Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(1); 144-149

ISSN: 0975-4873

Research Article

A Comparative Study of Prepared and Marketed Asokarista With Respect To Physicochemical Parameters and Phytochemical Markers

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Available Online: 1st February, 2015

ABSTRACT

In the present study, the quality control parameters specified in API were evaluated for two reputed commercial brands in comparison to laboratory formulated preparation. All the formulations were estimated for lyoniside and lyoniresinol content at various time intervals. The formulation was prepared by decocting the bark with water and fermenting it with *Woodfordia fruticosa* flowers. The samples were kept at ambient conditions. It was analyzed during processing (Decoction), at beginning of storage and then at 30, 60 and 90 days. The physicochemical parameters specified in API were evaluated and assay was done in terms of phytochemical marker (Lyoniside and lyoniresinol) of main component, *Saraca asoca* using validated HPLC method. The formulation completed in 8 days and it complied with pharmacopoeial limits. The physicochemical properties of the formulation modify with the progress in days. Lyoniside was present in decoction however its content decreased during fermentation. Upon storage, the content of lyoniresinol increased. The authors conclude that use of incubator produces identical formulation in lesser duration and during the processing, chemical marker of main component, *Saraca asoca* converts to its aglycone, Lyoniresinol. Also substantial quantity of elemental iron and calcium has been detected in the formulations, a reputed Gynecological medicine.

Keywords: HPLC; Fermentation; Arista; Physicochemical; Lyoniside; Lyoniresinol.

INTRODUCTION

Asokarista , a classical herbal Ayurvedic formulation is mentioned for Gynecological disorders. It contains *Saraca asoca* (Bark), *Woodfordia fruticosa* (Flower), *Cuminum cyaminum* (Fruit), *Cyperus rotundus* (Rhizome), *Zingiber officinale* (Rhizome), *Berberis aristata* (Stem), *Nymphea stellata* (Flower), *Terminalia chebula* (Fruit pericarp), *Terminalia belerica* (Fruit pericarp), *Phyllanthus emblica* (Fruit pericarp), *Mangifera indica* (Endosperm), *Adhatoda vasica* (Root) and *Santalum album* (Heartwood) along with jaggery. The quantity of Ashoka bark is 100 times then other herbal ingredients. It has been indicated for dysmenorrhoea, menorragia in ayurvedic treatises[1].Arista is example of self-fermented herbal or herbo-mineral preparation having improved stability due to the presence of alcohol. These formulations are formulated by fermenting the infusion / decoction using jaggery, *Woodfordia fruticosa* or *Madhuca indica*. The process employed in formulation of Arista is termed as Sandhan kalpana[2]. It consists of fermentation of substrate which is a herbal/ Herbomineral extract by means of traditional inoculum provided by flowers of *Woodfordia fruticosa*/ *Madhuca indica* [3]. The alcohol produced in this process acts as a solvent and as a preservative. In the present work the changes occurred in quality control parameters and phytochemical marker has been evaluated.

MATERIALS & METHODS

Material

All the plant materials were purchased from local market and authenticated by Dr. H. B. Singh, Raw Materials Herbarium & Museum, National Institute of Science Communication and Information Resources, New Delhi. A voucher specimen (PRL/JH/11/11) has been kept in Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi. All the chemicals used in analysis were from Merck. The weighing procedures were done using electronic balance from Citizen. Digital pH meter (Labindia) was used in pH determinations. Spectroscopic analysis was done using double beam spectrophotometer (UV 1800) from Shimadzu. HPLC analysis was performed using Shimadzu system equipped with LC-10AT VP pump, a SIL-10AF auto injector, an SPD-10A UV-VIS detector, and an SCL-10A VP system controller (Shimadzu, Japan). Marker compounds (Lyoniside and lyoniresinol, Purity > 95%) were procured from Chemfaces Pvt. Ltd, Wuhan, People's Republic of China.

