PHARMACEUTICOANALYTICAL STUDY AND STANDARDIZATION OF PANCHTIKTA GHRIKA

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ABSTRACT

In Ayurveda natural substances like herbs, minerals, fats, metals, animal and marine products are used for the management of various ailments. These natural products can be used directly or after specified procedure. Due to change in habitats and effect of environmental factors there may be change in chemical constitution of these products. For drug efficacy and its reproducibility of therapeutic results, it is essential to standardize the products. Skin diseases account for 10 - 20 % of all consultations in general practice. Most common skin complaints are Dermatitis, Eczema, Acne, Urticaria, Psoriasis, skin allergy etc. There are many therapeutic forms of drugs described in classical texts, like vati / gutika (tablets), churna (powder), asava and arista (self-generated alcohol based elixir), snehakalpana (processed medicated oil / skimmed milk fat (ghrita), etc. for skin disorders. Medicated ghrítakalpana (GK) are mainly used to alleviate and manage vitiated Vatadosha and Pittadosha. Panch Tikta Ghrita (PTG) is most commonly used GK preparation as Ayurvedic medicine for treatment of skin disorders, as herbs (panchiktika) are best among raktashodhaka (blood purifier) and ghrita is good for skin as well as to pacify vitiated pitta and Vatadosha. Three batches of PTG was prepared with Vasa (*Adhatoda vasica*), Nimba (*Azadirachta indica*), Guduchi (*Tinospora cordifolia*), Kantakari (*Solanum surattense*), Patola (*Tricosanthes dioica*) and ghrita (cow's milk fat) in accordance with Sharangdhar Samhita Madhyamkhanda 9/89-90. Analytical study for standardization was carried out on basis of classically illustrated organoleptic tests and modern parameters of physico-chemical properties like Loss on drying at 110°C, Refractive Index at 40°C, Acid value, Saponification value, Iodine value, Ester value, Peroxide value, Weight per ml, Specific gravity (melted), Melting Point (in °C) and Qualitative Tests was carried out through estimation of Carbohydrate, Protein, Alkaloid, Cardiac glycoside, Flavonoid, Anthraquinone glycoside, Tannin, Steroid, Triterpenoid and Thin Layer Chromatography was done. All test showed a significance changes after processing from raw ghrita. It shows that ghrita incorporates many properties of panchiktika herbs and in this way a range of parameter is established for re-production and therapeutically effectiveness of PTG in standard manner.

Keywords: Ayurveda, Panch Tikta Ghrita, skin diseases, standardization.

INTRODUCTION

Ayurveda is the oldest medical science, based on use of processed natural products like herbs, minerals and metal in therapeutic form. But for last few centuries, the practice of this medical science becomes reduced due to several factors and the prevalence of allopathic treatment has flourished in various countries due to numerous favorable aspects like quick symptomatic relief, palatability, easy availability and many more. However, multitudes of adverse effect of drugs are taken under consideration. Present scenario is changing; The Ayurvedic system of medicine is on the way to regain its past glory because of its preventive as well as curative nature, nil side effects and holistic approach with legal application of Good Agriculture and collection process (GACP), Good manufacturing process (GMP) and Good Laboratory Practice (GLP) in Ayurvedic pharmacy. These submissions give standardization of Ayurvedic medicines which results in validation of evidence based clinical effect as described in ancient Ayurvedic Texts. There are many therapeutic forms of drugs described in classical texts, like vati / gutika (tablets), Churna (powder), asava and arista (self-generated alcohol based elixir), snehakalpana (processed medicated oil / skimmed milk fat (ghrita), etc. for various ailments. Vata, Pitta and Kapha are the tripods for life. If they are in equilibrium then body will be healthy whereas if they are imbalance then body gets ill. Vatadosha is responsible for 80 types of ailment whereas Pittais responsible for 40 types of diseases¹. Medicated ghrítakalpana (GK) are mainly used to

