

# **Current Research in Pharmaceutical Sciences**

Available online at www.crpsonline.com



ISSN: 2250 - 2688

Received: 20/11/2021 Revised: 07/12/2021 Accepted: 10/12/2021 Published: 08/01/2022

#### Madhuri Sadashiv Pawar

Associate Professor
Department of Rasashastra Evum
Bhaishajyakalpana (Ayurvedic
Pharmaceutics and Pharmacology)
Bharati Vidyapeeth (Deemed to be
University), College of Ayurved, Pune,
India

#### Neelakantha Khadatar

Ayurvedic Physician
Department of Rasashastra Evum
Bhaishajyakalpana (Ayurvedic
Pharmaceutics and Pharmacology)
Bharati Vidyapeeth (Deemed to be
University), College of Ayurved, Pune,
India

#### Correspondence

# Dr. Madhuri Sadashiv Pawar

Associate Professor
Department of Rasashastra Evum
Bhaishajyakalpana (Ayurvedic
Pharmaceutics and Pharmacology)
Bharati Vidyapeeth (Deemed to be
University), College of Ayurved, Pune,
India

#### Email

madhuri.pawar@bharatividyapeeth.edu

DOI: 10.24092/CRPS.2021.110405

Website: www.crpsonline.com

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# Process Validation and GC Analysis of *Pathadisura*, a Fermented Ayurvedic Product used in *Shwas vyadhi* [Bronchial Asthma]

# Madhuri Sadashiv Pawar, Neelakantha Khadatar

#### **ABSTRACT**

Pathadisura [PS] is an ayurvedic fermented formulation, prescribed for respiratory disorders. It contains Suramanda prepared from (Rice grains), Guda (Jaggery) and herbal drugs viz Patha Murva, Rasna, Saral, Devdaru. The scientific preparation method and quality parameters of PS are not documented yet. Thus present study is aimed to develop and validate the preparation method and to generate quality standards for PS. Preparation of Sura, Suramanda and PS was done as per standard protocols of ayurvedic pharmaceutics blended with modern techniques. All herbal drugs, Sura and PS were tested with standard analytical methods. Obtained values for herbal drugs were within limits as per API standards, signify purity of plant materials. Study result provides organoleptic, physicochemical values and gas chromatography quality standards for Sura and Pathadisura. Standard operating procedure for preparation of Sura and Pathadisura is developed and validated by using standard methods of analysis. It could be used in further studies.

Key Words: Pathadi Sura, Sura, Annamanda, Fermentation, GC Analysis

# 1. INTRODUCTION

Sandhana kalpana is one of the herbal dosage forms wherein several formulations are prepared with fermentation technique. These poly herbal hydro-alcoholic formulations viz Sura, Asav-Arishta <sup>2</sup> are advocated in different disease conditions, owing to its greater shelf life, faster absorption and quick action. Pathadisura [PS] is ayurvedic fermented formulation prescribed in the treatment of respiratory disorders. It consists of Suramanda and herbal drugs viz Patha [Cissampelos pariera], Murva [Marsdenia tenacissima Wight.&Am], Rasna [Pluchea lanceolata Oliver & Hiem], Saral [Pinus roxburghii Sargent] and Devadaru [Cedrus deodara(Roxb)] 3. Sura is a type of alcoholic fermentation wherein rice grains are boiled in water and cooked nicely. Then kept for fermentation for specific time period and used for extraction of phyto-constituents from herbal drugs.

For preparation of *Pathadisura*, guidelines given in classical texts suggest to prepare intermediate fermented product [*Sura*] and keep powders of specific five herbal drugs in contact with supernatant liquid [*Suramanda*] for a whole night.<sup>4</sup> Application of this process might be helpful to extract water as well as alcohol soluble active bio-components of herbal drugs to achieve desired therapeutic effect of formulation. Till date no any publication on preparation and standardization is available on *Pathadisura*, though its therapeutics are documented in literature. Hence, the present study is aimed to prepare and validate the preparation method of *Sura* and *Pathadisura*. The method was developed and validated with physico-chemical tests and gas chromatography analysis.

#### 2. MATERIALS AND METHODS

Procurement of *Sahlishastik Tandula* [unpolished, reddish colored rice grains] and Guda [Kanak Organic jaggery] was done from authentic sources. Identification and selection of herbal drugs was done from ayurvedic physicians and botanists. Selected sample of each drug was authenticated using microscopic, physicochemical parameters as per API.