Methods

Preparation of arista: The composition given in Table 1 was followed. *Saraca asoca* bark was powdered and passed through 44 no. sieve. Specified quantity of double distilled water was added and decoction was prepared. It was then filtered using muslin cloth. Jaggery was powdered, dissolved in decoction and the resultant mixture was again filtered. All other herbal components (except *Woodfordia fruticosa*) were powdered, sieved through sieve no. 85 and added to the decoction. *Woodfordia fruticosa* flowers were rinsed in sterile water and then

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Table 1: Composition of formulation given in API and used for study

S.No.	Name of Plant	Botanical Name	Part used	Quantity prescribed	Quantity used
1.	Asoka API	*Saraca asoca*	Stem Bark	4.800 kg	480 g
2.	Water for decoction	-	-	49.152 L (reduced to 12.288 L)	4.9152 l (reduced to 1.288 L)
3.	Guda API	-	-	9.600 kg	960 g
4.	Dhataki API	*Woodfordia fruticosa*	Flower	768 g	76.8 g
5.	Ajaji (sveta jiraka) API	*Cuminum cyaminum*	Fruit	48 g	4.8 g
6.	Mustaka (Musta) API	*Cyperus rotundus*	Rhizome	48 g	4.8 g
7.	Sunthi API	*Zingiber officinale*	Rhizome	48 g	4.8 g
8.	Darvi(Daruharidra) API	*Berberis aristata*	Stem	48 g	4.8 g
9.	Utpala API	*Nymphea stellata*	Flower	48 g	4.8 g
10.	Haritaki API	*Terminalia chebula*	Pericarp	48 g	4.8 g
11.	Bibhitaka API	*Terminalia belerica*	Pericarp	48 g	4.8 g
12.	Amalaki API	*Phyllanthus emblica*	Pericarp	48 g	4.8 g
13.	Amrasthi API	*Mangifera indica*	Endosperm	48 g	4.8 g
14.	Sveta jiraka API	*Cuminum cyaminum*	Fruit	48 g	4.8 g
15.	Vasa API	*Adhatoda vasica*	Root	48 g	4.8 g
16.	Sveta candana API	*Santalum album*	Heart Wood	48 g	4.8 g

Table 2- An account of various physicochemical parameters in aqueous decoction, prepared formulation (PF-A) and commercial formulations (CF-1 and CF-2).

Day	pH	Specific gravity	Total solid content (%)	Total phenolic content	Total alcohol content	Calcium content (mg/100ml)	Iron content (mg/100ml)
Decoction	4.1 ±0.05	1.12 ±0.005	18.7 ±0.51	0.025 ±0.002	0	738±12	12.49±7.02
Prepared formulation (PF-A)	3.3± 0.05	1.074 ±0.004	11.2 ±0.42	0.082 ±0.006	8±0.5	3520±17	64.7±15
Commercial formulation-1 (CF-1)	4.2±0.05	1.02±0.002	12.2±0.46	0.066±0.004	8.5±0.76	3600±21	63.1±14
Commercial formulation-2(CF-2)	4.4±0.05	1.06±0.005	13.4±0.52	0.064±0.005	9.5±0.6	3767±22	61.0±1.35

Table 3: Quantitation of lyoniside and lyoniresinol in various samples

	ASE	PF-A	CF-1	CF-2
Lyoniside	13.98±0.4	1.42±0.04	5.01±0.05	4.2±0.08
Lyoniresinol	2.49±0.05	18.64±0.8	23.11±1.1	21.44±1.2

added to decoction mixture. The mixture was kept at 30±2° C in incubator for 8 days [1].

