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A DETAILED PHYSICOCHEMICAL AND PHYTOCHEMICAL STUDY OF PANCHATIKTA KSHEERA GHRITA (AN AYURVEDIC MEDICINAL PREPARATION)

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ABSTRACT

Ageing is a process of physical, psychological and social change in multi-dimensional aspects. Osteoporosis is one such age-related (generally), degenerative bone disorder with changes in biological material and consequent bone structural deterioration. Bone fragility and fracture are the worst outcomes; therefore, effective medical treatment and therapy are of the utmost need. In Ayurveda, it is mentioned that in *vardhakya avastha*, all *dhatus* undergo *kshaya* leading to *Dhatukshayajanya Vata prakopa Samprapti* which can be understood there is the involvement of *vatavyadhi* with ageing. In *Asthi vaha Srotodusti chikitsa*, *Panchatikta dravya siddha ksheera basti* and *Sarpi* are mentioned. *Panchatikta ksheera ghrita* is an Ayurvedic preparation consisting of *Nimba* (Azadirachta indica), *Patola* (Pteridosperm suaveolens), *Vasa* (Adhatoda vasica), *Guduchi* (Tinospora cordifolia), *Kantakari* (Solanum Xanthocarpum), *ghrita* (cow-ghee) and *go-Dugdha* (cow-milk). *Panchatikta dravyas* and *sneha dravyas* (ghee and milk) act as *rasayanas* for *Asthi dhatus* and *samana of vata*. This study highlights the results of standardization (identity, purity & strength) tests, preliminary phytochemical screening and TLC findings. All these said tests are conducted in State Drug Testing Laboratory, AYUSH, Guwahati, Assam. These studies are important in way of establishing the quality-control, efficacy & acceptability of herbal drugs.

Keywords: osteoporosis, *Vatavyadhi, Asthi Vaha Srotodusti, Panchatikta Dravya, Go-Ghrita, Ksheera*

INTRODUCTION

Osteoporosis is a condition of degenerative changes in bones that represent increased porosity of bones leading to resultant diminution of bone mass and increased bone fragility and susceptibility to fractures. In Ayurveda, it is mentioned that in vardhakya avastha, all dhatus undergo kshaya leading to Dhatukshayajanya Vata Prakop Samprapti which can be understood that there is the involvement of vata dosha with ageing.[1] Osteoporosis can be taken into consideration as kshaya of Asthi. Vata dosha residing in Asthi gets vitiated giving rise to symptoms like bone and joint pain. Around the world 1in 3 women and 1 in 5 men over the age of 45years suffer an osteoporotic fracture and osteoporotic changes much before that. Many research studies have been carried out in other medicinal systems and sciences to date to find out effective treatment for osteoporosis but there are many restrictions in finding the solution even after inventing a treatment protocol. In Ayurveda, in Asthi vaha Srotodusti chikitsa, Panchatikta dravya siddha ksheera basti and Sarpi are mentioned. Panchatikta ksheera ghrita is an Ayurvedic preparation consisting of Nimba (Azadiracta indica), Patola (Pteridosperm suaveolens), Vasa (Adhatoda vasica), *Guduchi* (Tinospora cordifolia), *Kantakari* (Solanum Xanthocarpum), *Ghrita* (cow-ghee) and *Go-Dugdha* (cow-milk). Panchatikta dravyas and sneha dravyas (ghee and milk) act as rasayanas for Asthi dhatus and samana of vata.[1] Panchatikta ksheera ghrita was subjected to pharmaceutical evaluation (evaluation of different Physicochemical and Phytochemical parameters) to prepare a profile of the formulation. The prepared Panchatikta ksheera ghrita used for evaluation in the study consists of Nimba (Azadiracta indica), Patola (Pteridosperm suaveolens), Vasa (Adhatoda vasica), Guduchi (Tinospora cordifolia), Kantakari (Solanum Xanthocarpum), ghrita (cow-ghee) and go-Dugdha (cow-milk), triphala (which was used as Prakshepa dravya

AIM AND OBJECTIVE:

1. To evaluate the quality of the drug by using different analytical techniques.
2. To prepare a profile of the drug.

The methodology used for Physico-chemical analysis of Pancha tikta Kshira ghrita was done by using various standard Physico-chemical parameters such as Refractive Index value, Saponification value, and Specific gravity at Drug Testing Laboratory, AYUSH. Physico-chemical analyses were carried out by following the standard procedure mentioned in API (Ayurvedic Pharmacopeia of India).

