Short Communication Physico-Chemical profile of *Puga Khanda:* A Preliminary Study

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### Abstract



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**Background:** Herbal medicines are the oldest known form of medicine in the world. However, the quality control and the assurance still remains a challenge because of the high variability of chemical components. Herbal drugs, singlely or in combinations, contain numerous compounds in complex matrices in which no single active constituent is responsible for the overall efficacy. This creates a challenge in establishing quality control standards and the standardization of finished herbal products. Many formulations have been mentioned in Ayurvedic text for *Vrushyatwa* (aphrodisiac). *Puga Khanda* is one among such formulations. **Aim:** To develop preliminary physico-chemical profile of *Puga Khanda*. **Materials and Methods:** *Puga Khanda* was prepared in three batches as per the classical reference mentioned in *Bhaishajya Ratnavali*. The formulation was subjected for physico-chemical analysis, phytochemical analysis and Thin Layer Chromatography (TLC). **Results and Conclusion:** The study revealed that organoleptic characters, pH and extractive values of all 3 samples were almost equal. All the samples had 60% of sugar needed for preservation and 2/3<sup>rd</sup> of it was non reducing sugar. The total alkaloids ranged from 0.002 to 0.004% w/w. In TLC study the entire samples showed similar pattern except the 2<sup>nd</sup> sample of *Puga Khanda*.

Key words: Khanda Kalpana, pharmaceutical standardization, Puga Khanda, quality control

### Introduction

Herbal medicines are promising choice over modern synthetic drugs that show minimum or no side-effects and are considered to be safe. Generally, herbal formulations involve the use of fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety, and efficacy of the herbal product.<sup>[1]</sup>

However, a key obstacle, which has hindered the acceptance of the herbal medicines in the developed countries, is the lack of documentation and rigorous quality control. There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort towards standardization of the plant based medicines.<sup>[2]</sup>

Traditional Ayurvedic texts claim that Ayurvedic formulations prepared according to the method given in Ayurvedic text will

Address for correspondence: Dr. Pramod C. Baragi, #748, Charakalaya Near Bus-Stand, Bagalkot Cross Road, Bijapur - 586 101, Karnataka, India. E-mail: ayupramod@yahoo.co.in be superior in quality, safety, and efficacy.<sup>[3]</sup> Ayurveda is a time tested, trusted world-wide plant-based system of medicine and consist of various Ayurvedic formulations such as solid dosage forms like *Churna* (powders), *Vati* (pills), liquid dosage forms like *Asavas* (self generated alcohol beverages) and semisolid dosage forms such as *Ghritas* and *Avaleha Kalpana* (linctus).<sup>[4]</sup>

*Puga Khanda* falls under the category of *Avaleha Kalpana* of Ayurvedic formulations consisting of 26 ingredients<sup>[5]</sup> [Table 1]. Method of preparation for *Avaleha Kalpana* is very tedious and plays a vital role in quality and efficacy of any formulations.<sup>[6]</sup>

Hence for the development of preparation and process validation, three batches of *Puga Khanda* were evaluated for organoleptic parameters, phytochemical analysis, and physicochemical parameters (such as pH, loss on drying, extractive values, amount of reducing, non-reducing sugars and total alkaloid estimation).

## **Materials and Methods**

#### Procurement and preparation of drugs

Most of the raw drugs were collected from the pharmacy of the Muniyal Institute of Ayurveda Medical Sciences, Manipal. Fresh *Puga* was collected from farm of Dr. Satyanarayan Bhatt,

Khanda			
Ingredient	Latin name	Parts used	Quantity
Suddha Puga	Areca catechu Linn.	Nut	384 g
Godugdha	Cows milk	-	1536 ml
Goghrta	Clarified butter	-	192 g
Khanda Sarkara	Sugar candy	-	2 kg 400 g
Tvak	<i>Cinnamomum</i> <i>zeylanicum</i> Blume.	Stem, bark	6 g
Ela	<i>Elettaria cardamomum</i> Maton.	Fruit, seed	6 g
Tamalapatra	<i>Cinnamomum tamala</i> Nees.	Leave	6 g
Nagakeshara	Mesua ferrea Linn.	Stamen	6 g
Shunthi	Zingiber officinale Rose.	Dry Rhizome	6 g
Maricha	Piper nigrum Linn.	Fruit	6 g
Pippali	Piper longum Linn.	Dry fruit	6 g
Lavanga	<i>Syzegium aromaticum</i> (L.) Merr and Perry.	Flower Bud	6 g
Chandana	Santalum album Linn.	Heart wood	6 g
Jatamamsi	Nardostachys jatamamsi DC.	Rhizome	6 g
Talisapatra	<i>Abies spectabilis</i> (D. Don) Spach.	Leave	6 g
Kamala Bija	<i>Nelumbium speciosum</i> Willd.	Seed pulp	6 g
Nilotpala	Nymphaea stellata Willd.	Flowers	6 g
Vamsalocana	<i>Bambusa arundinacea</i> Retz.	Bamboos Manna	6 g
Shrungataka	<i>Trapa bispinosa</i> Roxb.	Fruit	6 g
Vidarikanda	Pueraria tuberose Roxb.	Tuberous root	6 g
Shveta Jiraka	<i>Cuminum cyminum</i> Linn.	Fruit	6 g
Gokshura	Tribulus terrestris Linn.	Fruit	6 g
Shatavari	<i>Asparagus racemosus</i> Willd.	Tuberous root	6 g
Malati Puspa	Jasminum sambac Linn.	Flower	6 g
Amalaki	<i>Emblica officinalis</i> Gacrtn.	Pericarp	6 g
Karpura	<i>Cinnamomum camphora</i> Nees.	Sublimate (natural)	12 g

