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Shata- dhauta- ghrita – A case study

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Shata–dhauta–ghrita (SDG) is an *Ayurvedic* preparation, commonly prescribed for treatment of wounds, burns, chicken pox, scars, herpes, leprosy and other skin diseases. It is prepared by washing clarified butterfat (cow ghee) one hundred times with water. Having potential as a topical product for the treatment of skin diseases, it needs evaluation in modern scientific parameters. The study was carried out to evaluate the changes taking place while washing cow ghee one hundred times which makes it an important topical product.

Keywords: Cow ghee, Topical preparation, Skin hydration, Ointment base, GC/MS spectrum
IPC Int. Cl.[8]: A61K36/00, A61P17/00, A61P17/02, A61P17/08

Traditional system of medicine, evolved over the ages, had been completely looking after the healthcare of the world until the advent of allopathic system of medicine. As this system used knowledge of modern biology and chemistry, for both discovery and treatment, it found fast acceptability and now occupies significant space in the area of healthcare. In spite of this, the contribution of the traditional systems of medicine to healthcare continues to be enormous. The acceptability of traditional preparations, which are normally polyherbal, is increasing because of the general impression that these products are benign. The single molecule based drugs used in allopathic system can have severe adverse effects[1]. *Panchagavya,* i.e. 5 products obtained from cow (cow milk, curd, *ghee*, urine and dung) mentioned in *Ayurveda* have significant role in disease management[2]. Cow *ghee* is having various activities by itself and also by formulations developed using cow ghee. For example, effect of *Bramhi ghrita* on CNS, sedative and anticonvulsant activity of *Unmadnashak ghrita*, wound healing activity of *Darvhi ghrita*, wound healing activity of *Hingvadya ghrita*, antiinflammatory activity of *Jatyadi ghrita,* etc[3-8]. *Shata-dhauta-ghrita* (SDG) is 100 times washed (*shata* = one hundred, *dhauta* = washed) clarified butterfat. It is mentioned in traditional texts for the management of conditions like burns, chicken pox, scars, wounds, herpes, leprosy, and other skin diseases and as a vehicle for drugs to be applied externally[9,10]. In the study, the *Ayurvedic* preparation has been evaluated for its physicochemical parameters and changes occurring during washing were analyzed. An attempt is made to find out the rationale behind washing cow ghee 100 times with water.

Methodology

All the chemicals were purchased from E Merck, SD Fine Chemicals, Mumbai, Universal Laboratories and Qualigens Fine Chemicals. All the solvents were distilled before use. Solvents used for UV, IR and GC/MS studies were of spectroscopy grade. Cow ghee was procured from Go – Seva Anusandhan Kendra, Kusumba, Jalgaon. The copper vessels were purchased from local market. GC/MS was carried out on Shimadzu QP–2010 at Shimadzu Service Centre, Toshvin Analytical Laboratory, Mumbai. All the results are obtained by repetition of the each experiment at least 3 times. Cow ghee was observed for its organoleptic properties and analyzed for various physicochemical parameters (moisture content, viscosity, acid value, saponification value, iodine value, unsaponifiable matter, Rechert Meissl value, Polenske value, etc.) prescribed for lipids in Pharmacopoeias (IP, USP). Particle size and copper content were also determined[11–18]. For GC/MS analysis, Fatty Acid Methyl Esters (FAME) of the samples was prepared. About 25 ml of 10% solution

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of sample was prepared in n– hexane. In a suitable flask, 19 ml of this solution was treated with 1.25 ml of 3M methanolic potassium hydroxide and the flask was shaken for 30 min, the mixture was placed in a large centrifuge tube containing 10 ml of saturated solution of sodium chloride. The flask was rinsed several times with distilled water in to centrifuge tube (Use of water keeps eventual methanolic peaks in chromatograph to a minimum). Shaking for 30 min and then centrifuging brought the hexane solution containing the esters to the top of the tube. This hexane solution was transferred to a labeled, stoppered bottle. A GC/MS separation of the esters was performed[19]. Conditions for GC/MS included Model - Shimadzu QP – 2010, Column - 30 m/0.32 mm id/0.25 micron BP – 20, Column temperature - 50°C to 260°C at 5°C per minute, Injector temperature - 250°C, Split ratio – 40, Ion source temperature - 220°C, MS interference entioned in *Bhaishajya-kalpana-vidnayan*[9,10].

