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PHYSIOCO-CHEMICAL ANALYSIS OF *YASHTIMADHU*: GLYCYRRHIZA GLABRA

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ABSRACT

Time and demand are integral parts of any formulation. In due course of time, demand for phytochemicals of the plant extract increased, which led to cause adulteration and other mal-practices. To avoid these mal-practises, authentication and standardisation became inevitable in the process of preparing formulation. Following study is aimed at authentication and standardisation of one such essential drug - *Yashtimadhu*. Out of 5 different species, available in Mother Nature, *Glycyrrhiza glabra* is the most commonly used one. *Yashtimadhu* possesses multi-dimensional characteristics due to which, it plays vital role in curing various diseases and disease-conditions, and it can also be used in different forms, as *choorna, kwath, fanta, ghana*. With the help of available material and resources, basic, yet essential physico-chemical analyses were done in laboratory which included foreign matter, moisture content, extractive values with alcohol and water as media, total ash percentage, acid insoluble ash percentage, pH. Observed results were compared with authentic texts of Ayurvedic Pharmacopoeia of India. Results led us to the conclusion that the procured samples were authentic and standardised and can be used further in herbal or herbo-mineral formulations.

KEYWORDS: Yashtimadhu, Glycyrrhiza glabra, Physico-chemical analysis, Standardization.

INTRODUCTION

Ayurved is an ancient Indian medical science, evolved from the health needs of the society in due course of time. Initially the whole plant used to be taken into consideration for preparing

formulations. As the time advanced, our sages discovered that a particular part of plant is useful to cure a particular disease or a particular condition. In due course of time, as other medical pathies started invading human bodies, multi-dimensional research of a single part of the plant was initiated. Pharmacognosy and phytochemical components became new attraction of professionals. These phytochemicals from the part of the plant were extracted and used in the formulations to optimize dose and increase efficiency. On the other hand, adulteration and other mal-practices stepped into the industry since demand for phytochemicals was increased. This situation gave rise to separate and independent system of authentication and standardization of the drug.

Yashtimadhu (liquorice) is one such ayurvedic drug, which can be used in numerous diseases and different disease conditions in various forms like *choorna, kwath, fanta, ghana*[1] etc. There are 5 different species of liquorice in Mother Nature[2], out of which, *Glycyrrhiza glabra* is the most commonly used one. Liquorice is herbaceous perennial, growing to 1 metre in height, with pinnate leaves about 7–15 cm long, with 9–17 leaflets. The flowers are 0.8–1.2 cm long, purple to pale whitish blue, produced in a loose inflorescence. The fruit is an oblong pod, 2–3 cm long, containing several seeds.[4] The roots are stoloniferous.[5] According to classical texts of Ayurved, *rasapanchak* of *yashtimadhu* is as follows-***Rasa*** – *Madhur,* ***Veerya*** – *Sheet,* ***Vipak*** - *Madhur,* ***Guna*** – *Guru, Snigdha,* ***Karma*** – *Pittaghna, Vaataghna, Kaphavardhan, Keshya, Vedana-sthapan, Vrana-ropan, Shothaghna, Dahashamal.*[1]

Glycyrrhizin and glycyrrhizic acid are key contents of liquorice, which are found majorly in roots. These phytochemicals are governing major healing activities of *yashtimadhu*, such as immunomodulation, neuroprotective, wound healing, anti-oxidant, anti-cancerous, antimicrobial, anti-inflammatory, anticonvulsant, antidepressant, inhibitory actions in gastritis, asthma, bronchitis and the list will go on.[7] Thus, authentication and standardisation become need of hour to avoid adulteration of this drug. Current study is aimed at standardisation of *yashtimadhu choorna* collected from local markets. After procurement and authentication, the obtained samples of *yashtimadhu choorna* were proceeded for physico-chemical analysis in analytical laboratory. Comparative studies from authentic sources led to develop standards.

AIM AND OBJECTIVES

The necessity of authentication and standardization is highlighted in the introduction. Based on that, this research work is aimed at authentication and standardization of yashtimadhu choorna by performing basic physico-chemical tests.