alleviated and manage vitiated Vatadosha and Pittadosha. Ghrita is obtained from the classic Mammalia of the animal kingdom, especially cow. Ayurveda discriminates their particular features also and recommends the cow's skimmed milk fat as best and the best choice for both food and medicinal purpose². Bhavaprakasa has mentioned that it is a rasayana, good for health, stimulant for digestion, supports glow beauty, enhances memory and stamina, promotes longevity and protects body from various diseases³. GK is prepared by boiling ghrita with decoction of herbs integrated with paste of herbs. Acarya Caraka has said about ausadha gunas i.e. bahuta, yogyata, anekavidha kalpana and sampat. But Bhaishjyarnavali has used triphala kalka and mentioned specially ghrita murcana⁴; it was a new concept for addition of new properties along with coloration and purification of ghrita. Drug given in the form of ghee will not only be digested and absorbed fast, but will also be able to reach some of the most distant areas of body like the CNS. A water soluble drug is usually distributed in the extracellular spaces and it may not readily diffuse in to cerebrospinal fluid and other body cavities while the lipid soluble drugs are rapidly distributed throughout the intra and extracellular spaces. In 13th century Sharangadhara and Bhaishjya Ratnavali (B.R.)⁵ have mentioned Panchiktika ghrita (PTG). PTG is most commonly used GK preparation as Ayurvedic medicine for treatment of skin disorders. As herbs (panchiktika) are best among rakta shodhaka and ghrita is good for skin as well as to pacify vitiated pitta and Vatadosha, therefore to standardize

PTG present study was focused on pharmaceutical and analytical study of PTG.

MATERIAL AND METHODS

Present work is "Pharmaceutico-Analytical study and Standardization of PTG" was divided into 2 parts:

1. Pharmaceutical Study
2. Analytical study

Pharmaceutical Study

Preparation of PTG

PTG has been prepared according to Sneha kalpanaa⁶ procedure as per Sharangadhara Samhita Madhyamakhandha.

Vrushanimbamrutavyaghripatolanamsrutena cha.
Kalkenapakwasarpistunihinyadivisamajwaran.
Pandumkusthamvisarpa cha kruminarshasi nashayet.⁷
ar.sam.Mad.9 / 89-90

Kalka (Paste of Herbal drugs), panchtikta 1.850 Kg [Vasa (*Adhatoda vasica*), Nimba (*Azadirachta indica*), Guduchi (*Tinospora cordifolia*), Kankari (*Solanum surattense*), Patola (*Tricosanthes dioica*) each 370 g], Go-Ghrita (cow's milk fat) 11 Kg was purchased [from the local market of Indore and has been authenticated by Quality Control Laboratory] and 44 Liters decoction of panchtikta was taken. The ghrit with pest of Panchtikta was then added in this decoction and boiled over medium flame till complete evaporation of water content. After cooling PTG was filtered with double layer fine cotton cloth and packed in air tight jar. The process was repeated three times and three batches of samples were prepared. Each time the loss in the end product and the duration of process were observed.

Analytical study

Analytical parameters

Organoleptic characters

The organoleptic characters involved the testing of drugs using sensory organs. These are four subjective parameters – (i) color (ii) odour (iii) touch (iv) taste (v) Sound. The color was observed with naked eye in natural light, odor was smelt. The feeling of drugs was judged by touch and taste was tested with tongue.

Physico-chemical parameters

Loss on Drying at 110⁰ C

5 g of material was taken and heated at 105⁰ C in hot air oven. It was taken out and weighed again and again at regular interval till the consistent weight was achieved. The percentage of difference before and after subjecting the sample to heat was considered as loss on drying at that particular temperature⁸.

Refractive index

The Abbe's refractometer was used for the measurements of refractive index. To achieve accuracy, the apparatus was calibrated against distilled water at 40°C after cleaning the instrument with organic solvent followed by deionized water. The material was placed in between the prisms using pipette. After each sample, the refractometer was cleaned with petroleum ether followed by distilled water. The Refractive index was analyzed for all samples⁹.