## 2.1 Preparation of Sura

Clean, properly washed 100g rice grains were taken in container, 1400 ml potable water was added and heated (70°C) for appropriate cooking of grains. Standard guideline for addition of Guda in preparation of Sura was considered.<sup>5</sup> Thus, 468g powdered jaggery was added and dissolved in 1200 ml Annamanda [Rice scum]. That homogenous mixture was poured in a dry, clean, glass container [sterilized with campden sanitizing solution] and corked properly to become airtight. The container was kept at isolated place in a room for fifteen days. Temperature was maintained at 25 to 27 °C and humidity was maintained at 60 to 70 %. Samples for physico-chemical analysis were withdrawn daily at morning, in laminar chamber with precautionary measures. On fifteenth day product was observed and tested as per ayurvedic parameters<sup>6</sup> Fermented product [Sura] was then filtered with standard filtering aid [Muslin cloth]. With the similar procedure three batches of Sura [S1, S2, and S3] were prepared and analyzed to validate of preparation method. The liquid clear supernatant part i.e. Suramanda was collected after seven days.

# 2.2 Preparation of Pathadisura

Herbal ingredients of *PS* were cleaned and reduced separately to 80 mesh size powder. All powdered drugs were then mixed in equal quantity to formulate homogenous mixture. 50 g of mixture was mixed well with 500ml *Sura*manda to avoid clusters and poured in sterilized glass container. The container was sealed tightly and as per ayurvedic classical guideline, kept at isolated place for one night [7 pm to 7am]. Next day morning it was filtered with laboratory ordinary filter paper and was stored in amber colored glass bottles named as *Patahdisura*. Consecutive three batches of *PS* [P1, P2, and P3] were prepared with the similar method.

# 2.3 Analytical testing of Sura and Pathadisura

Estimation of pH, specific gravity, alcohol % and total reducing sugar % of three batches of *Sura* was carried out to determine the changes taken place during the fermentation process.

Obtained values are represented in figures. Viscosity and solid content of all three batches of *Sura* were evaluated using Ostwald's viscometer and water bath. All samples of Sura were further subjected to GC analysis using Agllient Model [6890 plus] instrument RTX [Stabilwax DA column 60m x 0.25 mm ID X 0.25 micron thickness].

Final product testing of *Sura* was carried out with organoleptic tests.

Likewise three batch samples of *Pathadisura* were tested with organoleptic tests and physico-chemical constants. Comparative analysis was carried out for three batch samples of *Sura* and *Pathadisura* 

## 3. RESULTS

The results of the physico-chemical analysis of raw herbal materials are shown in table 1.

Values were complied with API standards. pH and moisture content of all ingredients of PS are established in the study.

Analysis of [Guda] Jaggery: Sample of jaggery showed brownish color, characteristic taste and odor. It had negligible foreign matter, 3.80 % of moisture, 5.12 pH and 7% TRS.

Three samples of *Sura* showed alike characteristics, were transparent and clear, having faint brown color, sweetish pungent taste with mild alcoholic odour.

Analytical values for three batch samples of *Sura* has shown in figures. Gradual decrease in pH was noted from first day to tenth day and then was almost stabilized at 3.5 [Figure 1]. Values of specific gravity was changed from fourth day; it was not precisely noteworthy [Figure 2]. Gradual increase in the percentage of alcohol was seen upto tenth day and after that it became almost steady.[Figure 3].Change in total reducing sugar was observed from the first day of experiment, percentage of sugar was steadily decreased from first day up to fifteenth day. [Figure 4] Significant difference in percentage of total reducing sugar was not seen in three batch samples of *Sura*.

Viscosity for three samples was 7.76, 7.69 and 7.64; while values for solid content was 4.48, 4.50 and 4.55, didn't show major variation. Three batch samples of *Sura* exhibited comparable results for pH, total reducing sugar, specific gravity, alcohol %. Gas chromatography records of three samples of *Sura* are stated in Table 2 indicates the presence of eleven alcohols and eight volatile

acids. The difference in values for some of the alcohols and acids are noted here.

#### 3.1 Analysis of Pathadisura

It was observed that organoleptic characteristics and pH, specific gravity, solid content, viscosity percentages remained stable for all three samples of *PS*.