MATERIAL

Yashtimadhu samples	Measuring cylinder	Muffle Furnace	Weighing balance	Magnetic stirrer
Magnets	Iodine flask	Petridish	Watch glass	Beakers
Hot air oven	Tissue paper	Whatman no. 41	Crucible	Tongs
Desiccator	Tripod stand	Water bath	pH Meter	Dropper
Funnel	Forceps	Magnifying glass	Spatula	Stirrer

CHEMICALS

Distilled Water	Chloroform	Ethanol	Dilute HCl	pH 4 and pH7 buffer Solution

METHOD

1. Collection of samples

Five different samples of *upayuktang* of *Yashtimadhu* i. e. liquorice rhizomes were collected from local venders in the vicinity.

2. Authentication

Collected samples were handed unaltered to *Dravyaguna* department of the institute for authentication.

3. Standardization[3]

After authentication from *Dravyaguna* department standardization was done in the research laboratory of the institute. Macroscopic analysis, microscopic analysis, organoleptic examination and physico-chemical analysis were done.

3.1 Macroscopic Analysis

Stolon consists of yellowish brown or dark brown outer layer, externally longitudinally wrinkled, with occasional small buds and encircling scale leaves, smoothed transversely, cut surface shows a cambium ring about one-third of radius from outer surface and a small central pith, root similar without a pith, fracture, coarsely fibrous in bark and splintery in wood, odour, faint and characteristic, taste, sweetish.

3.2 Microscopic Analysis

Stolon- transverse section of stolon shows cork of 10-20 or more layers of tabular cells, outer layers with reddish-brown amorphous contents, inner 3 or 4 rows having thicker, colourless walls, secondary cortex usually of 1-3 layers of radially arranged parenchymatous cells containing isolated prisms of calcium oxalate, secondary phloem a broad band, cells of inner part cellulosic and outer lignified, radially arranged groups of about 10-50 fibres, surrounded by a sheath of parenchyma cells, each usually containing a prism of calcium oxalate about 10-35 μ long, cambium form tissue of 3 or more layers of cells, secondary xylem distinctly radiate with medullary rays, 3-5 cells wide, vessels 168 about 80-200 μ in diameter with thick, yellow, pitted, reticulately thickened walls, groups of lignified fibres with crystal sheaths similar to those of phloem, xylem parenchyma of two kinds, those between the vessels having thick pitted walls without inter-cellular spaces, the remaining with thin walls, pith of parenchymatous cells in longitudinal rows, with inter-cellular spaces. Root-transverse section of root shows structure closely resembling that of stolon except that no medulla is present, xylem tetrarch, usually four principal medullary rays at right angles to each other, in peeled drug cork shows phelloderm and sometimes without secondary phloem all parenchymatous tissues containing abundant, simple, oval or rounded starch grains, 2-20 μ in length.

Fig. 1: Liquorice Rhizome.

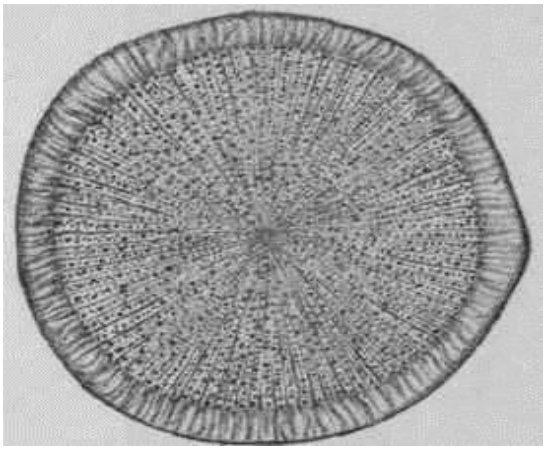

Fig. 2: T. S. of Liquorice root.

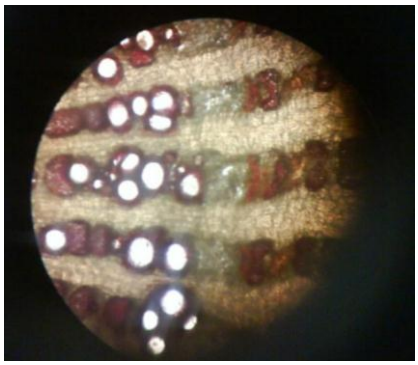

Fig 3: Microscopic view of T. S. of Liquorice root[8]

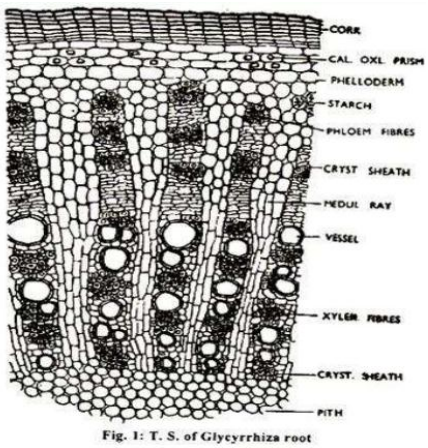

Fig. 1: T. S. of Glycyrrhiza root

Fig 4: Diagrammatic representation of T. S. of Liquorice.

