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Physico-chemical and Proximate Analysis of Poly Herbal Formula- Palakalyana Ghrita

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ABSTRACT

Background: Standardization is necessary in order to assess the quality of herbal formulations. Palakalyana Ghrita (PKG) is an herbal formula used in Ayurveda medical system to enhance fertility and immunity in both genders which consists nineteen medicinal plants with cow's ghee and milk. As per available literature PKG formula has not been standardized although it is a commonly used drug by traditional practitioners. Therefore, this study was carried out to evaluate the physio-chemical properties, nutrition composition and possible toxic elements and microorganisms of PKG formula. **Methodology:** Physico-chemical properties tested include refractive index, total ash content, acid in soluble ash content, water soluble ash content, moisture content, acid value, peroxide value, saponification value. Carbohydrate, protein, fatty acid, vitamin and mineral composition were assessed as nutritional parameters of PKG. Heavy metals and microorganisms were tested using standard protocols. **Results:** Unsaturated fatty acids namely Palmitoleic acid (0.26%), Oleic acid (40.36%) and Linoleic acid (9.19%) and saturated fatty acids; Capric acid (0.63%), Myristic acid (1.68%) Pentadecyclic acid (0.23%) Palmitic acid (42.25%) Margaric acid ((0.26%) and Stearic acid (4.85%) were present in the PKG formulation. The percentages of carbohydrate, protein and fat content of PKG were 0.04±0.0, 98.8±0.2 and 0.7 ± 0.0 respectively. Heavy metals and microbes were not detected. **Conclusion:** Results obtained could be utilized as references standard for quality assurance of PKG.

Keywords: Herbal formulation, Palakalyana Ghrita, Physico- chemical, Standardization.

INTRODUCTION

Palakalyana Ghrita (PKG) is used in traditional medical systemof Sri Lanka, to enhance fertility and immunity of both males and females. Roots of *Asparagus racemosus* is the main ingredient in PKG. It is known to possess medicinally important aphrodisiac, immunomodulatory, anti-ulcer and anti-anemic properties [1, 2, 3, 4]. PKG contains 19 medicinal plants along with cow's milk and cow's ghee (Table 1). Most of these herbal compositions are effective in practice, however are not scientifically validated. Therefore, standardization of PKG is essential to ensure efficacy and stability of its' finished product. In the present study, an attempt was done to standardize PKG using standard protocols.

MATERIAL AND METHODS

Plant materials

All plants materials with pasteurized cow's milk, and fresh cow's ghee were purchased from the Ayurveda pharmacy adjacent to the, Ayurveda teaching hospital, Borella, Sri Lanka and all were identified and authenticated by the Department of Materia Medica, in Institute of Indigenous Medicine, University of Colombo, Sri Lanka.

Preparation of the drug

Nineteen herbal ingredients in the formulation were washed and dried at 60 °C using an oven. Concentrated water extract of *Asparagus racemosus* was prepared using 600 g of the chopped roots infused in 20 L water, and boiled at 100 °C for 4-6 hours until reduced to 5 L volume of liquid. All the other herbal materials were separately washed and air dried to a constant weight and powdered using an electric grinder and passed through the 10 mesh sieve. Fifteen gram of each finely powdered herbal ingredients, 5 L of pasteurized cow's milk and 1280 g of fresh cow's ghee were added to the 5 L volume of liquid. The mixture with all ingredients was boiled until all the water evaporated. The preparation was stored in glass bottles at room temperature. This formulation was prepared as per the classical procedure [5] at the pharmacy attached to the Institute of Indigenous Medicine, University of Colombo, Sri Lanka.

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Physico-chemical parameters

Determination of physico-chemical parameters such as total ash, acid insoluble ash, and water soluble ash were done as per WHO guidelines 2011[6]. Moisture content, refractory index, saponification value and peroxide value were determined according to the method mentioned in SLS 340:1975[7]. Acid value was measured by using titrating method in an alcoholic medium with aqueous potassium hydroxide (KOH) solution.