Determination of Acid Value

10 g of the substance was weighed into a 250 ml flask and 50 ml of a mixture of equal volumes of alcohol and solvent ether was added to it, which was neutralized after the addition of 1 ml of solution of phenolphthalein. Gentle heating was done on a water-bath, until the substance got completely melted, and then it was titrated against 0.1 N potassium hydroxide shaking constantly until a pink color which persisted for fifteen seconds was obtained. Number of ml required was noted¹⁰. It was calculated as:

$$\text{Acid Value} = a \times 0.00561 \times 1000/W$$

Where, a = is the number of ml. of 0.1 N potassium hydroxide required and
W = is the weight in gm of the sample taken

Saponification value

First alcoholic potassium hydroxide solution was prepared. 1.2 liter alcohol was refluxed for 30 minutes with 10 g KOH and 6 granulated Aluminium or Al foil. Then, it was distilled and 1 liter was collected after discarding first 50 ml. Now 40 g of potassium hydroxide was dissolved in this 1 liter alcohol keeping temperature below 15°C. After this, it was allowed to stand overnight, and then the clear liquid was decanted and was kept in a bottle closed tightly with a cork. Now the sample was melted and was filtered through a filter paper to remove any impurities and the last traces of moisture and to make sure that the sample was completely dried. 2 g of dried sample was taken in to a 250 ml Erlenmeyer flask. 25 ml of the alcoholic potassium hydroxide solution was added into the flask. Similarly blank was also taken without introducing the material. Thereafter, the sample flasks and the blank flask were attached with condensers and were kept on the water bath. The boil was gentle and steady to complete the saponification (indicated by absence of any oily matter and appearance of clear solution). The time consumed was one hour of boiling. Now, the flasks and condenser were cooled and inside of the condenser was washed with about 10 ml of hot ethyl alcohol neutral to phenolphthalein. Now it was titrated against the excess potassium hydroxide with 0.5N hydrochloric acid, using about 1.0 ml phenolphthalein indicator¹¹. The saponification value was calculated as:

$$\text{Saponification Value} = 56.1 (B-S) N/W$$

B = Volume in ml of standard hydrochloric acid required for the blank
S = Volume in ml of standard hydrochloric acid required for the sample
N = Normality of the standard hydrochloric acid and
W = Weight in gm of the oil/fat taken for the test

Determination of Ester value

Ester value is difference of Saponification value and Iodine value.

Determination of Iodine Value

For determination of iodine value, Wij's method was used. First 10 ml of iodine monochloride was mixed in 1800 ml of glacial acetic acid and then it was shaken vigorously. 2 g of sample was taken in 500 ml Erlenmeyer flask. It was added with 25 ml of carbon tetrachloride. The content was mixed well. Now, 25 ml of Wij's solution was pipette in this and the glass stopper was replaced after wetting with potassium iodine solution. It was swirled for proper mixing and the flask was kept in dark for one hour. The same process was repeated for blank also. Then 15 ml of potassium iodide solution was added followed by 100 ml of recently boiled and cooled water. The rinsing was done for stopper too. Then

titration was done for liberated iodine with standardized sodium thiosulphate solution, using starch as indicator till the formed blue color disappeared after thorough shaking¹². The value was calculated as:

$$\text{Iodine value} = 12.69 (B - S) N / W$$

B = volume in ml of standard sodium thiosulphate solution required for the blank.

S = volume in ml of standard sodium thiosulphate solution required for the sample.

N = normality of the standard sodium thiosulphate solution.

W = weight in g of the sample.

Specific gravity and Weight per milliliter

First prior dried specific gravity bottles kept in hot air oven were taken in dessicator and weight of empty specific gravity (of capacity of 25 ml) bottles were taken. Now they were filled with distilled water boiled and cooled at 25° C. This weight of 25 ml of water gives the idea of weight of 1 ml of water. Now similarly, the weight of materials was taken in same bottles (25 ml capacity) after proper cleaning and drying in hot air oven. The weight per ml was determined for each sample after getting the weight of 25 ml of material. Then with reference to weight of water, specific gravity was determined¹⁴.

Specific gravity: weight of material/weight of water

Qualitative tests: Tests for different active principles

All samples of Panchtikta and murchhita ghrita were done with qualitative tests to reveal the presence or absence of various phytoconstituents as-

Carbohydrates

Materials were dissolved individually in 5 ml of distilled water and filtered; the filtrates were used to test the presence of carbohydrates.

Benedict's test: Filtrate were treated with Benedict reagent and heated on water bath, formation of an orange red precipitate, indicates the presence of reducing sugar.