3.3 Foreign Matter

100 grams of each sample were taken in a tray with plane white surface. With naked eyes and magnifying glass physical impurities like stones, sand and soil particles were separated and measured.

3.4 Organoleptic Examination

3.4.1 Taste - Sweet

3.4.2 Odour - Sweet

3.4.3 Colour - Wood brown, ochre

3.4.4 Appearance – Wooden sticks of 5-8cm length with serrated, darker outer bark and lighter inner portion.

3.5 Loss on drying

5 grams of fine powder of sample was measured in a pre-conditioned petridish. It was then kept in hot air oven for 5 hrs. After removing and desiccating, it was measured. The procedure was repeated until constant reading was obtained.

3.6 Alcohol Soluble Extractives

5 grams of fine powder of sample was measured and poured in iodine flask containing magnet and 100 ml ethanol was added after accurate measuring. Cork was fixed. 6 hrs. intermittent stirring was done in magnetic stirrer and then it was kept stand still for next 18 hrs. After 24 hrs. the mixture was filtered with filter paper and 25 ml of it was poured in pre-conditioned petridish. This petridish was then subjected for evaporation over water bath at

70-80°C, until all the liquid got evaporated. After cooling petridish in desiccator, the residue was measured and calculations were done.

3.7 Aqueous Soluble Extractives

5 grams of fine powder of sample was measured and poured in iodine flask containing magnet and 100 ml chloroform water was added after accurate measuring. Cork was fixed. 6 hrs. intermittent stirring was done in magnetic stirrer and then it was kept stand still for next 18 hrs. After 24 hrs. the mixture was filtered with filter paper and 25ml of it was poured in pre-conditioned petridish. This petridish was then subjected for evaporation over water bath at 100°C, until all the liquid got evaporated. After cooling petridish in desiccator, the residue was measured and calculations were done.

3.8 Total Ash Percentage

2 grams of fine powder of sample was taken in pre-conditioned silica crucible. This silica crucible was then kept in Muffle furnace for 5hrs. After desiccating crucible, it was measured in weighing balance. The procedure was repeated until carbon-free ash is obtained. After obtaining carbon-free ash calculations were done.

3.9 Acid Insoluble Ash Percentage

This ash is weighed and subjected to just boiling 25ml of dilute HCl and allowed to pass through ash-free filter paper i. e. Whatman filter paper no. 41. The ash-free filter paper along with its contents is kept in Muffle furnace for 5hrs. After desiccating crucible, it was measured in weighing balance. The procedure was repeated until carbon-free ash is obtained. After obtaining carbon-free ash calculations were done.

3.10 pH

1% solution of the sample was prepared by adding 50ml of distilled water to 500mg of powder. This solution was kept stand still for 4 hrs. covered by watchglass. After calibrating pH meter by 4pH and 7pH solutions, desired sample solution was kept in contact with the electrode and reading was noted.

OBSERVATIONS AND RESULTS

Table 1: Comparative analytical values from 5 different samples of *Yashtimadhu*.

	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5
LOD%	9.45	9.26	8.85	9.45	4.45
ASE%	15.92	18.89	20.03	15.92	10.62
WSE%	27.44	31.74	23.02	27.44	24.64
TA%	6.38	4.75	8.39	6.38	7.28
AIA%	0.24	0.1	1.81	0.24	0.45
pH	5.15	5.76	5.18	5.15	5.36
Foreign Matter	Nil	Nil	Nil	Nil	Nil

Table 2: Mean with standard deviation of 5 different samples of *Yashtimadhu*.

