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Standardization of Selected Asavas and Arishtas

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In the present investigation and standardization of selected asavas and arishtas have been done by preliminary phytochemical, chromatographic and analytical studies. The preliminary phytochemical study revealed the presence of phenolic compounds in all the selected asavas and arishtas and no flavanoids have been detected. Out of the nine solvent systems used for the chromatographic study, only the solvent *n*-butanol : glacial acetic acid : water system showed a good separation of asavas and arishtas and in other solvent systems streaking was observed. Aravindasava, asokarista, dasamularista and kumaryasava gave positive results for Borntrager reaction. Analytical study revealed the lowest pH 3.42 in kumaryasava and highest pH 4.8 in amritarista; specific gravity ranges in betwecn 1.0177 (aravindasava) and 1.2612 (dasamularista) and lowest alcohol content was observed in amritarista (3.6%) and highest in asokarista (10.5%).

Key Words: Standardization, Asavas, Arishtas.

INTRODUCTION

Asavas and arishtas are a group of ayurvedic drugs which are prescribed for various diseases[1]. Asavas and arishtas are prepared by a process of fermentation. Jaggery, honey or both along with powdered drugs are mixed in decoction or plant juices in an earthen pot and buried in the ground or a heap of husk for a period of 30–40 days. During this period alcoholic fermentation takes place and active ingredients are extracted by water[2]. In the formulations of asavas and arishtas, there are a variety of ingredients which serve as natural carriers of the fermenting organisms that bring about ethanolic fermentation[3]. In the present study, a total of four asavas and six arishtas such as aravindasava, chandanasava, kumaryasava, lohasava, amritarista, asokarista, aswagandharista, balarista, dasamularista and kutajarista were prepared in accordance with the textual procedure and an attempt has been made to standardize the asavas and arishtas by preliminary phytochemical, chromatographic and analytical studies.

EXPERIMENTAL

Preparation of asavas and arishtas

Pure and authentic ingredients were used for the preparation of asavas and

arishtas. The asavas and arishtas were prepared as per the Ayurvedic formulary of India[4].

Preliminary phytochemical study of asavas and arishtas

Asavas and arishtas were concentrated to 1/10th of their volume and preliminary phytochemical study was carried out[5,6].

Thin layer chromatography

Asavas and arishtas were used directly without any treatment for the TLC study. TLC plates were prepared as per the procedure described by Stahl[7]. 10 μL of the sample was loaded on TLC plates and eluted in the following solvent systems:

1. Toluene : Ethyl acetate : Diethylamine7 : 2 : 1
2. Ethyl acetate : Methanol : Water100 : 13.5 : 10
3. *n*-Butanol : Glacial acetic acid : Alcohol : Water2 : 1 : 1 : 0.5
4. *n*-Butanol : Ethylacetate : Formic acid : Water3 : 5 : 1 : 1
5. *n*-Butanol : Glacial acetic acid : Methanol : Water4 : 4 : 1 : 1
6. *n*-Butanol : Glacial acetic acid : Alcohol : Water2 : 1 : 1 : 0.5
7. Alcohol : Acetic acid : Water 2 : 4 : 1
8. Methanol : Chloroform95 : 5
9. *n*-Butanol : Glacial acetic acid : Water4 : 4 : 2

The plates were dried at room temperature and detected under UV light at 365 nm. The plates were sprayed with 5% alcoholic KOH[8]. Colour of the spots in visible light, UV light and after spraying the reagent were noted and R_f value of each spot was calculated.

RESULTS AND DISCUSSION

Preliminary phytochemical study of asavas and arishtas

Results of preliminary phytochemical study is tabulated in Table-1. Preliminary phytochemical study of amritarista showed the presence of phenols and