 Table 1: Crude drugs used for preparation of Puga

 Khanda

Manipal. Malati Pushpa (Jasminum sambac Linn.) was purchased from Canara flower stores, Manipal. Fresh cow's milk and ghee were purchased from Nandini dairy, Manipal.

The authenticity of the species of herbs was checked and confirmed. The plant material was cleaned by sorting out using a cloth duster to remove dust and air blowing to remove minute sand particles. *Puga Khanda* was prepared by selecting raw materials of the same sources, by using similar methods in three batches.

#### Puga shodhana

Prior to the preparation of Puga Khanda, Puga was subjected for

Shodhana to remove the undesired constituents. 1 kg of Fresh *Puga* was taken and cut into small pieces. A clean cloth was taken and *Puga* pieces were kept in cloth and *Pottali* (bolus) was prepared. The *Pottali* was hanged up with the help of a rod in a steel vessel containing equal quantity of water (2 L) and milk (2 L). *Swedana* (boiling) was carried out on *Mandagni* (mild heat) for 3 h. After *Swedana*, *Puga* pieces were collected and washed with hot water and then dried in sunlight. Then the pieces were powdered in the *Khalva Yantra* and sieved through 60 no. sieve to obtain *Shodhita Puga Churna* (purified areca powder) [Figures 1 and 2].

#### Preparation of Puga Khanda

At first, fine powders of raw materials numbered 1, 5 to 23, 25 and 26 of formulation composition mentioned at Table 1 were prepared and kept aside. Malati Pushpa Kalka (paste of Jasminum sambac) was prepared. Shodhita Puga Churna (500 g) was taken in a large steel vessel and 2 L of Godugda (cow's milk) was added and kept on Mandagni on a gas stove, maintaining the temperature between 90°C and 95°C until a semisolid consistency is obtained. This is fried with 192 g Goghruta (cow's ghee) until it becomes dark color and ghee starts to ooze from the mass. In the meanwhile, 2 kg 400 g Khanda Sharkara is added with little quantity of water and Paka (cooked) was prepared on Mandagni of 2 thread consistency. To this Paka, the fried mass is added and mixed well with constant stirring. Heating was stopped when the Paka Siddha Lakshanas (features of perfection) appeared. However, stirring was continued until the Khanda Lakshanas (features of candy) appeared, i.e., 3-4 thread consistency. Then the fine powders of ingredients were added one by one and stirred well to get a homogenous mixture [Figure 3]. This Puga Khanda was analyzed by employing various analytical parameters.

#### Analytical study

- A. Organoleptic parameters: *Rupa* (colour), *Rasa* (taste), *Gandha* (odour) and *Sparsha* (touch)
- B. Physico-chemical parameters: pH of 5% aqueous solution, loss on drying at 110°C, methanol soluble extractive<sup>[7]</sup>
- C. Estimation of sugars: Total sugars, reducing, and non-reducing sugars<sup>[8]</sup>
- D. Estimation of total alkaloids<sup>[9]</sup>
- E. Chromatographic analysis: Thin Layer Chromatography (TLC).<sup>[9]</sup>

### 1<sup>st</sup> method for TLC

Methanol soluble extracts of the samples.

- Stationary phase: Silica gel GF 254 pre coated plates.
- Solvent system: Toluene: Ethylacetate: Diethylamine (70:20:10).

#### 2<sup>nd</sup> method for TLC

Chloroform soluble extract used for the estimation of total alkaloids.

- Stationary Phase: Silica gel GF 254 precoated plates
- Solvent system: Toluene: Ethylacetate: Diethylamine (70:20:10).

The developed plate were visualized by exposing to iodine vapors, short ultraviolet (UV) (254 nm), long UV (366 nm). The Rf values were recorded.



Figure I: Ashodhita Puga Phala



Figure 2: Shodhita Puga Phala



Figure 3: Puga Khanda

### **Results**

Comparative organoleptic characters were placed in Table 2, while physico-chemical observations were placed at Table 3.

