo – nonlabile / heat stable or inorganic constituents. The total ash value in the sample was 8.43%. The acid-insoluble content which indicates the presence of siliceous matter and heavy metals in the sample foundat 1.31%. Water-soluble ash estimates the inorganic water-soluble salt was found 6.78%in sample.Qualitative analysis of inorganic matter showed the presence of carbohydrate, alkaloid, amino acid, protein, saponin, glycosides, phenolic compound, steroid and tannin in *Glycyrrhizaglebra* (Linn.)powder. Thin-layer chromatography establishes the phytochemical fingerprint profiling in drug for identity.

IV. CONCLUSION

Glycyrrhizaglebra (Linn.)is a well-known *Ayurveda* plant. After performing the work, it was found that the phytochemical screening confirmed the presence of various phytochemical constituents such as carbohydrates, amino acid, protein, tannin, phenolic compound, saponin, glycoside, steroid and alkaloid. Different physicochemical parameters such as loss on drying value, pH value, water and alcohol soluble extract value, total ash, acid insoluble ash, water-soluble ash, and R_f value were observed. These values can be useful to detect the purity, safety and efficacy of the drug. All studied standardization parameters like pharmacognostic, phytochemical and physicochemical analysisprovide the knowledge in the identification and authentication of *Glycyrrhizaglebra* (Linn.).

ACKNOWLEDGMENT

I acknowledge my gratitude to my mother's institute National Institute of Ayurveda, Jaipur (Laboratory of *DravyagunaVigyan*) for permitting me and the facilities provided me to carry out this study.

REFERENCES

[1]. P. C. Sharma, M. B. Yelne, T. J. Dennis etc. Datbase on Medicinal Plants used in Ayurveda and Siddha Vol.3, Central Council for Research in Ayurveda and Siddha, 2001, page no. - 561

[2]. Agnivesha, CharakaSamhita with AyurvedDipika commentary by Chakrapanidutta, YadavjiTrikamji, editor. Varanasi, ChaukhambaSurbharatiPrakashan, Sutra Sthana 25/40, 2017, page no. - 132

[3]. P. C. Sharma, M. B. Yelne, T. J. Dennis etc. Datbase on Medicinal Plants used in Ayurveda and Siddha Vol.3, Central Council for Research in Ayurveda and Siddha, 2001, page no. – 561

[4]. General Guidelines for Drug Development of Ayurvedic Formulations, CCRAS, Dept. of Ayush, govt. of India, New Delhi, Vol. 1,First Edition – 2018, page no. 79

[5]. General Guidelines for Drug Development of Ayurvedic Formulations, CCRAS, Dept. of Ayush, govt. of India, New Delhi, Vol. 1, First Edition – 2018, page no. 84

[6]. General Guidelines for Drug Development of Ayurvedic Formulations, CCRAS, Dept. of Ayush, govt. of India, New Delhi, Vol.1, First Edition – 2018, page no. 79

[7]. General Guidelines for Drug Development of Ayurvedic Formulations, CCRAS, Dept. of Ayush, govt. of India, New Delhi, Vol. 1, First Edition – 2018, page no. 78