Three batches of *PS* showed comparable characteristics, possesses faint brown color, sweetish astringent taste and mild aroma. pH values ranged between 3.84 to 3.95, specific gravity was alike [1.02], viscosity values were in between 5.14 to 5.23 while solid content for all samples were almost comparable.

## 3.2 Comparative analysis of Sura and Pathadisura:

Comparison in organoleptic characteristics of *Sura* and *Pathadisura* revealed change in color and odor. Light brown color and mild alcoholic odor of *Sura* was changed to dark brown and mild aromatic in *PS*. Change in percentage of solid content in *PS* as compared to *Sura* is found noticeable.

#### 4. DISCUSSION

Consensus method used to identify raw herbal drugs, is believed to be fruitful. Analytical values for each herbal drug were within standard limits, suggestive of identity and purity of all ingredients. In our previous experiment we observed contamination during the preparation of fermented product, thus -instead of clay pots, glass containers and autoclaved sterilized instruments were used, at the same time temperature and humidity was maintained throughout the procedure. Aforementioned applications have assisted to formulate non contaminated product *Sura* and it attained ideal taste and faint brown color.

pH is the factor which strongly controls chemical and biological stability of fermented product. The acidic nature of *Sura* [3.85] might be playing important role to protect the said product from contamination. Rate of fermentation reaction, survival of specific microorganisms and alcohol percentage of formulation depends on sugar concentration of the mixture. Here, it was noticed that fermentation process begins by addition of 39 % jaggery to rice scum. However, it was also observed that without addition of wild or chemical yeasts, fermentation process was initiated in first two -three days and completed within fifteen days. It can be said that starches of rice scum and sugars in jaggery are considerably required as a source of nutrition and food for growing of specific microorganisms. Thus quantity of jaggery used in this study helped to formulate self-generated alcohol in acceptable limit in hydroalcoholic product [Sura].

Gradual reduction in sugar percentage was seen in three samples of *Sura*, contrary to this, alcohol percentage was gradually increased over the period of fermentation and become stable Decrease in sugar percentage as well as specific gravity and increase in alcohol percentage seems to be reciprocal to each other which were resulted in the study.

Alcoholic products contain a wide range of volatile compounds, including alcohols and short-chain aldehydes. Gas chromatography (GC) is a powerful tool in the analysis of alcoholic products to determine the volatile profile of a product. In present study, GC analysis of three samples of *Sura* showed presence of twelve alcohols and eight acids. Carbonyl compounds are the most volatile substances found in alcoholic preparations. Acetaldehyde is the principal carbonyl compound has been found in these preparations at (0.1–16.4 mg/l) and 37.2 mg/l levels. In earlier works, 2, 3-butanedione content of alcoholic preparations formulated from malt has been determined and it varies from 0.01 to 0.2 mg/l, and as high as 0.63 mg/l. Thus it can be interpreted that the variation seen in values of acetaldehyde and 2,3 Pentanedione of three samples of *Sura* are not varying remarkably.

Volatile and non -volatile acids were estimated in this study for *Sura*, however we couldn't find any rational explanation for variation seen in the quantitative data for Propionic Caproic, iso-Caproic, Heptonic acids. According to WHO (2014), methanol concentration of 6-27 mg/l in alcoholic products prepared from malt, corn, rice and hops is not harmful. It is also said that a small amount of methanol is found in alcoholic products as a part of the natural fermentation process. This small amount of methanol does not cause any harmful effect. Methanol, found in *Sura* samples was within permissible limit [1.0 ppm (1mg/l)]. Hence it can be inferred that *PS* formulation prepared with this specific method may not produce any untoward effects when consumed with standard ayurvedic protocol[ in diluted form, formulation mixed equal quantity of with water]

It is summarized that the thought put by ayurvedic physicians for designing of *Pathadisura* is replicated as a unique method of preparation. This method resembles with preparation of tinctures where herbal extracts made by soaking the bark, leaves (dried or fresh), or roots from one or more plants in alcohol. <sup>11,12</sup> It is also reviewed that hydro-alcoholic formulations probably results into transformation of several phyto-chemicals of herbal drugs and facilitate dilation of vessels and rapid absorption before metabolism. <sup>13</sup> Thus, it can be said that *Pathadisura* formulated with this specific method may produce cumulative effects to cure respiratory ailments [*Shwas* (Bronchial asthma) , *Kasa* (cough)] and show restorative actions.