For physicochemical analysis, the following parameters are evaluated. Saponification Value, Specific Gravity, Refractive Index, For phytochemical analysis, the following parameters are evaluated, Reducing Sugar, Alkaloids, Glycosides Phytosterols And Triterpenoids, Phenolic Compounds And Tannins, For detection of phytoconstituents in the drug, Thin Layer Chromatography

DETERMINATION OF SAPONIFICATION VALUE

The saponification value is the number of mg of potassium hydroxide required to neutralize the fatty acids, resulting from the complete hydrolysis of 1 g of the oil or fat, when determined by the following method: Dissolve 35 to 40 g of potassium hydroxide in 20 ml water, and add sufficient alcohol to make 1,000 ml. Allow it to stand overnight, and pour off the clear liquor. Weigh accurately about 2 g of the substance in a tared 250 ml flask, add 25 ml of the alcoholic solution of potassium hydroxide, attach a reflux condenser and boil on a water-bath for one hour, frequently rotating the contents of the flask cool and adding 1 ml of a solution of phenolphthalein and titrate the excess of alkali with 0.5 N hydrochloric acid. Note the number of ml required (a). Repeat the experiment with the same quantities of the same reagents in the manner of omitting the substance. Note

the number of ml required (b) Calculate the saponification value from the following formula: —

Saponification Value = (b–a) × 0.02805 × 1.000/ W

Where 'W' is the weight in g of the substance taken

Specific gravity: The specific gravity of a liquid is the weight of a given volume of the liquid at 250 (unless otherwise specified) compared with the weight of an equal volume of water at the same temperature, all weighing being taken in air. Method obtained as described under API. Obtain the specific gravity of the liquid by dividing the weight of the liquid contained in the pycnometer by the weight of water contained, both determined at 250 unless otherwise directed in the individual monograph.

REFRACTIVE INDEX: The refractive index (η) of a substance concerning air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance. It varies with the wavelength of the light used in its measurement. Unless otherwise prescribed, the refractive index is measured at 250 (±0.5) concerning the wavelength of the D line of sodium (λ 589.3 nm). The temperature should be carefully adjusted and maintained since the refractive index varies significantly with temperature. The Abbe's refractometer is convenient for most measurements of the refractive index but another refractometer of equal or greater accuracy may be used. Commercial refractometers are normally constructed for use with white light but are calibrated to give the refractive index in terms of the D line of sodium light. To achieve accuracy, the apparatus should be calibrated against distilled water which has a refractive index of 1.3325 at 250 or against the reference liquids. This parameter is used to evaluate the QC parameters of oil formulation.

DETERMINATION OF PHYTOCHEMICAL ANALYSIS

Reducing sugar: Took 2 ml of syrup sample to make it to 100ml with distilled water. Took 50ml from it and add 2ml conc. HCL, boil and neutralize with sodium carbonate and make 250ml with distilled water (Solution A) Tool 5ml of falling solution A and B each in titration flask and titrate with solution A using methylene blue as an indicator, boil while titrating the endpoint blue to brick red.

$$\% \text{ Of sugar } \% = \frac{\text{factor of fehling solution x 100 x 250}}{\text{t}-\text{reading x xwt.Of sample x 50}}$$

Detection of Alkaloids: About 50 mg of solvent-free extract was stirred with little quantity of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloid tests viz., Mayer's Test, Wagner's Test, Hager's Test, Dragendroff's Test

Detection of Glycosides: For detection of glycosides, about 50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hrs on a water bath, filtered and the hydrolysate was subjected to the Glycoside test viz., Bontrager's Test, Legal's Test.