Nutritional values

VitaminB1, B2, and B12 were detected using ROCHE Basle method. Vitamin C was evaluated according to US Pharmacopeia 22nd Edition 2004[8]. Vitamin A and E were detected according to a standard protocol using HPLC [9].Fatty acid composition was analyzed using BF3 /Methanol fatty acid ester derivertization [10]. Kjeldahl method was used for the detection of protein [11]. Carbohydrate was detected using Molish's test [12]. Total dietary fiber was evaluated using enzymatic gravimetric method. Crude fat content was analyzed using the method described in AOAC (2000) and the Soxtherm manual [13].

Heavy metals

Heavy metals such as Mercury, Arsenic, Lead and Cadmium were estimated by hydride generation technique (cold vapor atomic absorption spectrometry) [13].

Iodine value

This was estimated according to SLS 313 Part 2, section 2; 2014[14].

Microbiological limits

Aerobic plate counts, yeast and moulds were observed according to SLS 516/1 and SLS 516/2 [15] methods. Coliforms/ *Escherichia coli*, and *Staphylococcus aureus* were observed using methods as described in SLS/3 [16] and SLS/516/6 [17] respectively.

TLC fingerprinting

Extraction procedure for the sample

Sample (10 g) was refluxed with water (50 ml), added to a separating funnel containing hexane, shaken well and kept for 15 min. After that removed the hexane layer and active ingredients were extracted into dichloromethane and concentrated.

Extraction procedure for the active ingredients

Plant ingredients mentioned in Table 1, mixed in a ratio of 1:1 (w/w) and extracted into dichloromethane and concentrated. Both sample (5 µl) and mixture of raw materials (5 µl) were spotted on the same TLC plate. A mixture of solvent system consist of methanol, cyclohexane and dichloromethane in a ratio of 0.2:2:9.8 v/v was used as the mobile phase.

RESULTS AND DISCUSSION

Very minor quantities total ash, water soluble ash or acid insoluble ash were present in PKG (Table 2). That indicates fewer impurities in the final product of PKG. Less moisture content (0.12% w/w) shows that there is less chance of microbial growth. Acid value of the amount of potassium hydroxide in milligrams required to neutralize the acid present with any formulation. During the process of oxidation acidity increases because triglycerides are converted into fatty acids and glycerol. Releasing of fatty acid is result of hydrolysis, and thermal effects. Further there is direct relationship of acid value with rancidity. The less acid value (1.8mg/KoH/g Oil) indicate better quality and suitability for human use. Further these results are supported to previous studies on standardization of Ghrita preparations in Ayurveda medicine [18] (Table 3).

Table 1: Medicinal plants in Palakalyana Ghrita

Plant Ingredients	Family	Part of the plant
Terminalia chebula(Retz)	Combretaceae	fruits
Terminalia bellirica (Roxb)	Combretaceae	fruits
Phyllanthus emblica (L[1])	Phyllanthaceae	fruits
Picrorhiza kurroa (L[1])	Plantaginaceae	rhizomes
Curcuma domestica (L[1])	Zingiberaceae	rhizomes
Rubia cordifolia(L)	Rubiaceae	roots
Vitis vinifera(L)	Vitaceae	fruits
Nymphoides cristata (Roxb)	Menyanthaceae	flowers
Santalum album (L)	Santalaceae	wood
Saussure lappa (Decne)	Asteraceae	roots
Withania somnifera (L) Dunal	Solanaceae	roots
Sida cordifolia (L)	Malvaceae	roots
Ipomoea peniculata (L)	Convolvulaceae	tubers
Pterocarpus santalinus (L.f)	Fabeceae	wood
Coscinium fenestratum (Goetgh)	Menispermaceae	stem
Sacchrum officinarum (L)	Poaceae	stem
Glycyrrhiza glabra (L[1])	Fabeceae	rhizomes
Vernonina cineria (H Rob)	Asteraceae	Whole plant
Asparagus recemosus (willd[1])	Asparagaceae	roots