TABLE-1
RESULTS OF PRELIMINARY PHYTOCHEMICAL STUDIES OF ASAVAS AND ARISHTAS

S.No.	Asavas/Arishtas	Phenols	Flavanoids	Terpenoids	Glycosides	Steroids	Alkaloids
1.	Amritarista	+ve	–ve	+ve	–ve	–ve	–ve
2.	Aravindasava	+ve	–ve	–ve	+ve	+ve	+ve
3.	Asokarista	+ve	–ve	–ve	–ve	+ve	+ve
4.	Aswagandharista	+ve	–ve	+ve	–ve	+ve	+ve
5.	Balarista	+ve	–ve	+ve	–ve	+ve	+ve
6.	Candanasava	+ve	–ve	+ve	–ve	+ve	–ve
7.	Dasmularista	+ve	–ve	+ve	+ve	–ve	+ve
8.	Kumaryasava	+ve	–ve	–ve	+ve	+ve	–ve
9.	Kutajarista	+ve	–ve	–ve	–ve	+ve	+ve
10.	Lohasava	+ ve	–ve	+ve	–ve	–ve	–ve

terpenoids. Aravindasava showed the presence of phenols, glycosides, steroids and alkaloids. Asokarista showed the presence of phenols, alkaloids and steroids. Steroids, alkaloids, tannins and choline hydrochloride have been reported from asokarista[9, 10]. Aswagandharista showed the presence of phenols, terpenoids, steroids and alkaloids. Steroids and alkaloids are reported from aswgandharista[9]. Balarista showed the presence of phenols, terpenoids, steroids and alkaloids. Chandanasava showed the presence of phenols, terpenoids and steroids. Phenols, terpenoids, glycosides and alkaloids were detected in dasamularista. Alkaloids have been reported from dasamularista[11]. Kumaryasava showed the presence of phenols and glycosides. The presence of saponins, steroids, O-glycosides and C-glycosides has been reported from kumaryasava[12, 13]. In kutajarista phenols, steroids and alkaloids were detected. Lohasava showed the presence of phenols and terpenoids. The presence of gallic acid, quercetin, proanthocyanidin, traces of saponins, steroids, terpenoids and phenolic compounds have been reported from lohasava[14].

Phenolic compounds were detected in all the selected asavas and arishtas and no flavanoids were detected in any of them. The presence of phenolic compounds has been reported in almost all the asavas and arishtas[9]. *Aspergillus niger* has converted gallotannin from *Woodfordia fruticosa* flower, an important ingredient of all the asavas and arishtas, into gallic acid[15]. Gallic acid and tannins are reported from most of the asavas and arishtas[16, 17].

Thin layer chromatography

TLC study of asavas and arishtas was carried out in nine different solvent systems. All the asavas and arishtas showed separation in solvent system *n*-butanol : glacial acetic acid : water (4 : 4 : 2) and in other solvent systems, streaking was observed. The spots of each asava and arishta were detected under UV at 365 nm and sprayed with 5% alcoholic KOH. The colour of bands in visible light, UV and after spraying with 5% alcoholic KOH with respective R_f values are summarized in Table-2.

Amritarista showed single spot with yellowish brown colour and under UV it showed fluorescent bluish green colour with R_f value 0.44.

Aravindasava resolved into three spots, *viz.*, yellowish grey, grey and violet and showed fluorescent bluish green and two brown spots under UV region with R_f values 0.7, 0.77 and 0.91 respectively. After spraying 5% alcoholic KOH, violet spot turned to red with R_f value 0.91.

Asokarista showed three spots: yellowish grey, grey and violet with R_f values 0.54, 0.66 and 0.84. Violet spot showed brown colour under UV and after spraying with 5% alcoholic KOH turned to red with R_f value 0.84. It has been reported that the benzene extract of asokarista resolved into four spots with R_f values 0.27 (violet), 0.41 (violet), 0.64 (purple) and 0.78 (violet) in the solvent system chloroform and methanol after spraying with ethanol, acetic anhydride and sulphuric mixture. The chloroform-soluble base showed single spot with R_f value 0.68 (orange) when subjected to TLC in the solvent system butyl acetate, butanol, acetic acid and water and after spraying with Dragendorff's reagent[9].

Aswagandharista resolved into three spots: yellow, yellowish violet and violet and under UV they showed blue, yellowish brown and brown with R_f values 0.57,

0.72 and 0.9 respectively. Earlier study revealed that benzene extract of aswagandharista when subjected to TLC in the solvent system chloroform and methanol and after being sprayed with ethanol, acetic anhydride and sulphuric mixture and dried at 110°C gave four spots with R_f values 0.21(violet), 0.38 (purple), 0.71 (brown) and 0.80 (violet). Chloroform soluble base gave three orange spots with R_f values 0.22, 0.40 and 0.64 with Dragendorff's reagent[9] in the solvent system butyl acetate, butanol, acetic acid and water[9].

