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PHYSICOCHEMICAL ANALYSIS AND DRUG STANDARDIZATION OF PUGA PAKA

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ABSTRACT

Introduction: Pugapaka is a formulation mentioned in Ayurveda mentioned under Prameha chikitsa adhyaya with additional indications like menstrual disorders, Jwara, Raktapitta, Agnimandya and Female infertility. **Aims and objective:** This work was carried out to standardize the finished product of Pugapaka. **Materials and methods:** Pugapaka was prepared according to standard procedure of Avaleha kalpana and analyzed for the following parameters like physico-chemical analysis and High performance thin layer chromatography (HPTLC). **Discussion:** Infertility cases increasing drastically due to various reasons with an increase in demand for infertility treatment. Among several treatment options through Ayurveda; Pugapaka was selected. **Result:** On the basis of observations and experimental results, this study may be used as reference standard in the further quality control researches.

KEYWORDS: Ayurveda, Avaleha, Female Infertility, HPTLC, Physico-Chemical analysis, Pugapaka, Standardization.

INTRODUCTION

Pugapaka[1] is a formulation consists of Puga, Amalaki, Shatavari, Goksheera, Goghruta as main ingredients with Jeerakadvaya, Musta, Ela, Nagakesara etc. as Prakshepaka drugs. The benefits of this formulation mentioned as "*Yogo Garbhakarahapram gadaharahastreenamasrukdoshajit*" i.e.; the formulation is beneficial in alleviating menstrual disorders and bestows fertility in women. It is useful in Jwara, Raktapitta, Agnimandya, Prameha and is also Shukraprada. The ingredients of this yoga possess Tikta, Katu and Madhura rasa, Ushna veerya, Deepana-Pachana properties and acts as Vatanulomaka, Kaphahara, Srotoshodhaka. Thus the yoga acts on Jataragni, by this it helps in Dhatuposhana and stimulates the Beejarupi Artava Utpatti for conception.

In the present day detailed evaluation and standardization of finished product is necessary to bring uniformity in all the finished products and to meet the demands of ever increasing urbanization and more dependence on readymade preparations.To meet the challenges of new millennium with increasing demand and to capture the world market,standardization of Ayurveda formulation is very necessary. Hence analytical parameters are essential as a measure of quality control and standardization of the finished product. Analytical study improves the therapeutic applicability of the drug based on its composition.In addition analytical parameters provide a proof of drug identity, purity and substantiate the strength of finished product.

AIM AND OBJECTIVE

The objective of the study was to prepare the Pugapaka and to standardize it on the basis of organoleptic and physico-chemical parameters.

MATERIALS AND METHODS

Collection, Identification and authentication of raw drugs

In the present study two batches of Pugapaka were prepared and further studied for organo-leptic, physico-chemical and chromatographical evaluation. The raw drugs for the study were procured from the Sri

Dharmasthala Manjunatheshwara Ayurveda Pharmacy, Udupi. The ingredients were identified and authenticated in the department of Dravya Guna, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan.

The ingredients and part used are listed below:

Table 1: Ingredients of Pugapaka with officinal parts.

Drug	Botanical name	Family	Officinal Parts
Puga	Areca catechu	Arecaceae	Fruits
Amalaki	Emblica officinalis	Euphorbiaceae	Fruits
Shatavari	Asparagus recemosa	Liliaceae	Root
Goksheera	Cow's milk	-	-
Goghrita	Cow's Ghee	-	-
Nagakesara	Mesua ferra	Guttiferae	Stamens
Musta	Cyperus rotundus	Cyperaceae	Root
Chandana	Santalum album	Santalaceae	Bark
Shunti	Zingiber offinale	Zingiberaceae	Rhizome
Maricha	Pipper nigrum	Piperaceae	Fruits
Pippali	Pipper longum	Piperaceae	Fruits
Priyala	Buchanania longifolia	Anacardaceae	Fruits
Amalaki	Emblica officinalis	Euphorbiaceae	Fruits
Katuki	Picrorrhiza kurroa	Scrophulariaceae	Root
Lajjalu	Mimosa pudica	Leguminosae	Whole plant
Patra	Cinnamom tamalum	Lauraceae	Leaf
Twak	Cinnamom zeylenica	Lauraceae	Bark
Ela	Elettaria cardmum	Scitaminae	Fruits
Krisnajeeraka	Carum carvi	Umbelliferae	Fruits
Shwetajeeraka	Cuminum cyminum	Umbelliferae	Fruits
Jatiphala, Javithri	Myristica fragrance	Myristicaeae	Fruits
Lavanga	Syzgium aromatic	Myrtaceae	Stamen
Dhanyaka	Coriandrum sativum	Umbelliferae	Seed