Observations of TLC were mentioned in Tables 4-7.

## **Discussion**

Khanda Kalpana is a semisolid preparation intended for internal administration. It is considered as a variant of Avaleha Kalpana.

#### Table 2: Organoleptic parameters of Puga Khanda

		-	
Characters	Sample-1	Sample-2	Sample-3
Colour	Dark brown	Dark brown	Dark brown
Taste	Madhura+++ Kashaya+	Madhura+++ Kashaya+	Madhura+++ Kashaya+
Odour	Characteristic of <i>Karpura,</i> <i>Khanda,</i> <i>Dugdha</i>	Characteristic of <i>Karpura,</i> <i>Khanda,</i> <i>Dugdha</i>	Characteristic of <i>Karpura,</i> <i>Khanda,</i> Dugdha
Consistency	Semisolid	Semisolid	Semisolid

#### Table 3: Physico-chemical parameters of Puga Khanda

Parameters	APC	SPC	S-1	S-2	S-3
pH at 5% Aqueous soln.	-	-	5.00	5.85	5.52
Loss on drying at 110°C (%w/w)	-	-	8.92	11.34	9.40
Extractive values (%w/v)					
Methanol soluble extractive	13.2	13.58	6.61	6.18	6.49
Chloroform soluble extractive	10	4.5	5.36	5.19	5.17
Sugars (%w/w)					
Total			65	64	62
Reducing			20	18	17
Non-reducing			45	46	45
Estimation of total alkaloids (%w/w)	0.04		0.002	0.004	0.004
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APC: Ashuddha Puga Churna, SPC: Shuddha Puga Churna, S-1: Sample 1, S-2: Sample 2, S-3: Sample 3

## Table 4: TLC of methanol soluble extractives under short wave UV (366 nm)

APC (Rf)	SPC (Rf)	S-1 (Rf)	S-2 (Rf)	S-3 (Rf)
0.058	0.058	0.058	0.058	0.058
0.117	-	0.117	0.117	0.117
0.176	-	-	-	-
-	-	0.382	0.382	0.382
-	-	0.441	0.441	0.441
-	-	0.617	0.617	0.617
0.676	-	-	-	-
-	-	0.823	0.823	0.823

TLC:Thin layer chromatography, UV: Ultravilot, APC: Ashuddha Puga Churna, SPC: Shuddha Puga Churna, S-1: Sample 1, S-2: Sample 2, S-3: Sample 3, Rf: Retention factor

## Table 5: TLC of methanol soluble extractives under short wave UV (254 nm)

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A.P.C (Rf)	S.P.C (Rf)	S-1 (Rf)	S-2 (Rf)	S-3 (Rf)
0.058	0.058	0.058	0.058	0.058
0.117	-	0.117	0.117	0.117
0.176	-	0.176	0.176	0.176
0.382	-	0.382	0.382	0.382

TLC:Thin layer chromatography, UV: Ultravilot, APC: Ashuddha Puga Churna, SPC: Shuddha Puga Churna, S-1: Sample 1, S-2: Sample 2, S-3: Sample 3, Rf: Retention factor

*Khanda Kalpana* have other beneficial aspects in addition to palatibility.<sup>[10]</sup> These preparations have comparatively longer shelf life mainly due to less moisture, presence of sugar, ghee, and honey.<sup>[11,12]</sup>

All the three samples of *Puga Khanda* were subjected to analyse by using the selected parameters. The organoleptic parameters form the basic criteria for selecting a raw drug and also to confirm the finished product. All the three batches had similar desirable organoleptic features. The samples were of dark brown in color with semisolid consistency hence that they can be cut into pieces, they were aromatic with sweet and astringent taste. These preliminary findings indicate that in all three batches the products full fills the basic standards of *Khanda Kalpana*.

Sample  $1^{st}$  and  $3^{rd}$  had shown very similar moisture content, where as,  $2^{nd}$  sample showed a bit higher moisture. However, a range between 8.9 and 11.35% can be fixed as a desirable loss on drying.

The pH conventionally represents the acidity and alkalinity, samples had shown almost similar pH values ranging between 5 and 6, which is slightly acidic.

Methanol soluble extractives of all the 3 samples are very close to each other, which ranged between 6.18 and 6.61%. Chloroform soluble extractives were also almost uniform in all the 3 samples.