Physicochemical characterization

The pH was determined by using calibrated digital pH meter. The specific gravity was determined by weight difference method using 25 ml sample. Twenty five ml water was taken into a clean, dry pycnometer and weight

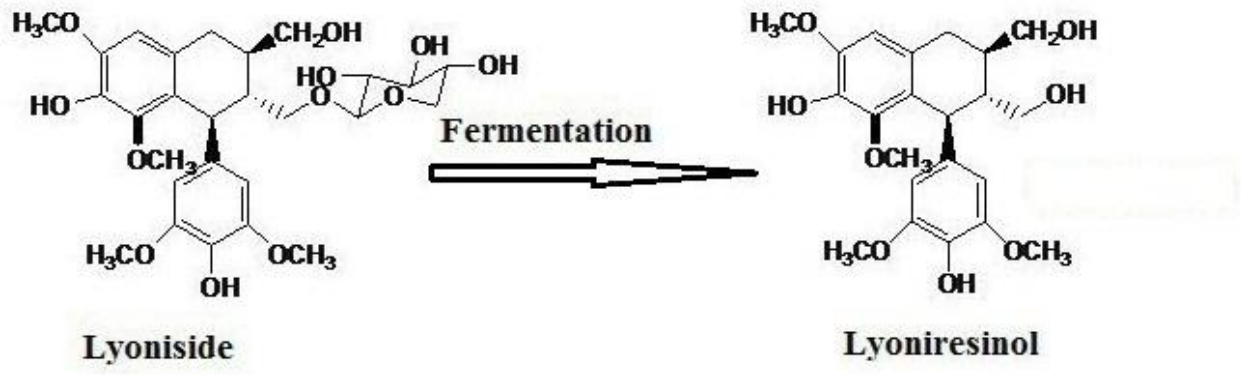

Figure 1. Conversion of Lyoniside to lyoniresinol

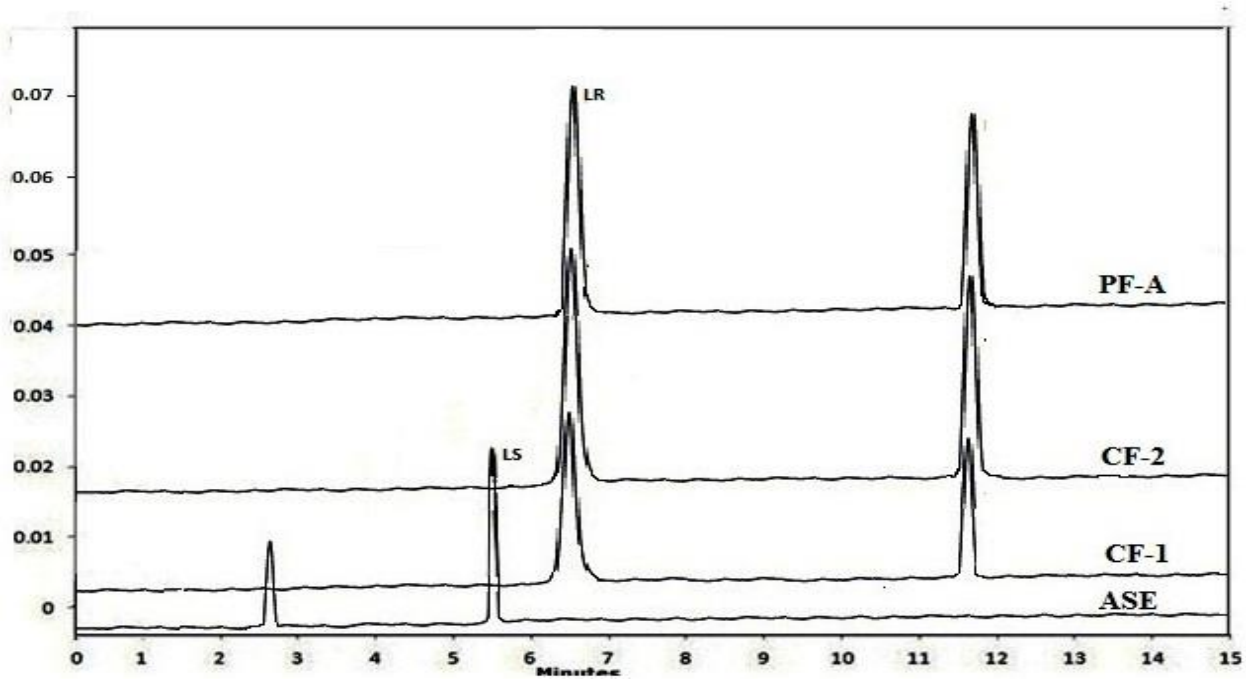

Figure 2. HPLC chromatogram of Aqueous decoction (ASE), prepared formulation (PF-A) and commercial formulations (CF-1 and CF-2)