Detection of Protein and Amino acids (Millon's Test)

The hydro-alcoholic extracts were treated with 2 ml of millon's reagent. The Formation of white precipitate which turns to red upon heating indicates the presence of proteins.

Detection of Alkaloids

Materials were extracted in hydro-alcoholic solution and those extracts were dissolved individually in dilute HCL and filtered. The filtrates were tested carefully with alkaloid reagent.

Dragendroff's test

Filtrates were treated with Dragendroff's reagent (solution of potassium bismuth iodide) formation of red precipitate indicates the presence of alkaloids.

Keller Killiani Test

Glacial acetic acid followed by 5 % FeCl₃ was added to drug with water. Then it was added with concentrated H₂SO₄. Reddish-brown color at the junction of two liquid and bluish-green at top of layer indicates presence of cardiac glycoside.

Test for Flavonoids (Alkaline reagent test)

The hydro-alcoholic extracts were treated with few drops of sodium hydroxide separately; formation of intense yellow color which becomes colorless on addition of few drops of dilute hydrochloric acid indicates the presence of flavonoids.

Test for Anthraquinine glycosides (Borntrager's test)

Hydrolysate is treated with chloroform and the chloroform layer is separated, to this equal quantity of dilute ammonia solution is added, color change in the ammonia layer shows the presence of glycosides.

Detection of Phenol and Tannins (Ferric chloride test)

The hydro-alcoholic extract was treated with few drops of neutral ferric chloride solution; the formation of bluish color indicates the presence of phenol nucleolus.

Detection of Phytosteroids (Salkowski's Test)

The hydro-alcoholic extracts of sample was treated with Chloroform and filtered separately. The filtrates were treated with few drops of concentrated H₂SO₄, shaken and allowed to stand if the lower layer turns red sterols are present; if lower layer turns golden yellow triterpenes are present.

Detection of Triterpenoids

Chloroform solution of drugs was taken. Then it was added with acetic anhydride and concentrated H₂SO₄. The appearance of green, orange, blue or purple red color indicates the positive test.

Thin Layer Chromatography

Thin layer chromatography was run for all samples of Panchtikta and murchhita ghrita using solvent system Toluene: Diethyl ether: Ethyl acetate: Acetic acid:: 80: 10: 10: 0.2. The spraying agent used was Anisaldehydesulphuric acid. The R_f of spots exhibited was measured using scale.

Observation

Pharmaceutical work

The loss during the preparation of PTG was observed as showed in Table- 1

Table 1: showing date of preparation, amount, loss, % of loss, average % of loss

Products	Started	Completed	Before preparation	After preparation	Loss	% of Loss	Average % of Loss
Murchitaghrita	01.06.12	02.06.12	35 kg	33.5 kg	1.5 kg	4.28 %	7.42 %
Panchtikta ghrita I	05.06.12	06.06.12	11 kg	10.3 kg	700 g	6.36 %	
Panchtikta ghrita II	07.06.12	08.06.12	11 kg	10.1 kg	900 g	8.18 %	
Panchtikta ghrita III	09.06.12	10.06.12	11 kg	10.15 kg	850 g	7.72 %	

Organoleptic Characters**Table 2: Showing Organoleptic Characters**

	PG 01	PG 02	PG 03	MG
Color	Yellowish green	Yellowish green	Yellowish-green	Yellow
Odor	Characteristic	Characteristic	Characteristic	Characteristic
Appearance	Sticky	Sticky	Sticky	Sticky
Taste	Bitter	Bitter	Bitter	Bitter

Physico-chemical Parameters**Table 3: Physico-chemical Parameters**

	PG 01	PG 02	PG 03	MG
Loss on drying at 110°C	0.12 %	0.11 %	0.09	0.25 %
Refractive Index at 40°C	1.4525	1.4535	1.4524	1.4483
Acid value	1.56	1.55	1.65	0.45
Saponification value	224.28	228.80	234.50	224
Iodine value	32	35.25	36.22	28
Ester value	222.72	227.25	232.85	223.55
Peroxide value	9.23	10.12	10.22	6.34
Weight per ml	1.010	0.912	0.915	0.922
Specific gravity (melted)	0.9100	0.9053	0.8990	0.8963