Table 2: Rasapanchaka of main ingredients of Pugapaka.

Prakshepaka Dravyas	Rasa	Guna	Veerya	Vipaka	Karma
Puga[2]	Kashaya, Madhura	Guru, Ruksha, Vikasi	Sheeta	Katu	Kaphapittahara, Deepana
Amalaki[3]	LavanavarjitaPancha rasa	Ruksha, Laghu	Sheeta	Madhura	Vrushya, Rasayana
Shatavari[4]	Madhura	Guru , Snigdha	Sheeta	Madhura	Vrushya, Rasayana
Goksheera[5]	Madhura	Sheeta, Snigdha	Sheeta	Madhura	Vrushya, Rasayana
Goghruta[6]	Madhura	Guru	Sheeta	Madhura	Balya, Vrushya, Deepana

Method of Preparation of Pugapaka

Before preparing the medicine, Purification of Areca catechu was done by Swedana (fomentation) with cow's milk for 6 hours and dried under sunlight.[7] All ingredients made into fine powder form. Puga churna (400gms), Shatavarichurna (500gms) were added with 3 liters of milk and boiled over mild flame until it becomes concentrated. 350gms of Ghee was taken and added into same vessel. Paka was fried until it appears brownish red colour. Meanwhile Sugar (350gms) was taken in separate vessel; required quantity of water was added and boiled over mild fire with frequent stirring. When it attained Paka lakshana above said fried Paka and Amalaki churna(500gms) were added and boiling was continued. When appropriate Lehya pakalakshanas (3-4thread consistency) were observed, all powdered Prakshepaka dravyas were added, stirred well to homogeneous mixture[8]

Analytical study[9]

The analytical study, was carried out at S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi to determine the following physico-chemical parameters like reducing sugar, non reducing sugar, rancidity, water soluble extracts and organoleptic characteristics were a evaluated and are tabulated below(Table No 3 & 4).HPTLC analysis was carried out using Toluene: Ethyl Acetate (9.0:1.0) as solvent system.

Reducing and non-reducing sugar

10 g of sample was taken in a 250 ml volumetric flask and 200 ml of water was added. Slight excess solid basic Lead acetate was added to remove tannins and made up to the mark without disturbing the solution by adding water, shaken and filtered. Slight excess of solid Sodium oxalate was added to remove excess of basic Lead acetate, shaken and filtered. This filtrate was used for the estimation of reducing sugar.

Reducing sugar: The sugar solution was taken in a 50 ml burette.

Preliminary titration: 10 ml of Fehling's solution was pipetted into a 250 ml conical flask, from the burette, 15 ml of the sugar solution was added. The liquid boiled on asbestos-covered gauze and further quantities of the sugar solution was added (One ml at a time) at 10 to 15 second intervals to the boiling liquid until the blue colour is nearly discharged. 3-5 drops of aqueous Methylene blue solution (1%) was added and continued the titration until the indicator is completely decolourised.

Accurate titration: The titration repeated, before heating, almost all of the sugar solution required to effect reduction of copper added. It was gently boiled for two minutes. 3-5 drops of Methylene blue indicator was added and the titration was completed within a total boiling time of three minutes. At the end point all the blue colour should be discharged and the liquid should be red.