was recorded. Then it was emptied and dried. Further 25 ml decoction (Fermented/non fermented) was filled into it. Its weight was recorded. The specific gravity was calculated by dividing density of test sample at 25^0C by density of water at 25^0C.

Total solid content was determined by evaporating 50 ml sample to a thick extract ,extracting with four equal quantities (10 ml) of ethanol. The extract was evaporated, dried Diatomite (1 g) was added and the mixture was dried at 105 ºC till constant weight. The solid content was obtained by deducting the weight of diatomite and calculating with reference to the volume taken[1].

For determination of total phenolics, suitable aliquot was taken and diluted with water. Then, 0.25 ml Folin-Ciocalteu reagent (1N) and 1.25 ml of the sodium carbonate solution (20%) were added; the mixture was vortexed and kept for 40 min. It was then analyzed spectrophotometrically at 725 nm taking tannic acid as standard [1]. The alcohol content was determined by pharmacopoeial method. 25 ml sample was diluted with 150 ml water and distilled to obtain approximately 100 ml distillate. The distillate was saturated with sodium chloride and partitioned with n-hexane (100 mlX1). Hexane layer was washed with saturated solution of sodium chloride and washings were added to partitioned distillate. Then it was made alkaline with 1M sodium hydroxide, diluted with water and distilled to give 90 ml distillate. Then relative density of distillate was determined and alcohol content was determined[1,4].

Total reducing sugar was determined in terms of dextrose using nelson-somogyi method. 0.5 mg / ml solution of dextrose was prepared (Solution A) and aliquots of 1ml to 10 ml were taken. It was diluted to 10 ml with water (Solution B). From this, 1 ml sample was taken from each tube and 1 ml of alkaline reagent was added. This mixture was heated for 20 min and then cooled. Further, 1 ml of arsenomolybdic acid reagent was added and diluted to 10 ml with water. The mixture was then analyzed spectrophotometrically at 520 nm [1].

Non reducing sugar was determined by subtracting reducing sugar from total sugar. Total sugar was determined by hydrolyzing all sugar content using acid and