Test Performed	Mean Value	Standard Deviation
LOD%	8.292	1.933
ASE%	16.276	3.261
WSE%	26.856	2.972
TA%	6.636	1.198
AIA%	0.568	0.631
pH	5.32	0.233
Foreign Matter	Nil	Nil

Table 3: Standard Values from Ayurvedic Pharmacopoeia of India.

Parameters	Prescribed Standard Values
LOD%	Not more than 10%
ASE%	Not less than 10%
WSE%	Not less than 20%
TA%	Not more than 10%
AIA%	Not more than 2.5%

DISCUSSION

All the 5 samples of *Yashtimadhu* were procured in different seasons, in order to understand the seasonal effect on the plant. Some very basic yet essential physico-chemical tests were performed in order to standardize the drug.

Liquorice rhizome is evidently hygroscopic.[6] Thus, determination of moisture content was one of the important tests. After calculating values for loss on drying, sample-5 showed the least value which indicated that it had least moisture content. The mean was 8.292 with SD +/- 1.933 making range to be approximately 6 to 10. API standards state that this value should not exceed 10%. Hence, all the collected samples were within the standards for moisture content parameter.

Useful part of liquorice plant is rhizome, since major phytochemicals glycyrrhizin and glycyrrhizic acid are present in rhizome with high concentration. These phytochemicals are governing major healing activities of *yashtimadhu*, such as immunomodulation, neuroprotective, wound healing, anti-oxidant, anti-cancerous, antimicrobial, anti-inflammatory, anticonvulsant, antidepressant, inhibitory actions in gastritis, asthma, bronchitis[7] and the list will go on. Thus, percentage solubility of these phytochemicals is one of the essential characteristics. Out of various media, alcohol and water were selected to calculate the extractive values. Average alcohol soluble extractive value came out as 16.27 with SD +/- 3.26 making approximate range to be 13-19. Though sample-3 and sample-5 were not falling within the range, they satisfied the API standards which state that alcohol soluble extract should always be more than 10%. Whereas, water soluble extractive having mean 26.85 with SD +/-2.97, only sample-2 was out of range. But, because API standards state that water soluble extractive value should not be less than 20%, sample-2 was not rejected. Therefore all 5 samples were standardized with respect to alcohol and water solubility.

Carbon contents, purity and quality parameters are determined by total ash percentage. API standards state that, this percentage should not exceed 10%. If total ash percentage is exceeding 10% then, it indicates adulteration and low quality of sample. In this case, mean value of total ash percentage was 6.63 with SD +/- 1.19 and all 5 sample values being less than 10% ensured adequate purity and quality of samples.

Siliceous impurities were calculated by further processing the ash with dilute HCl. The test is named as acid insoluble ash percentage. This test helps in determination of impurities other than carbon contents and components not soluble in acid. Resultant mean after performing test came out as 0.568 with SD +/-0.631. According to the standards mentioned in API, this value of acid insoluble ash should not be more than 2.5%. Since all the obtained values came under standard, all 5 samples are considered as standard.

Determination of acidity or basicity was performed with the help of pH meter. Average pH value was 5.32 with SD +/- 0.23. This proved moderate acidic nature of rhizome. This got cross-verified, as *Glycyrrhiza glabra* contained glycyrrhizic acid in its rhizome.[7]

To rule out any possibility of physical impurities and adulteration, visible to naked eyes, foreign matter was checked with the help of magnifying glass. There was no significant amount of foreign matter resulting into samples being standardized and authentic.

CONCLUSION

1. Efficacy and effectiveness of any drug is negatively hampered due to adulteration. Thus, authentication and standardization of any drug prior to its use in any formulation is essential.
2. Authentication and standardization of any Ayurvedic drug is carried out based on standards prescribed by API. These standards are nothing but, threshold values of physico-chemical tests as per their respective significance for any specific drug.
3. In this case, total 7 physico-chemical tests were conducted on 5 different samples of *yashtimadhu choorna*, collected from local markets. After the statistical analysis on the observed test results, all the samples were found to be as per API standards. Hence, all the 5 samples were authentic and standardized.
4. Now, since all these samples are upto standards, they can be used for further research work that involves *yashtimadhu choorna* and can directly be used for herbal and herbo-mineral formulation.
5. Similar research work should be performed with respect to every single drug that is used in all classical and proprietary herbal and herbo-mineral formulations. This will promote research-based formulations and certainly enhance reliability of Ayurvedic drugs.

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