A Comparative Pharmaceutico-Analytical Study of Punarnasava and Punarnavarishta

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Abstract: Asava and arishta are parts of Sandhan kalpana mentioned with Ayurvedic texts. Differences in pharmaceutical processing and biochemical changes of these two formulations are not studied. Present study shows mild differences in pH, quanities of alcohol, reducing sugar etc. But differences are not so much to suggest for commercial applications. Changes in chemical differences are observed as calcium percentage varied from 2.52% to 2.84% for selected formulations as Punarnavasava and Punarnavarishta respectively.

Keywords: Asava, Arishta, Punarnavasava, Punarnavarishta, Reducing sugar, pH, calcium, alcohol.....

Sandhan kalpana is one of panchvidha kashaya kalpana. It is process of ornamentation of drugs with fermentation to alcohol[1]. Asava and arishta are preparations of sandhan kalpana where fermentation occurs. Sharandhara samhita, madhyam khanda (10/2) differentiates asava and arishta as asava is prepared by fermentation of ambu (water) of pakwa drugs i.e., swarasa while arishta is fermented preparation of decoction of drugs[2]. Many of formulations are there in both categories as *Vasarishta-Vasasava, Patolasava-Patolarishta, Punarnavasava-Punarnavarishta* etc. with or without changes of components. Both are fermented products. Fermentation is a biochemical process for preservation[3]. The phenomenon occurring in the process is ethanolic fermentation in which ethanol is generated in two steps using TPP and $NADH_2$ as coenzymes[4]. In Both cases of asava and arishta sugar is converted to alcohol. They contain functional components either originating from the ingredients or generated during the process of fermentation. Fermentation also increases the digestibility, bioavailability, micronutrient contents etc[5]. *Punarnavasava* and *Punarnavarishta* both are used for abdominal problems, inflammation, liver problems etc. and they can be prescribed in multiple of other complaints as additional medicines along with principle medicines. Present work is selected with view of understanding that if basic forms of drugs viz., swarasa or decoction interfere with process of development of asava and arishta when the components of both asava and arishta remain same.

Materials and Methods: Punarnasava was prepared by process mentioned with Bhaisajya Ratnawali[6] whereas Punarnavarishta was formulated using same ingredients of Punarnavasava according to Anuktmaan vidhi mentioned with Sharangdhar Samhita Madahyam khanda 10/3-4[2]. Only difference in preparation was use of decoction of drugs in Punanavarishta.

A. Ingredients: 1 pala each of shunthi, pippali, maricha, haritaki, bibhitaki, amalaki, brihati, kantakari, daruharidra, gokshura, vasa, eranda moola, katuki, gajapippali, punarnava, nimba, guduchi, shushka mulaka, durlabha and patola; 16 pala of draksha; 1 and ½ tula of sita and madhu respectively and 2 drona of jala.

B. Process of Preparation: All mentioned drugs of pharmacopeial qualities were taken. Fumigation of sandhan pot (40 litres capacity) was done with 10 gm each of agaru, chandana, maricha, haridra, mamsi, guggulu and karpura. The lepana with ghrita was done on inner side of pot. Sandhan patra was kept inside dark room previously cleaned and fumigated in between uniformly distributed husk. Mentioned quantity of draksha was first washed and

frenched in water overnight (12 hours). Next day they were grinded to fine paste. The paste was dissolved in drava dravya. The coarse powder of other mentioned drugs were taken in given quantity. Before that previously boiled and cooled water was taken. Given quantity of khanda sharkara was dissolved in water and it was filtered later. Thereafter, coarse powder of drugs was added and stirred. After cooling for sometimes, it was added with noted quantity of madhu. It was stirred well and later dhataki in given quantity was added on top and it was mixed well.

C. Covering and sealing of pot: The mouth of pot was tied with previously cleaned and dried double layered cotton cloth. The mouth was closed with its lid and it was sealed air-tight with help of mud plastering technique. Boiled and cooled water was used during plastering to avoid contamination.

D. Observation: Colour, odour, quantity of dravya before and after addition of sandhaan dravya were checked before sealing and later pot was left undisturbed. For checking the progress of rate of fermentation, candle test and lime water test were used. Observations were done in four stages viz., initial stage of preparation, before onset of fermentation, after onset of fermentation and after the completion of fermentation. After completion of fermentation, supernatant liquid was filtered using double layered cotton cloths and it was brought to chemical analysis in quality control laboratory of ALNRMAMC, koppa.

Chemical Analysis: The chemical analysis was done under three headings as:

a. Physicochemical analysis
b. Preliminary phytochemical tests
c. Quantitative test

A. Physicochemical analysis[7]:

i. **pH determination:** pH meter *Chemiline CL-120* was used for the purpose. It was calibrated using buffer of pH 4.00 and pH 9.20 (*Nice Chemicals*). Then both samples were observed for pH.
ii. **Specific gravity:** It was determined using specific gravity bottle at 25ºC.
iii. **Total solid:** 50 ml of both drugs were taken in previously weighed evaporating dishes. They were applied with heat on water bath till liquid completely evaporates. The remaining solid weights were taken and percentage for both was calculated w/v.

B. Preliminary phytochemical tests[7,8,9,10,11]:

The tests were performed for carbohydrate, protein, alkaloids, flavonoids, anthraquinone glycosides, saponins, tannin and terpenoids.