Thin Layer Chromatography**Solvent System**

Toluene: Diethyl ether: Ethyl acetate: Acetic acid:: 80: 10: 10: 0.2

Spraying agent

Anisaldehydesulphuric acid

R_f (Retention factor)

M: 0.2 (yellow colored in visible light), 0.43 (bluish grey in visible light), 0.54, 0.61 (light-grey in visible light), 0.64, 0.70 (grey colored)

1: 0.20, 0.40, 0.54, 0.61, 0.63, 0.73

2: 0.20, 0.41, 0.54, 0.61, 0.70

3: 0.21, 0.40, 0.49, 0.54, 0.58, 0.70

DISCUSSION

GK one of important pharmaceutical preparations mentioned in Ayurvedic Pharmaceutical Sciences involves the mechanism of complex/compound with lipid, where lipid and other compounds of ghee react with compounds of kalka or/and of kwath drugs. Basically, ghee is made up of esters of fatty acids and alcohol or polyols and its physical character is very much affected by its molecular composition. Actually triacylglycerols (TAGs) constitute the bulk of the lipid mass in fats along with other minor lipids and lipid-soluble components. They altogether determine the behavior of fat¹⁻³. Over 400 different fatty acids have been identified in milk fat⁴ which may be saturated or unsaturated, branched or linear, and short or long-chained. Saturated fatty acid chains tend to adopt a straight-chain configuration, with the long carbon chains adopting an in-plane zigzag pattern. In the case of unsaturated fatty acids, a bend occurs at the position of the double bond and leads to a decrease in packing density.

Mechanism of Processes involved with PTG

It is seen that saturated fatty acids have higher melting temperatures than their corresponding unsaturated fatty acids in the *cis* configuration due to their dense structural packing. Heat, mass, kinetics, momentum transfer etc. Determine the rheological properties of fats. The esterification also reduces the grainy composition of fats. At molecular level thermal energy induces the increasing kinetic energy which invites more complex formation or free radicals of various byproducts. In case of PTG, hydroxyl groups from added

water becomes free and also helps in the dissolution of active principles into the Ghrita, enhancing the therapeutic value. Ghrita, which is basically glycerides of fatty acid interacts with liquids of ingredients and undergoes hydrolysis resulting in the formation of fatty acid and glycerol. So formed fatty acids are Amphipathic in nature which comprises of a hydrophobic exterior and hydrophilic interior. The water soluble constituent of the ingredients interacts with the hydrophilic end of the fatty acid and the oil soluble constituent interacts with the hydrophobic end. This amphipathic lipid gets oriented at oil-water interface with the polar groups in the oil phases. When a critical concentration of these amphipathic lipids is present in an aqueous media, they form micelles. The driving force for micelle formation is hydrophobic end of amphipathic lipid. Since the hydrophilic end cannot break the hydrogen bonds between water molecules, they cluster together in close proximity. The continuous heating and agitation during the manufacturing process of PTG enhances the extraction process by weakening the bonds thereby separating the hydrophobic component from hydrophilic component. This hydrophobic matter will act as a surface active agent which gets soluble in fatty material after the evaporation of water. Hence with micelle formation, the finished ghrita is found likely to contain oil soluble as well as water soluble active principles. Due to continuous stirring, homogenous distribution of active constituent in the solvent occurs simultaneously reducing the concentration gradient, thereby reducing boundary wall thickness. The continuous boiling and circular movement of the liquid by agitation decreases the diffusion co-efficient.

Discussion on Pharmaceutical study

Murchita ghrita Concept of murchana samskara for snehas was first established by Govind Das Sen. This is not mentioned in old classical texts. The present research work says that by murchana, Oxidation process can be checked. Antioxidants are added to sneha through murchana dravyas. Snehas are chemically stable and quickly absorbable in to the system. Sneha have good penetrating capacity and it can better dissolve active constituents of drugs used for murchhana and also when further treated with Specific drug for specific purposes. According to some other Sag due to murchana, taila and ghrita will be free from aama dosha. It will be able to catch the all properties of herbs added during preparation.