# 5. TABLES AND FIGURES

Table 1: Analytical values of contents of Pathadisura

Sr.No	Test	Shastik a shali Tandul a	Patha	Murva	Rasna	Saral	Deva daru
1.	Foreign Matter	Negligi ble	Negligi ble	Negligi ble	Negligi ble	Negli gible	Negli gible
2.	Moisture content	2%	8%	8.5%	9%	7.5%	7%
3.	Total ash	4.56%	6%	4%	18%	1%	1.5%
4.	Acid insoluble ash	3.2%	0.86%	0.39%	6.39%	0.31%	0.07%
5.	Alcohol soluble extract	2.3%	14.40%	8%	11.2%	6.4%	10.4%
6.	Water soluble extract	2%	16%	15.2%	24.8%	1.6%	2.4%
7.	pH value	6.02	6.36	6.5	6.68	6.31	6.39

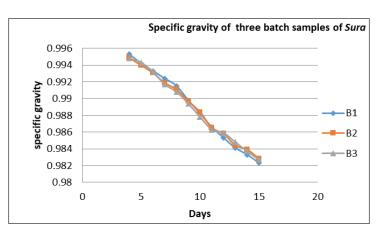


Figure 2: Comparative determination of Specific Gravity of three samples of *Sura* 

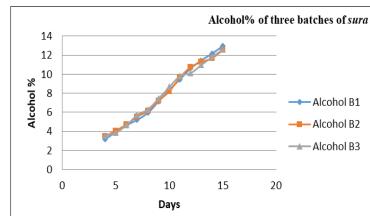


Figure 3: Comparative determination of Alcohol percentage for three samples of *Sura* 

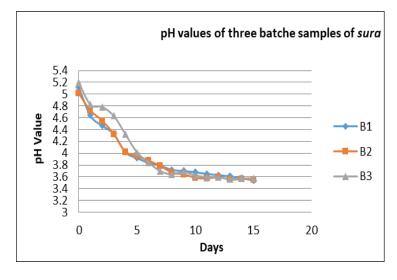


Figure 1: Comparative determination of pH of three samples of *Sura* 

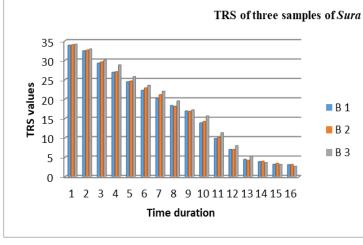


Figure 4: Comparative determination of TRS values of three samples of *Sura* 

Table 2: Gas chromatography analysis of Sura

Sr.	Alcohols in ppm		Batch 2	Batch 3	
No.		Batch 1			
1	Acetaldehyde	8.7	12.6	3.5	
2	Acetone	0.39	0.57	0	
3	Ethyl acetate	0.3	0.28	0.28	
4	Methanol	1.1	0.72	1.07	
5	2-Butanol	0.1	0.059	0	
6	1-Propanol	2.31	2.5	1.9	
7	2,3 Pentanedione	0.0	2.8	0	
8	n-butyl acetate	0.3	0.17	0	
9	Iso-butanol	14.61	14.03	11.99	
10	1-Butanol	0	0.11	0.21	
11	Iso-amyl alcohol	87.28	84.29	82.03	
12	1-pentanol I	108	106	106	
No.	Volatiles Acids in		Batch 2	Batch 3	
	ppm	Batch 1			
1	Acetic acid	51.39	46.80	48.84	
2	Furfural	00	0.62	00	
3	Propionic acid	8.65	4.3	1.95	
4	n-butyric acid	3.1	3.4	4.52	
5	iso-caproic acid	0.74	00	3.47	
6	Caproic acid	4.4	00	6.57	
7	Heptonic acid	0.9	3.49	00	

# 6. CONCLUSION

Standard operating procedures for *Sura* and *Pathadisura* have been developed and validated using laboratory methods. The study provides preliminary quality control data for *Sura* and *Pathadisura*. The demonstrated method of preparation would be taken up for the manufacturing of *Pathadisura* for the specific quoted reference.

#### 7. FURTHER SCOPE OF STUDY

Preclinical and clinical experiments would be carried out to assess safety and efficacy of the formulation.

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