Standardization of a polyherbal Siddha formulation, *Amukkara Choornam*

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Most of the traditional systems of medicine are effective but they lack of standardization. So, there is a need to develop a standardization technique. Central Council of research in Ayurveda and Siddha has given preliminary guidelines for standardizing these conventional formulations. For the uniformity of batches in production of herbal formulations it is necessary to develop methods for evaluation. In the paper, attempt has been made to evaluate Amukkara choornam, a Siddha formulation. Four samples were procured from different manufacturers and were subjected to physiochemical screening, High Performance Thin Layer Chromatography (HPTLC) finger printing, microscopic characterization was compared using authentic ingredients as reference. It was observed that all commercial samples matched exactly with that of authentic standards after performing the standardization.

**Keywords**: Amukkara choornam, Siddha drugs, Standardization, Chromatography

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India having a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha and Unani. Botanicals constitute of major part of these traditional medicines. The development of these traditional systems of medicines with the perspectives of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural products in the healthcare1, 2. The plant species mentioned in the ancient texts of Ayurveda and other Indian Systems of Medicine may be explored with the modern scientific approaches for better leads in the healthcare. Standardization of herbal formulations is essential in order to assess the quality of drugs3-5. The main problem in polyherbal herbal formulation is that the presence of each ingredient has to be established. The microscopic characters of each ingredient are very difficult to identify and also some times these are overlapping with the character of other ingredient. The paper presents development of methods for the evaluation of *Amukkara Choornam*, a Siddha formulation which is used for gastritis, spleen enlargement, leucorrhoea, hiccup, anemia, tuberculosis and kappa diseases. It consists of fine powders of *Lavangam* (clove)- *Syzygium aromaticum* -10 parts, *Sirunaga poo* (cinnamon)- *Cinnamomum wightii* -20 parts, *Ela arisi* (cardamom)- *Elettaria cardamomum* -40 parts, *Milagu* (back pepper)- *Piper nigrum* -80 parts, *Thippili* (long pepper)- *Piper longum* -160 parts, *Chukku* (dry ginger)- *Zingiber officinale* -320 parts, *Amukkara* (*Withania*)- *Withania somnifera* -640 parts, sarkarai (cane sugar) -1280 parts6,7.

**Methodology**

The samples were collected from the physicians and manufacturers of Aravindh Herbal Laboratories (P) Ltd (Sample A), Rajapalayam (South India) (Sample B), SKM Siddha and Ayurvedic Medicines India (P) Ltd, Erode (Sample C), which are being used for the treatment of gastric troubles, spleen enlargement, leucorrhoea, hiccup, anaemia, tuberculosis and kappa diseases. For in-house preparation, the ingredients were purchased from the local raw materials traders and tribal belt of Jharkhand, which was authenticated (Sample D) and used as control. Organoleptic characters and particle size of all the samples were recorded. Quantitative analysis for total ash, water soluble ash, acid insoluble ash, ethanol soluble extractive value, water soluble extractive value and loss on drying at 70°C were carried out in triplicate in all four samples of *Amukkara Choornam* according to the prescribed methods and CCRAS guidelines. Phytochemical analysis, fluorescence analysis, and HPTLC finger printing profile were also determined in all the samples. For microscopic analysis, all the ingredients were studied individually by preparing three slides for each ingredient one in water, stained with iodine and mounted in glycerin, second one in chloral hydrate.

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mounted with glycerin and third one in phloroglucinol and concentrated hydrochloric acid. Preliminary tests were carried out on methanolic extract for the presence/absence of phytoconstituent like alkaloids, carbohydrates, flavonoids, glycosides, saponins, sterols, terpenes and tannins. Fluorescence analysis were carried out in 1N sodium hydroxide in methanol, 1N sodium hydroxide in water, 50% HCL, 50% HNO₃, 50% H₂SO₄, petroleum ether and chloroform at 254 nm and 366 nm.

For HPTLC, 5 gm of each sample was extracted with 30 ml of toluene by a sonicator for 20 minutes, filtered and concentrated. The chromatograph was performed by spotting standards and extracted samples on pre-coated silica gel aluminum plate 60F-254 (10 cm×10 cm with 250 µm thickness) using a Camag Linomat IV sample applicator and a 100 µl Hamilton syringe. The samples, in the form of bands of length 5 mm, were spotted 15 mm from the bottom, 15 mm from left margin of the plate and 10 mm apart, at a constant application rate of 150 nl/s using nitrogen aspirator. Plates were developed using a mobile phase consisting of toluene–ethyl acetate (9:3 v/v). Linear ascending development was carried out in 10 cm×10 cm twin trough glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 15 min at room temperature. The length of chromatogram run was 7 cm. 10ml of the mobile phase (5 ml in trough containing the plate and 5ml in the other trough) was used for each development, which required 8 min. Subsequent to the development, TLC plates were dried in a current of air with the help of an air-dryer. The slit dimension settings of length 5.00 mm and width 0.45 mm, and a scanning rate of 20 mm/s was employed. The monochromator band width was set at 20 nm. Densitometric scanning was performed on Camag TLC scanner III in the absorbance/reflectance mode at 260 nm and operated by win CATS Planar chromatography version 1.1.4.0. The source of radiation utilized was halogen tungsten lamp.

Result and discussion

Amukkara churna samples of different manufacturers and in house preparation were subjected to analysis. All the samples brown in colour were with pleasant odour. All samples were very fine. Results of quantitative analysis for total ash, water soluble ash, acid insoluble ash, ethanol soluble extractive value, water soluble extractive value and loss on drying at 70°C were calculated (Table 1). Microscopic analysis of four samples show the presence of identifying diagnostic character, which are not overlapping with the character of other ingredient that are epidermal cells (EC) of Elateria cardamomum (Fig. 1), endosperm fragments (EF) of Piper longum (Fig. 2), U-shaped stone cells (U-SC) of Cinnamomum wightii (Fig. 3), stone cells (SC) (nearly isodiametric with intercellular space) of piper nigrum (Fig. 4), group of stone cells (SC) (nearly isodiametric with intercellular space) of piper nigrum (Fig. 5), brownish matter (BM) of Syzygium aromaticum (Fig. 6), starch grains (Eccentric hilum) (SGE) of Zingiber officinalis (Fig. 7), and starch grains (concentric hilum) (SGC) of Withania somnifera (Fig. 8).

Phytochemical analysis (Table 2) and Fluorescence
analysis (Table 3) were done. The densitometric scanning was performed on developed toluene extract with Camag TLC scanner III in the absorbance/reflectance mode at 260 nm. (Fig. 9).

**Conclusion**

After analysis of different samples of *Amukkara Choornam* by different parameters such as total ash, water soluble ash, acid insoluble ash, ethanol soluble extractive value, water soluble extractive value and loss on drying at 70°C, phytochemical analysis, fluorescence analysis and HPTLC chromatogram shows good co-relation between them. The study of microscopic characters of different samples shows the presence of diagnostic identifying characters for presence of each
ingredient. So it can be concluded that these parameters can be used for the evaluation of Amukkara Choornam.

References
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