It was seen that total sugar content was above 60% in all the samples, which is sufficient for consistency and the preservation of the formulation and it was also observed that

Table 6: TLC of total alkaloidal fractions under long wave UV (366 nm)

APC (Rf)	SPC (Rf)	S-1 (Rf)	S-2 (Rf)	S-3 (Rf)
0.058	0.058	0.058	0.058	0.058
0.117	-	0.117	0.117	0.117
-	-	0.205	0.205	0.205
0.294	-	-	-	-
0.323	-	-	-	-
-	-	0.470	0.470	0.470
0.558	-	-	-	-
0.676	-	-	-	-
0.823	-	-	-	-

TLC:Thin layer chromatography, UV: Ultravilot, APC: Ashuddha Puga Churna, SPC: Shuddha Puga Churna, S-1: Sample 1, S-2: Sample 2, S-3: Sample 3

Table 7: T	LC of total	alkaloidal	fractions	under short
wave UV (	(254 nm)			

APC (Rf)	SPC (Rf)	S-1 (Rf)	S-2 (Rf)	S-3 (Rf)
0.058	0.058	0.058	0.058	0.058
0.176	-	0.176	0.176	0.176
0.264	-	0.264	0.264	0.264
-	-	0.708	0.708	0.708
0.764	-	-	-	-
0.823	-	0.823	0.823	0.823
0.852	-	0.852	0.852	0.852
0.900	-	-	-	-
0.923	-	0.923	0.923	0.923
0.976	-	0.976	0.976	0.976

TLC:Thin layer chromatography, UV: Ultravilot, APC: Ashuddha Puga Churna, SPC: Shuddha Puga Churna, S-1: Sample 1, S-2: Sample 2, S-3: Sample 3

about 1/3 was the reducing sugar and 2/3 was non-reducing sugar.

While observing the total alkaloids, all the three samples were compared with the *Shuddha* and *Ashuddha Puga Churna*. It was observed that all the samples had a small amount of total alkaloids ranging from 0.002 to 0.004%; however, surprisingly the samples of *Shuddha Puga Churna* did not yield any amount of total alkaloid. Hence, it is difficult to say that the alkaloids observed in *Puga Khanda* are of *Puga* mainly, but may be due to the presence of other ingredients. However, *Ashuddha Puga* showed the presence of alkaloids.

The methanol soluble extractives and the total alkaloidal fractions were subjected to TLC study by similar conditions. *Ashuddha* and *Shuddha Puga Churna* were also used for comparison. This can be considered as the reference standard for validating this formulation in future [Figures 4 and 5; Tables 4-7].



Figure 4:Thin Layer Chromatography of total alkaloidal fractions under long wave ultraviolet (366 nm).APC=Ashuddha Puga Churna; SPC=Shuddha Puga Churna; SI, Puga Khanda sample I; S2=Puga Khanda sample 2; S3=Puga Khanda sample 3



Figure 5:Thin Layer Chromatography of total alkaloidal fractions under short wave ultraviolet (254 nm) APC= Ashuddha Puga Churna; SPC=Shuddha Puga Churna; SI=Puga Khanda sample 1; S2=Puga Khanda sample 2; S3=Puga Khanda sample 3

## Conclusion

The study revealed that sufficient quality control parameters were maintained throughout the process of the preparation of *Puga Khanda* in all the three batches. All the three prepared samples were comparatively analyzed by following parameters such as organoleptic characters, physico-chemical parameters, chromatographic study, estimation of sugar, and alkaloids. The results obtained were very similar in each parameter in every sample. The chromatogram obtained in both methanol extractives and the total alkaloid fractions have indicated that TLC can be very useful parameter to fix the standards to *Puga Khanda*.

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# पूग खण्ड का गुणवत्ता नियंत्रण एवं मानकिकरण परीक्षण

## प्रमोद सी. बारगी, उमापति सी. बारगी, सत्यनारायण भट्ट, प्रदीपकुमार प्रजापति

काष्ठ औषधियों का ज्ञान पूर्वकाल से चला आ रहा है। इसका प्रयोग प्राचीन काल से ही रोग के निवारण हेतु एवं स्वास्थ्य संरक्षण के लिये किया जाता है। लेकिन गुणवत्ता नियंत्रण और गुणवत्ता परीक्षण अभी भी एक चुनौती बनी हुई है। हर्बल औषधियों मे एकल एवं कल्प औषधियों मे बहुत से रासायनिक घटक मिले होते है, उसमे समग्र प्रभाव के लिए एक सक्रिय घटक कारण नहीं होता है। यह गुणवत्ता नियंत्रण या मानकिकरण के लिए एक चुनौती है। आयुर्वेदीय ग्रन्थों में वृष्यत्व के लिए कई योगों का उल्लेख किया गया है। पूग खण्ड एक वृष्य योग है। वर्तमान अध्ययन में पूग खण्डका शास्त्रीय पद्धति से निर्माण कर उसका आयुर्वेदीय व आधुनिक परिक्षण किया गया। पूग खण्ड का मानकीकरण तीन चरणों में किया गया, और प्रत्येक प्रक्रिया के दौरान व पश्चात गुणवत्ता एवं मानकीकरण परीक्षण किया गया।