TABLE-2
THIN LAYER CHROMATOGRAPHY STUDIES OF ASAVAS AND ARISTHAS

S.No.	Asavas/ Arishtas	Visible	Colour of band		R_f value
			UV (365 nm)	After spraying 5% KOH	
1.	Amritarista	Yellowish brown	Fluorescent bluish green	—	0.44
2.	Aravindasava	Yellowish grey	Fluorescent bluish green	—	0.70
		Grey	Brown	—	0.77
		Violet	Brown	Red	0.91
3.	Asokarista	Yellowish grey	—	—	0.54
		Grey	—	—	0.66
		Violet	Brown	Red	0.84
4.	Aswagandharista	Yellow	Blue	—	0.57
		Yellowish violet	llowish brown	—	0.72
		Violet	Brown	—	0.90
5.	Balarista	Yellow	Bluish green	—	0.56
		Greyish violet	Light brown	—	0.71
		Violet	Brown	—	0.90
6.	Candanasava	Yellowish grey	—	—	0.73
		Grey	Brown	—	0.81
		Violet	Brown	—	0.86
7.	Dasmularista	Yellow	Fluorescent bluish green	—	0.53
		Violet	Brown	Red	0.91
8.	Kumaryasava	Yellowish grey	Bluish green	—	0.71
		Yellowish violet	Brown	—	0.79
		Violet	Brown	Red	0.90
9.	Kutajarista	Yellow	Fluorescent bluish green	—	0.67
		Violet	Brown	—	0.91
10.	Lohasava	Violet	Brown	—	0.71
		Grey	Brown	—	0.87

Balarista eluted into three spots: yellow, greyish violet and violet and under UV they showed bluish green, light brown and brown with R_f values 0.56. 0.71 and 0.9 respectively.

Chandanasava showed three spots: yellowish grey, grey and violet with R_f values 0.73, 0.81 and 0.86. Under UV grey and violet spots showed brown color with R_f values 0.81 and 0.86.

Dasamularista resolved into two spots: yellow and violet and under UV showed fluorescent bluish green and brown with R_f values 0.53 and 0.91 . Alam *et al.*[11] reported the alkaloid separation from dasamularista. It showed five spots in the solvent system ethyl acetate : benzene : methanol : water with R_f values 0.55, 0.61, 0.68, 0.72 and 0.95. In the solvent system chloroform : methanol : water, it resolved into two spots with R_f values 0.55 and 0.95 and in the solvent system methanol : water it resolved into two spots with R_f values 0.55 and 0.68. But the separation of alkaloid was poor in the tested solvent system. In the polar solvent system, the spots were moving along with the solvent front while in the nonpolar solvent system the mobility and resolution of spots was very poor. There was streaking in the polar system.

Kumaryasava eluted into three spots: yellowish grey, yellowish violet and violet and under UV spots were found to be bluish green and two brown spots with R_f values 0.71, 0.79 and 0.9. After spraying with 5% alcoholic KOH violet spot turned to red with R_f value 0.9.

Kutajarista showed two spots: yellow and violet and under UV they were found to be fluorescent bluish green and brown with R_f values 0.67 and 0.91.

Lohasava resolved into two spots: violet and grey. Under UV both the spots showed brown colour with R_f value 0.71 and 0.87. The dark yellow residue of lohasava when subjected to TLC resolved into five spots of which three were phenolic in nature. Two of them were compared with gallic acid and quercetine[14].

In aravindasava, asokarista, dasamularista and kumaryasava the appearance of red colour spot after spraying with 5% alcoholic KOH (Borntrager reaction) indicates the presence of anthraquinone derivatives.

Analytical study of asavas and arishtas

The data of analytical study is tabulated in Table-3. Amritarista showed acidic pH 4.8, specific gravity 1.225 and alcohol content 3.6%. Earlier analytical study

TABLE-3
ANALYTICAL STUDIES OF ASAVAS AND ARISHTAS

S.No.	Asavas/Arishtas	pH	pecific gravity	Alcohol content (%)
1.	Amritarista	4.80	1.2251	3.6
2.	Aravindasava	3.50	1.0177	9.6
3.	Asokarista	4.07	1.1445	10.5
4.	Aswagandharista	3.90	1.1833	9.8
5.	Balarista	3.74	1.1782	6.0
6.	Candanasava	3.43	1.0265	6.6
7.	Dasmularista	4.20	1.2612	8.2
8.	Kumaryasava	3.42	1.1246	6.9
9.	Kutajarista	3.54	1.0766	7.2
10.	Lohasava	3.46	1.1174	9.0

of amritarista revealed pH 4.4, specific gravity 1.23 and alcohol content 7.5%[18]. The standard values are pH 3.5–5, specific gravity 1.15–1.22 and alcohol content 2–4%[6].