The copper vessels were cleaned thoroughly and rinsed with distilled water. 2.5 kg of previously standardized cow ghee was taken in copper vessel. 1.5 L of distilled water was added to it. With the help of manual copper agitator, the mixture of cow ghee and water were triturated for 5–8 minutes. The contents in copper vessel were allowed to settle. Water was decanted carefully, avoiding loss of ghee. The fresh slot of 1.5 L distilled water was added in previously washed cow ghee and similar procedure was repeated. This operation was carried out one hundred times to obtain SDG. Samples were collected after each washing and stored in plastic containers at room temperature for analysis. Analysis of SDG as per Pharmacopoeial standards for oils and fats was carried out based upon physicochemical parameters. Being a fatty product, the tests applied for fats and oils were carried out to know the physical and chemical properties of finished product (SDG). Tests carried out for standardization of raw material (organoleptic properties, moisture content, viscosity, acid value, saponification value, iodine value, unsaponifiable matter, Rechert Meissl value, Polenske value particle size and copper content, etc.) were performed for evaluation of SDG[11-18]. GC/MS analysis was carried out to find out the changes occurred in fatty acid composition. FAME were prepared and subjected for GC/MS analysis using method similar to that used for raw material.

Results and discussion

A comparison of physicochemical parameters of cow ghee and SDG has been done (Table 1, Fig. 1). GC/MS spectra for FAME of cow ghee and SDG were obtained (Figs. 2&3) and comparison of the area per cent of major peaks was done (Table 2, Fig. 4). From the study carried out, some facts behind

Table 1— Comparison of physicochemical parameters of cow ghee and SDG

Parameter	Cow ghee	SDG	Change
Organoleptic properties			
Colour	Golden yellow	White	
Odour	Characteristic, Pleasant	Odorless	
Taste	Characteristic	Tasteless	
Texture	Granular, oily	Smooth, non - oily Homogeneous mass	
Physical parameters			
Moisture. content (%)	0.27	0.8	↑ 2.98 times
*P*h	4.6	5.9	↑ 1.28 times
Particle size (μ)	179.02	59.99	↓ 2.98 times
Viscosity (cp) at 20 rpm for 30 sec	8000	9770	↑ 1.22 times
Copper content (ppm)	0.13	1.2	↑ 8.86 times
Chemical parameters			
Acid value	0.84	0.11	↓ 7.59 times
Saponification value	234.26	25.96	↓ 9.02 times
Iodine value	35.59	2.82	↓ 12.62 times
Unsaponifiable matter	0.78	0.04	↓ 18.72 times
RM value	21.67	0.22	↓ 98.50 times
P value	1.1	0.1	↓ 11 times