The proportions of the various sugars, equivalent to 10 ml of Fehling's solution were taken from the table.

Total Sugar: 20ml of reducing sugar solution was taken and 10ml of Concentrated Hydrochloric acid was added and kept aside over night.

Neutralized with approximately 1M Sodium hydroxide solution or with solid sodium carbonate and made up to 100 ml in a volumetric flask. The total sugar content was determined by the titrimetric method described above. Repeat the experiment twice and take the average value.

Rancidity

Mixed 1.0ml of melted fat of the sample and 1.0ml of Conc. HCL in a test tube, add 1.0ml of 1% Phloroglucinol in diethyl ether and mixed thoroughly with the fat acid mixture. A pink color indicates that the fat is slightly oxidized, while a red color indicates that the fat is definitely oxidized.

Water soluble extractive

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled water, shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours. Cool in a desiccator and weigh. Repeat the experiment twice. Take the average value.

Table 3: Organoleptic parameters for Pugapaka.

Parameters	Results
Color	Dark brown
Odour	Characteristic
Taste	Sweet and Savory

Table 4: Standardisation parameters for Pugapaka.

Parameters	Results
Total sugar (%)	92.46
Reducing sugar (%)	13.28
Rancidity	Not oxidized
Water soluble extractive value (%)	78.83

High performance thin layer chromatography (HPTLC)

One gram of powdered samples were dissolved in 10 ml ethanol and kept for cold percolation for 24hours and filtered. 4, 8 and 12µl of the above samples of were applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (9.0:1.0). The developed plates were visualized in UV 254, 366 nm and then derivatised with Vanillin Sulphuric acid reagent and scanned under UV 254nm, 366 nm and 620nm. Rf, colour of the spots and densitometric scan were recorded.

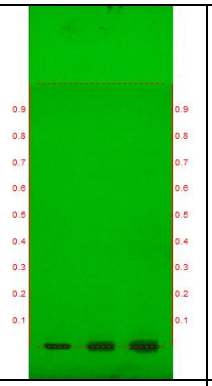	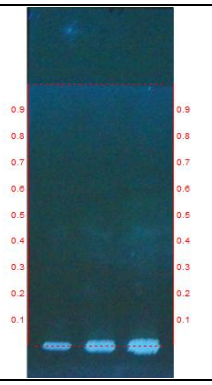	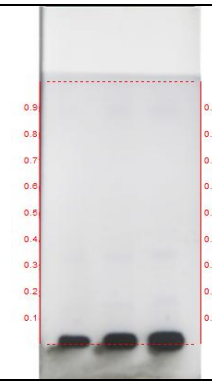	**Track 1Pugapaka -– 4µl** **Track 2Pugapaka-– 8µl** **Track 3Pugapaka-– 12µl** **Solvent system–Toluene: Ethyl Acetate (9.0: 1.0)**
Nil	**0.43 (F.blue),** **0.62 (F. blue)**	**0.15 (Purple),** **0.34(Purple),** **0.89(Purple)**	**R_f values** ***F – Fluorescent; L –Light; D – Dark**
Short UV	**Long UV**	**Post derivatisation**	

Figure 1: HPTLC photo documentation of methanol extract of Pugapaka.

DISCUSSION

The Pugapaka was prepared as per preparation of Avaleha kalpana. Puga by virtue of Vikasiguna, has potential to penetrate Sukshma Srotas (minute channels) and thereby reach the target organ. Shatavari consists of phytoestrogens which helps in endometrial bed development and cervical gland proliferation. It also has folliculogenesis action. Hence the Pugapaka was found beneficial in female infertility. Prepared Pugapaka was standardized through analytical study of physico-chemical and chromatographic parameters. Analytical constants ascertain the quality of product.

CONCLUSION

The analytical data generated here may be considered for the development of standard parameters for the formulation. Further studies may be carried out on Pugapaka based on identification and separation of active ingredients with the help of various biomarkers.On the basis of observations and experimental results, this study may be used as reference standard in the further quality control researches.

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