C. Quantitative test[9]:

i. **Alcohol content:** 100 ml of each sample was taken. Now they were added with sufficient quantity of NaCl. Now they were added with light petroleum and were shaken for 2-3 minutes. To confirm the alkalinity of solution, it was tested using phenolphthalein as indicator. After 30 minutes, 100 ml of water was added and it was distilled. 90 ml of distillate was collected. The distillate was measured for specific gravity. The percentage of alcohol was measured after matching with table given with book corresponding to specific gravity.

ii. **Reducing sugar:** First both samples were clarified by adding 10 ml of solution I (21.9 gm zinc acetate in 3 ml glacial acetic acid making up to 100ml) and 10 ml of solution II (10.6 patassium ferrocyanide in 100 ml of water). 10 ml of Fehling's solutions were taken in conical flask. It was added with sample in burette drop wise. After addition of every ml, it was heated to boil over asbestos covered with gauge. It was continued till copper was reduced and brick red colored appeared. Then 3-5 drops of methylene blue as indicator was added and was titrated till permanent brick red colour appeared. The proportion of reducing sugar was calculated from table.

iii. **Total Sugar:** An aliquot of clarified solution was taken with 15 ml HCl diluted to 150 ml. It was boiled for 2 minutes and was cooled. Neutralization was tested using phenolphthalein as indicator by 10% NaOH. Now the experiment was repeated as done for reducing sugar.

iv. **Estimation of calcium, sodium and potassium:** Flame Photometer, Systronic 128 and FPM Compressor 126 was used for determination**.** 1000 ppm of standard solutions was prepared by sodium chloride, potassium hydroxide and calcium chloride(Chemicals used for standard were of Nice chemicals). Sodium and Potassium were prepared for calibration at concentration of 80 ppm, 60 ppm, 40 ppm and 20 ppm while calcium was prepared with concentration 80 ppm, 60 ppm and 40 ppm. After calibration, the solution containing different concentrations of sodium chloride, potassium chloride and calcium were introduced to find the intensity of the emitted light of each solution. A calibration graph between concentration and intensity of the solutions were drawn for each case. Finally the samples of Punarnavasava and Punarnavarishta were brought to find out the intensity of emitted radiation. From the intensity displayed with screen, the concentration of calcium, sodium and potassium were determined.

Result:

Observation:

Both Punarnavasava and Punarnavarishta revealed similar results except in colour as it was darker in arishta.

	Initial stage of preparation	**Before onset of fermentation**
Colour	Dark brown	Dark brown
Taste	Bitter, astringent, sweet	Bitter, astringent, sweet
Temperature	28°C	28°C
	After onset of fermentation	**After completion of fermentation**
Colour	Dark brown	Dark brown
Odour	Mild alcoholic	Alcoholic
Taste	Bitter, astringent, sweet	Sweet, bitter, astringent
Effervescence	Positive	Positive
Burning candle test	Stopped burning	Continued burning
Lime water test	Milky white	No milky white

Temperature	28.5°C	28°C

Total yield, percentage of loss with respect to drava dravya

	Yield	Percentage of loss
Punarnavasava	: 32.26 liters	7.00%
Punarnavarishta	: 31.24 liters	10.37%

Days taken for completion of fermentation

Punarnavasava	: 30 days
Punarnavarishta	: 35 days

Chemical Analysis

A. Physicochemical analysis

	Punarnavasava	Punarnavarishta
pH	3.87 $\pm$ 0.10	3.90 $\pm$ 0.10
Specific gravity	0.9963	1.1140
Total Solid	12.60%	29.88%

B. Preliminary phytochemical tests

	Punarnavasava	Punarnavarishta
Carbohydrate	Present	Present
Protein	Absent	Present
Alkaloid	Present	Present
Anthraquinone glycoside	Present	Present
Cardiac glycoside	Present	Present
Tannin	Present	Present
Flavonoids	Present	Present
Saponin	Present	Present
Terpenoids	Present	Present

C. Quantitative tests

	Punarnavasava	Punarnavarishta
Total alcohol	14.82%	13.60%
Total sugar	11.92%	9.17%
Reducing sugar	11.11%	8.45%
Calcium	2.52%	2.84%
Sodium	0.012%	0.015%
Potassium	0.16%	0.16%

Basic differences between asava and arishta lie with using of forms raw materials. Swarasa is sap of plants and the sap contains chemical constituents in its original forms while decoction is aqueous extract obtained heat extraction method. Hence, chances of denaturation or conversion of forms of compounds are present in decoction. Detailed study with nature of changes and effects of these changes on therapeutic effects are still under questions. Studies reveal the increased bioavailability of fermented products and both of these are fermented formulations[5,12,13]. Actually process of fermentation is temperature dependent. Optimum temperature accelerates the rate of fermentation. Increased rate also invites biochemical changes for number of new compound formation with basic moieties or sometimes with change of them too[14].

In both cases, *Woodfordia* flowers were used as agent of fermentation. A study shows the presence of yeast (*Saccharomyces cerevisiae*) with flowers. The same study has shown the isolation along with biochemical and genetic characterization of yeasts from *Woodfordia* flowers[15]. The reason is apparent why in *Ayurvedic formulations*, this flower is suggested to bring the process of fermentation.

pH after fermentation decreases and such decline in pH is more observed when pot is sealed and kept in dark with optimum temperature required for process[16]. Decreasing pH (acidic) invites better solubility of minerals[17]. Both asava and arishta are rich in minerals and vitamins and have acidic pH too. pH is not even too acidic to harm the gastrointestinal environment. Hence for better absorption and actions, both products can be used in place of churna or other forms. No much differences could be observed based on selected parameters for comparative study, still options with details of process and chemical compounds are always there as newly formed compounds were not examined in this study.

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