Table 2: Medicinal plants in Palakalyana Ghrita

	Test	Result
1.	Refractive index	1.4666 at 40 °C
2.	Total ash	0.03% w/w
3.	Acid insoluble ash	Not detected < 0.01%
4.	Water soluble ash	Not detected < 0.01%
5.	Moisture content	0.12% w/w
6.	Acid value	1.8 mg/KOH/g Oil
7.	Peroxide value	7.5 milliequvillent/kg
8.	Saponification value	212.0
9.	Iodine value	52.6 g per 100

n = 6

PKG consists with Capric acid (0.63%) and Myristic acid (1.68%) (Table 3). Medium chain fatty acids possess antioxidant properties which counter negative results of premature gray hair, macular degeneration and sagging of the pores and skin. Both unsaturated and saturated fatty acids included in PKG are coronary heart pleasant fatty acids, increases the fee of metabolism that helping in weight loss ,increase the extent of true cholesterol (high density lipoproteins) and decrease the bad cholesterol (low density lipoproteins) in human frame .Those are great sources of energy[19]. Microbes such as aerobic microbes, yeast and moulds, coliforms/*Escherichia coli* and *Staphylococcus aureus* were not detected in PKG.

Table 3: Fatty acid composition of Palakalyana Ghrita

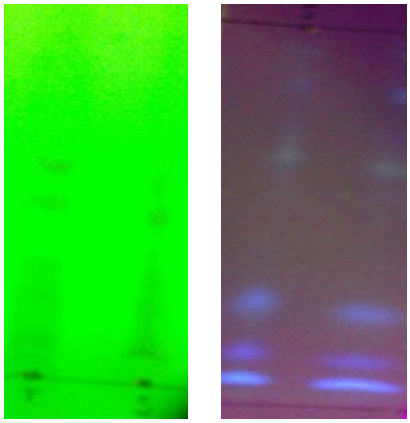
	Name of the Fatty acid	percentage
1.	Capric acid	0.63%
2.	Myristic acid	1.68%
3.	Pentadecyclic acid	0.23%
4.	Palmitic acid	42.25%
5.	Palmitoleic acid	0.54%
6.	Magaric acid	0.26%
7.	Stearic acid	4.85%
8.	Vaccenic acid	40.36%
9.	Linoleic acid	9.19%

n = 6

Table 4: Nutritional composition in Palakalyana Ghrita

	Phytoconstituents	Results (%)
1.	Carbohydrate	0.04± 0.0 %
2.	Fat	98.8±0.2 %
3.	Protein	0.7 ± 0.0 %
4.	Vitamin A	1.0 (mg/100g)
5.	Vitamin E	2.5 (mg/100g)
6.	Vitamin B12	0.041 (mg/100g)

n = 6



254nm 366nm

Figure 1: TLC profile of Palakalyana Ghrita (PKG)

TLC fingerprint profile is showed in (Figure 1). The sample has similarities in terms of Rf values and colour of PKG sample to the Rf values and colour of the raw materials. It is proved homogeneity of a final product.

Nutritional analysis indicates good amount of vitamins as vitamin A, E and B12. Due to high oily base in PKG enhance the absorption of vitamins A and E which are fat soluble vitamins consisted. Ancient physician were prescribed PKG as fertility enhancing treatment. Nutritional analysis of PKG shows evidence to prove that claim (Table 4). Mercury, lead and cadmium were not present in PKG. Results have shown that concentration of arsenic was 0.3 mg/kg in PKG. However, the observed arsenic concentration was within the WHO recommended limits. These evidences confirm the nontoxic effect of PKG. The iodine value of PKG was 52.6 g per 100. Higher iodine value indicates the grater the number of unsaturated fatty acids. R_f values of sample and mixture of raw materials were similar to each other (0.06, 0.15, 0.18, 0.21, 0.31, 0.41, 0.46, 0.48, 0.53, 0.62, 0.75 and 0.82).

CONCLUSION

Palakalyana Ghrita was standardized for the first time using standard protocols and output of the present study could be utilized as references standard for quality assurance of Palakalyana Ghrita.

Conflict of interest- None declared

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