Note ↑: increase, ↓: Decrease

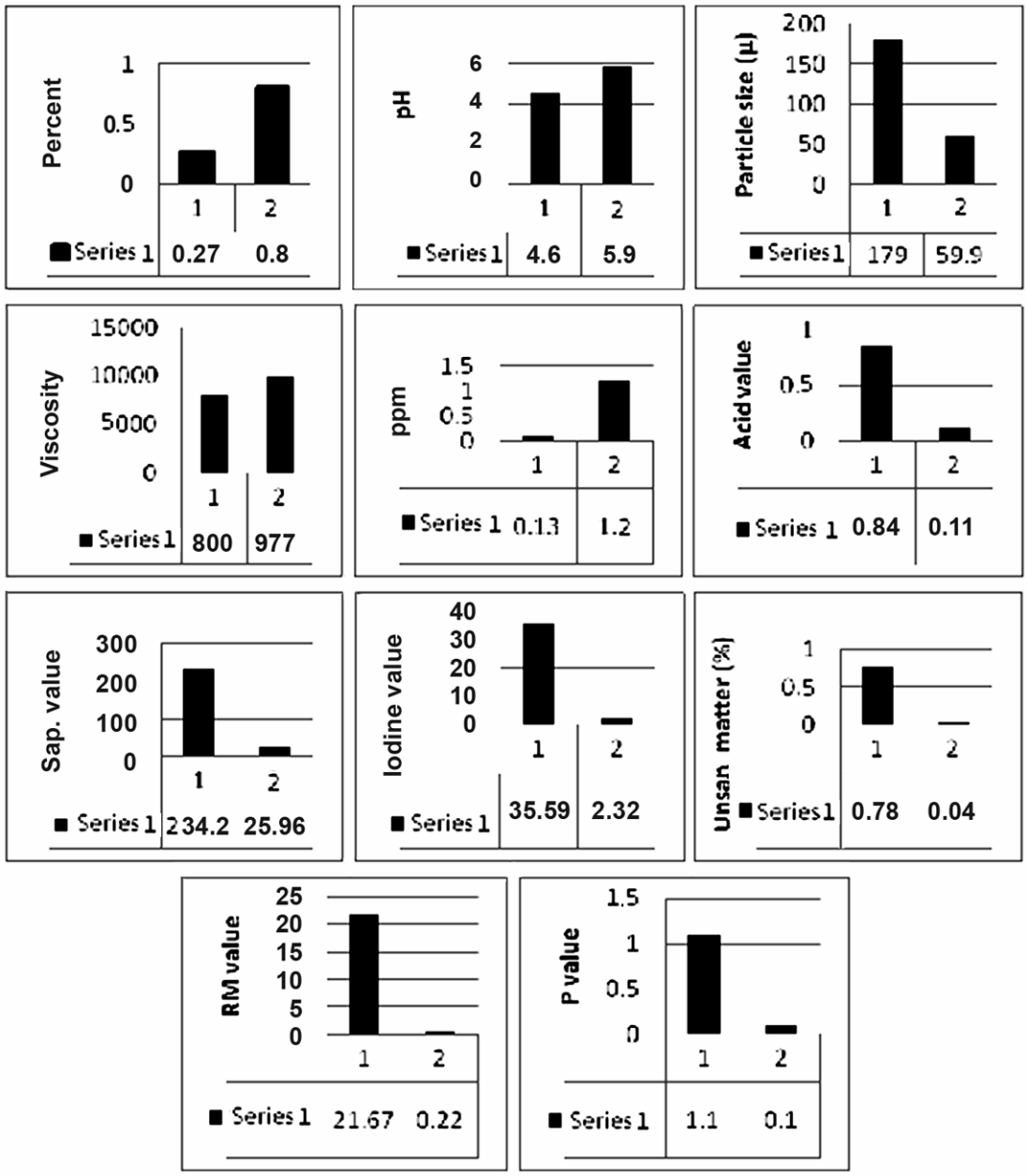

Fig. 1— Physicochemical parameters of cow ghee and SDG

preparation of SDG can be illuminated. Initially, the pure lipid phase, i. e. cow *ghee* comes in contact with aqueous phase. Due to trituration, it results in formation of w/o type of emulsion as lipid phase (cow *ghee*) is a major phase. As the washing continues, due to pressure applied during trituration, particle size of fat granules gets reduced (as evident from decrease in particle size). Eventually with successive washings, aqueous phase becomes predominant over lipid phase (evident from increase in moisture content). This results in the phenomenon of phase inversion resulting in o/w type of emulsion. Chances are there that it may result in complex system like w/o/w emulsion. The characteristic odor and granular, oily consistency in cow ghee remains no longer in SDG making it homogeneous, smooth, non-oily product, which is easier to apply, thus improves patient compliance.

Increase in moisture content may be useful for skin hydration and cooling effect, which can justify its use for the treatment of burns[20]. *p*H change from acidic to neutral makes it beneficial to prevent skin irritation.

Table 2— Fatty acid composition of cow ghee and SDG (Area % of GC/MS peaks)

Fatty acid	Cow ghee	SDG
Butyric	0.65	38
Caproic	1.13	0.66
Caprylic	0.77	0.41
Capric	1.68	0.84
Lauric	2.03	0.16
Myrestic	7.17	0.31
Palmetic	18.74	0.24
Stearic	11.8	0.18
Oleic	14.3	0
Linoleic	1.7	0.63
Linolenic	0.43	0