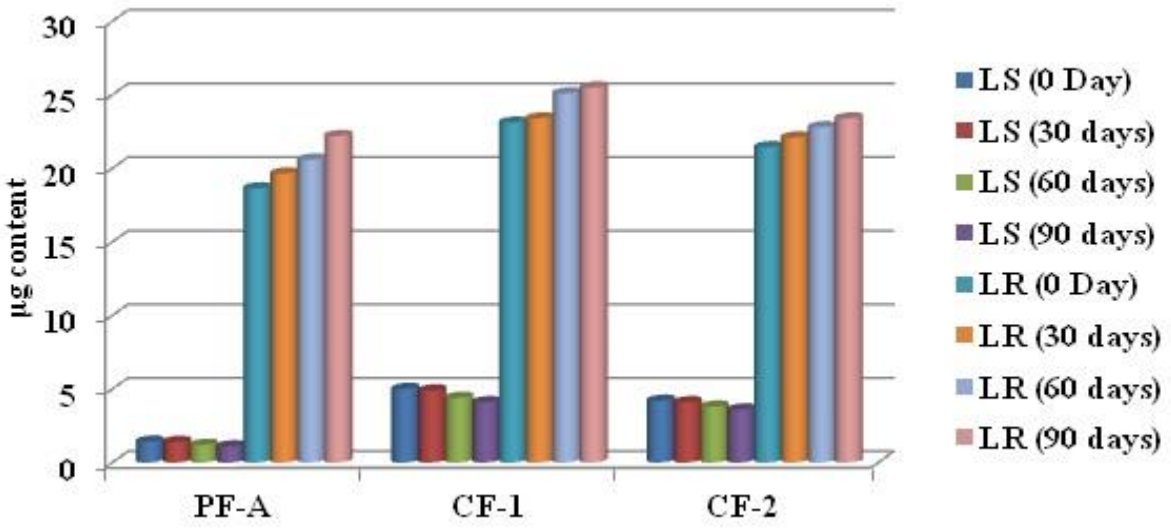

Figure 3. Content of Lyoniside and lyoniresinol in various samples at different storage time

then analyzing total reducing sugar content. 50 % hydrochloric acid was added to the sample and the mix was allowed to stand at room temperature for 24 hr. It was then neutralized with 5N sodium hydroxide and diluted with water. This solution was then processed for determination of reducing sugar[1].

Iron and Calcium content

Sample preparation- The sample was ignited at 500-550ºC and moistened with 5-10 ml of HCl. It was then boiled for two min and diluted with 30 ml of water. This mixture was heated and filtered. It was then washed with water & volume was made up[1].

Iron content: The sample was diluted with water and methyl red indicator and ammonium chloride were added. Further, dilute Ammonia solution was added till brown precipitate occurs. The solution was boiled with precipitate for 4-5 minutes. It was then cooled and filtered. The residue was dissolved in dilute hydrochloric acid and washed with hot water. It was then made upto 100 ml and boiled. Stannous chloride solution was added to it in excess. It was then cooled and 10 ml 10 % mercuric chloride solution was added along with 25 ml acid mixture and 2-3 drops of diphenylamine barium sulphonate indicator. This mixture was then titrated against standard potassium dichromate solution till violet end point[1].

1 ml of 1N potassium dichromate solution ≡ 55.85 mg Iron

Calcium content: Twenty percent aqueous solution of triethanolamine was added to the filtrate obtained after sample preparation. A pinch of Hydroxylamine hydrochloride and 20% solution of potassium hydroxide were added. Then 4-5 drops of Paton and Reeder's indicator was added to the solution and titrated against EDTA[1].

1 ml of 1M EDTA solution ≡ 40.08 mg Calcium

Preparation of extracts

The formulation (Prepared/Commercial) (50 ml) was heated to remove the ethanol. It was then diluted with distilled water and sequentially fractionated with n-hexane and ethyl acetate. The ethyl acetate extract was dried and 20 mg ethyl acetate extract was dissolved in 2 ml methanol. The resultant solution was filtered using 0.2 µm membrane filter and injected into the HPLC column.

Chromatographic conditions: Method development and validation

The column was C-18 with column specifications as 25 cm, 4.6 mm id, 5µm particle size (Phenomenex). The injection volume, detection wavelength and flow rate were 20 µl and 220 nm respectively. Initially, several solvent ratio were tried i.e. 50:50, 60:40,40:60, 30:70, 15:85 respectively of methanol and water with flow rates at 1.5,1.2,1,0.8,0.6,0.4 ml/min. Finally, isocratic solvent system 15:85 methanol and water was used at 0.6 ml/min. The method was validated for specificity, sensitivity, accuracy and precision. The specificity was determined by peak purity (UV spectra) and sensitivity was estimated in terms of detection and quantitation limit. Also linearity range was determined for quantification. The precision was determined as inter- and intra day repeatability. For accuracy determination, recovery of known quantity was determined.