Detection of Phytosterols and triterpenoids: Tested by Liebermann - Burchard's and Salkowski test

Detection of Phenolic Compounds and Tannins: Tested by Ferric chloride test, Gelatin test, Lead acetate test, Alkaline reagents, and Shinoda test or Magnesium - Hydrochloric acid reduction

FOR DETECTION OF PHYTOCONSTITUENTS IN THE DRUG

Thin Layer Chromatography

Thin Layer Chromatography of sample was done by using standard procedures and is mainly used for the detection of the nature of phytoconstituents present.

TLC is an important analytical tool in the separation, identification and estimation of different classes of natural products. In this technique, the different components are separated by the differential migration of solute between two phases - a stationary phase and a mobile phase. Here, the principle of separation is adsorption and the stationary phase acts as an adsorbent. Depending on the particular type of stationary phase, its preparation and use with different solvents, separation can be achieved based on a partition or a combination of partition and adsorption.

OBSERVATIONS AND RESULTS

Physicochemical Screening	
Refractive Index	1.121
Saponification Value	79
Specific Gravity	0.93

Phytochemical Screening	
Test For Reducing Sugar	+Ve
Test For Alkaloids	+Ve
Test For Glycosides	+Ve
Test For Foam	+Ve
Test For Tannis	+Ve
Test For Tarpenoids	+Ve

PHARMACOGNOSTIC EVALUATION		
Organoleptic evaluation	Color	Green
	Odor	Ghee like
Thin-layer chromatography	TLC of hexane extract was carried out with Tolene: Ethyl acetate as solvent. Three spots were identified under UV 365 nm 7 R_f values were determined.	

Name of sample : Pancha Tikta Kshir Ghrita

SL. NO.	NAME OF TEST	OBSERVATION	
1	Physical Evaluation	Touch	Viscous
		Types of sample	Liquid
	PHARMACOGNOSTIC EVALUATION		
2	Organoleptic Evaluation	Colour	Green
		Odor	Ghee like
3	Thin Layer Chromatography	TLC of hexane extract was carried-out with Tolene:Ethyl acetate as solvent.Three spots were identified under UV 365 nm & R_f values were determined.	
	PHYTOCHEMICAL SCREENING		
4	Phyto-Chemical Screening	Test for reducing sugar	+ve
		Test for Alkaloids	+ve
		Test for Glycosides	+ve
		Test for foam	+ve
		Test for Tannins	+ve
		Test for Tarpenoid	+ve
	PHYSICO-CHEMICAL EVALUATION		
5		Refractive index	1.121
		Specific gravity	0.93
		Saponification value	79
		Rancidity test	Fat is not oxidised

DISCUSSION

The drug constituents were collected from a local market, the herbal garden of Govt. Ayurvedic College, Guwahati. The plants were then identified by the Dravyaguna Department of our college which were then subjected to production in our State Ayurvedic Pharmacy. About 2kgs of the drug were produced. From there, a sample of 200ml was taken to Drug Testing Laboratory, AYUSH. The Pharmacognostical evaluation showed that the sample was green in colour, ghee like odour. In phytochemical screening, tests for reducing sugar, alkaloids, glycosides, foam, tannins, terpenoids were all positive. In physicochemical evaluation, the refractive index was 1.121, specific gravity of 0.93, saponification value was 79 and during rancidity test, fat is not oxidised. Thus, the values of the resultant sample were within normal limits with no foreign residue.

CONCLUSION

The fundamental concepts of Ayurveda are very complicated, and a complete understanding of this science is difficult. Thus, extensive and continuous research work is necessary to establish its understanding of the basic concepts. A systematic study of the crude drug is essential in the present era for quali-

ty control and analysis of pharmaceuticals derived from them. From this study, we have been able to gather important information regarding Panchatikta ksheera ghrita which has ascertained its purity as a drug and simultaneously establishes its basic chemical profile. The authors hope that the information provided by the present study can be useful for further studies on Panchatikta ksheera ghrita.

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