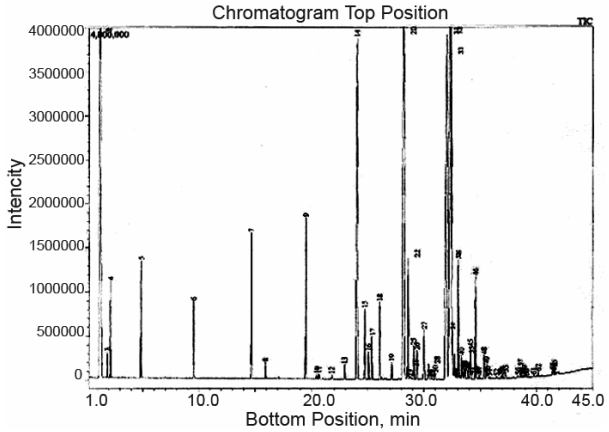

Fig. 2— GC/MS spectrum of FAME of cow ghee

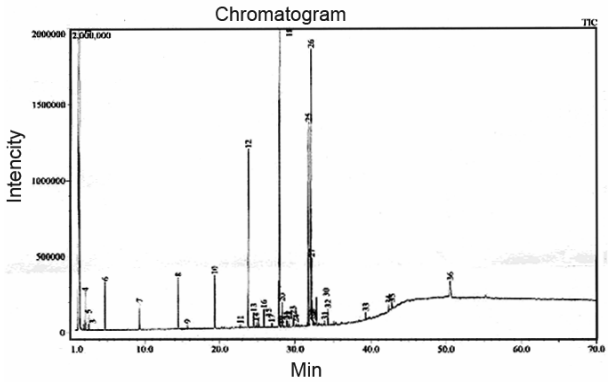

Fig. 3— GC/MS spectrum of FAME of SDG

So, the preparation can be applied on open wounds. Reduction in particle size makes the product non-granular, non-sticky, homogeneous, which makes it easy to apply on skin and may result in increased rate of absorption through skin. Viscosity was found to be increased. Washing results in homogeneous mass of oil in water emulsion with better consistency and viscosity which is helpful in its topical application. Increase in copper content of the preparation makes it

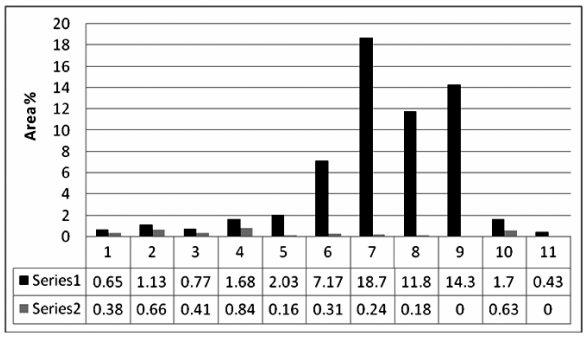

Fig. 4— Fatty acid composition of cow ghee and SDG

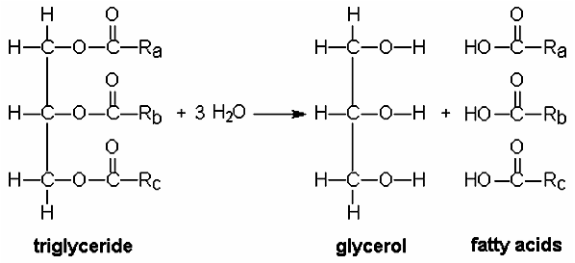

Fig. 5— Fat splitting process

significant in removal of scars and as an antiinflammatory agent[10,21]. Decrease in iodine value indicates decrease in degree of unsaturation, which eventually reduces chances of rancidity thus increase stability of the product[16,17]. Where as decrease in RM and P value indicate decrease in steam volatile water soluble and water insoluble fatty acids, respectively. These mainly include short chain fatty acids[18]. Decrease in unsaponifiable matter is the indicator of removal of matter other than fatty acids[15].

GC/MS spectrum of cow *ghee* (Fig. 2) showed its composition which lies in the standard values prescribed[2]. In GC/MS spectrum of SDG (Fig. 3), percentage of almost all fatty acids was found to be decreased. Decrease in degree of unsaturation can be supported by absence of unsaturated fatty acids, viz. oleic acid and linolenic acid in SDG. Preparation of SDG can be correlated with the phenomenon of fat splitting. Fat splitting is the process in which, fat is hydrolyzed in the presence of water to yield free fatty acids and glycerols (Fig. 5)[22]. The reaction proceeds step by step and is reversible. If reactants and products are not removed from the sphere of the reaction, an equilibrium depending upon the concentrations of the former will eventually be reached. In practical fat splitting, a high degree of hydrolysis is ensured by using a large excess of water and repeatedly withdrawing the glycerol rich aqueous phase and replacing it with fresh water. High

temperature and pressure accelerates aqueous hydrolysis. In preparing SDG, temperature is not increased but there is repeated and prolonged tritutation of the fat and water mixture. Thus, the pressure factor may be contributing for fat splitting in this case. After each trituration, aqueous phase is withdrawn and replaced by fresh slot of the same in case of fat splitting. It has also been found that splitting is accelerated by presence of mineral acids, certain metal oxides and sulphonic acids. SDG has been mentioned to be prepared in copper vessels. Thus, incorporation of copper metal in the mixture during trituration was indicated by increase in copper content in SDG. Copper can act as a catalyst to promote fat splitting. The above hypothesis of fat splitting by using large excess of aqueous phase can be correlated with the idea of washing cow ghee 100 times with water. It can be pointed out that by washing cow *ghee* 100 times with water, the triglycerides are splitted into glycerol and free fatty acids. The decrease in acid value can be correlated with the fact of fat splitting in to glycerol and fatty acids, which are removed along with the aqueous phase. From the present work, it can be concluded that changes taking place in cow *ghee* while washing it with water 100 times to prepare *Shata-dhauta-ghrita*, makes it an elegant and suitable product for topical application. However, there is a need to carry out further study on its stability, pharmacological evaluation as an effective topical product and its formulation development.

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