RESULTS & DISCUSSION

In the present study, the quality control parameters specified in API were evaluated for two reputed commercial brands in comparison to laboratory formulated preparation. All the formulations were estimated for lyoniside and lyoniresinol content at various time intervals. The physical appearance of prepared formulation (PF-A) was similar to commercial formulations (CF-1 and CF-2). The odor (Alcoholic-acidic) and taste (Sour-sweet) were identical. The pH of decoction was 4.1 which became 3.3 when formulation is completed as various acids liberated during fermentation. Specific gravity of the decoction (1.12) was higher than finished formulation (1.07). The solid content decreased from 18.7 to 11.2 % due to fermentation. Phenolic content increased by four times, from 0.025 to 0.082 %. *Saraca asoca* bark has been reported to contain tannins and other phenolic compounds

which may be hydrolyzed during fermentation to release more quantity of phenolic compounds[5,6]. Phenolic compounds are antioxidants and their increased content indicate towards increased antioxidant potential of arista. Apart from antioxidant, *Saraca asoca* bark has also been reported to contain, DNA binding property[7], Menstrual cycle regulatory activity[6], and DNA topoisomerase inhibitory property[8].

Arista is an alcoholic preparation and has a specified limit of ethanol. The alcohol content was 8% in prepared formulation which is within limit. The prepared formulation was evaluated for iron and calcium content since presence of organic iron and calcium compound in *Saraca asoca* was suggested in earlier literature. Prepared formulation contained 3767 mg calcium and 61 mg elemental iron in 100ml dosage. Increase in the content of elemental calcium and iron after fermentation also highlights the role of fermentation in overall quality of studied gynecological formulation. The physicochemical parameters for tested commercial formulations are shown in Table 2.

Quantitation of Lyoniside and lyoniresinol

Sadhu et al reported the presence of lignan glycosides in *Saraca asoca* bark [9]. Lyoniside is the major component of *S.asoca* bark so it was estimated in decoction and different formulations (PF-A, CF-1 and CF-2). It is reported that microbes from Woodfordia flowers contain β-glucosidase enzyme which might be responsible for conversion of lyoniside into lyoniresinol [10]. Lyoniside is a glycoside which yields lyoniresinol on deglycosylation so the content of lyoniresinol was also evaluated. Figure 1 shows the conversion of lyoniside to lyoniresinol and figure 2 shows the chromatogram showing major presence of lyoniside in decoction however it was almost negligible in either prepared formulation or in any commercial formulation. In all the formulations, lyoniresinol was present in good quantity which means that major portion of lyoniside was converted to lyoniresinol during fermentation. It has been reported that *Woodfordia fruticosa* flowers contain microorganisms which produce glycosidase so it can be postulated that the conversion of lyoniside to lyoniresinol is deglycosylation rather that hydrolysis. Bhondave reported that mobilization of active phytoconstituents was independent of alcohol generation and perhaps microbial biotransformation plays more important role[11]. Table 3 shows the content of lyoniside and lyoniresinol in various samples. Figure 3 depicts the content of lyoniside and lyoniresinol during storage for 1,2,3 months.

The developed HPLC method was validated in terms of specificity, sensitivity, accuracy and precision. The method was found to be specific since it clearly resolves the peaks of LS and LR in various samples. Calibration curves were plotted for both, LS and LR. The respective calibration curves were depicted by Y=534812x+769433 and Y=2233656x+349161 in the range of 50-350 ng. The % relative standard deviation was within limits. The method was precise as % RSD values were 0.25 and 0.22 respectively.

The authors want to report here that traditional fermentation takes almost 30-37 days for similar changes to take place but in our study it has been done in 8 days. This study establishes the presence of calcium and iron in a traditional gynecological formulation. The chemical marker for main component, *Saraca asoca*, gets modified during fermentation and its aglycone represents the chemical milieu of traditional formulation. On storage, the chemical composition was not modified significantly and it retains its original character.

CONCLUSION

Arista is self fermented ayurvedic formulation and fermentation helps in improving the quality of preparation. However fermentation being a vital process, there is vital need to control its composition. Present work documents the physicochemical changes during processing of ayurvedic formulation, Asokarista and establishes its chemical marker. The study also validates its claim of being effective in gynecological disorders.

ACKNOWLEDGMENTS

One of the authors (SM) is thankful for financial support from DST under WOS-A scheme (SR/WOS-A/LS-119/2011).

CONFLICT OF INTEREST

The authors report no conflict of interest. The authors solely are responsible for content and writing of the article.

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