MICROSCOPICAL CHARACTERS OF MURCHHITA GHRITA AND DIFFERENT SAMPLES OF PANCHTIKTA GHRITA

Under Low Power (4x)

Murchhita Ghrita

Panchtikta Ghrita-1

Panchtikta Ghrita-2

Panchtikta Ghrita-3

Under High Power (40x)

Murchhita Ghrita

Panchtikta Ghrita-1

Panchtikta Ghrita-2

Panchtikta Ghrita-3

**THIN LAYER CHROMATOGRAPHY OF MURCHHITA GHRITA
AND DIFFERENT SAMPLES OF PANCHTIKTA GHRITA**

Under Visible Light

Under Long UV

Before Derivatization

Under Visible Light

Under Long UV

After Derivatization

Discussion on Analytical study

Loss on Drying

The parameter indicates the presence and percentage of moisture content with the product. With minor differences, the moisture content of murchhita ghrita and all three samples of PTG were below 1 % which is quite acceptable and suggests the quality of products as good.

Refractive Index

It is the ratio of velocity of light in vacuum to the velocity of light in the oil or fat. It is the ratio between the Sine of angle of incidence to the Sine of angle of refraction when a ray of light of known wave length (usually at 589.3 nm, the mean of D lines of Sodium) passes from air into the oil or fat. All fats are composition of different types of fatty acids and their derivatives in various ratios and with processing in presence of temperature or any other ingredients, the basic constitution of particular fat changes. Such change may also occur due to the any impurities added or adopted after any direct or indirect reason. Refractive index varies with temperature and wavelength. It means to say that with temperature, changes in basic constitution may occur due to breakdown or activation of certain compounds while with wavelength angle of refraction changes.

Acid Value

Acid value is also called as neutralization number. This is the amount of Potassium hydroxide in milligrams required to neutralize the acid present with any compound. Hence, it is in present case reaction of potassium hydroxide with carboxylic acids present with fatty acids during the process of titration. The acidity increases with oxidation because triglycerides are converted in to fatty acids and glycerol. Liberation of fatty acid is also result of hydrolysis, thermal effects and lypolytic enzyme (lipase). So, there is direct relationship of acid value with rancidity. In present work, acid value of all samples was below 2 which indicate the better quality. The value increases in Panchtikta, when it is compared with various samples of Panchtikta. This may be due to more conversion of triglycerides to fatty acids due to further processing as murchhita ghrita was used for preparation PTG.

Saponification Value

Saponification value or Saponification number is the number of milligrams of potassium hydroxide required to saponify 1 g of fat in specified condition. This is exactly the reaction of Potassium hydroxide with triesters of fatty acids. The length of chain of fatty acid is inversely proportional to the Saponification number. The long chain fatty acids found in fats have a low Saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of the fat as compared to short chain fatty acids. The observations show medium chain fatty acids as main component. Medium-chain triglycerides are generally considered as good biologically inert source of energy that the human body finds reasonably easy to metabolize, MCTs passively diffuse from the GI tract to the portal system (longer fatty acids are absorbed into the lymphatic system) without requirement for modification like long-chain fatty acids or very-long-chain fatty acids. In addition, MCTs do not require bile salts for digestion.

Iodine Value

The iodine number or iodine value is the quantification of unsaturation of fatty acid in form of double bonds which react with iodine. Iodine number is directly proportional to the number of unsaturated fatty acids. The unsaturation of fatty acids inheriting with various samples of PTG might have increased due to more heat treatment in comparison to murchhitaghrita. Heat increases the kinetic of molecules which may lead to dissociation and unsaturation.

Peroxide Value

Peroxide values are milli equivalents of peroxide per kg of fat. Peroxides (O₂) are intermediate products of fat oxidation and breakdown rapidly to Aldehydes, Ketones and other products. This value is indicator of fat oxidation. It is used to assess the stability or rancidity of fats used as feed ingredients, by measuring lipid peroxides and hydroperoxides formed during the initial stages of oxidation. The value of peroxides formed was less in murchhita ghrita which further went undergone treatment involving heat and water. This change in peroxide value may be directly correlated with its moisture content, which is the main reason for oxidation of fatty acids.

Specific Gravity

Specific gravity is ratio of weight of material in reference to weight of water for constant volume. The weight of lipid material is affected by basic constitution, dissolved constituents used in processing of formulation, or any other compound which may be formed during the process etc. In case of fat, it may also change with thermal effects.

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