Aravindasava showed pH 3.42, 1.1246 specific gravity and 6.9% alcohol content. The standard values are pH 3.5–5, specific gravity 1.10–1.50 and alcohol content 4–7%[6].

Asokarista showed pH 4.07, specific gravity 1.1445 and alcohol content 10.5%. Saxena[19] reported the analytical study of four commercial asokaristas which showed pH ranging between 3.9–4.2, specific gravity 1.020–1.070 and alcohol content 8.4–12%. It has also been reported that asokarista has pH 3.89, specific gravity 1.0907 and alcohol content 5%[10]. The standard values are pH 3.5–5, specific gravity 1.05–1.15 and alcohol content 5–6%[6].

Aswagandharista showed pH 3.95, specific gravity 1.1833 and alcohol content 9.8%. Earlier study on three commercial samples of aswagandharista showed alcohol content ranging in between 6.4–8.86%[19]. The standard values are pH 3.5–5, specific gravity 1.05–1.15 and alcohol content 4–7%[6].

Balarista showed acidic pH 3.74, specific gravity 1.1782 and alcohol content 6%. The standard values are pH 3.5–5, specific gravity 1.10–1.15 and alcohol content 3.5–6.5 %[6].

Chandanasava showed pH 3.43, specific gravity 1.0265 and alcoholic content 6.6%. The standard values are pH 3.5–5, specific gravity 1.01–1.02 and alcohol content 5–7%[6].

Dasamularista showed pH 4.2, specific gravity 1.2612 and alcohol content 8.2%. Pandey[20] reported the analytical study of dasamularista which revealed pH 3.65, specific gravity 1.1704 and alcohol content 2.68%. Alam *et al.*[2] have reported analytical study of dasamularista with acidic pH 3.9, specific gravity 1.13 and alcohol content 8.20%. A further analytical study on dasamularista by Alam *et al.*[11] has shown pH 3.92, specific gravity 1.154 and alcohol percentage 4.05. The standard values are pH 3.5–6, specific gravity 1.10–1.15 and alcohol content 3–7.5%[6].

Kumaryasava showed pH 3.42, specific gravity 1.1246 and alcohol content 6.9%. Bhavsar *et al.*[12] have reported the standardization study on kumaryasava. They have prepared four samples of kumaryasava by keeping all the other ingredients constant and changing only kumari rasa component in each and its analytical report has shown almost same values with pH varying from 3.65–3.7, specific gravity 1.1147–1.1185 and alcohol content found to be 2.4% in all four samples. The analytical study of market samples by them has shown pH ranging from 3.7–4.2, specific gravity 1.0409–1.0682 and alcohol content 2.4–9.84%. An analytical study on four samples of kumaryasava showed specific gravity ranging in between 1.086–1.200 and alcohol 0.18–8.8%[19]. The standard values are pH 3.5–5, specific gravity 1.02–1.05 and alcohol content 2.8–4.1%[6].

Kutajarista showed pH 3.54, specific gravity 1.0766 and alcohol content 7.2%. An analytical study on three samples of kutajarista revealed the specific gravity ranging between 1.022–1.072 and alcohol content 7.4–10%[19]. The standard values are pH 4–5, specific gravity 1.10–1.12 and alcohol content 5–6%[6].

Lohasava showed pH 3.46, specific gravity, 1.1174 and alcohol content 9.0%.

The standard values are pH 4–5, specific gravity 1.01–1.02 and alcohol content 5–6%[6].

From the above discussion, we can conclude that the prepared asavas and arishtas have shown almost similar results with that of the reported. These parameters are useful for the authentication of asavas and arishtas. A further study is needed to confirm the presence of active principles in the asavas and arishtas by spectral and advanced chromatographic studies.

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