Simple Spectrophotometric Methods for Standardizing Ayurvedic Formulation

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Traditional medicines are effective but the standardization of Ayurvedic formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles. Department of AYUSH has given preliminary guidelines for standardizing these conventional formulations, for uniformity of batches in production of Ayurvedic formulation and it is necessary to develop methods for evaluation. The present work is an attempt to standardize *asav-arishta*, the traditional Ayurvedic formulation using simple, non-expensive spectrophotometric methods. The various parameters performed included total phenolics, total flavonoids, total alkaloids and total saponins, also included pH, sugar %, alcohol content and specific gravity. The results obtained may be considered as tools for assistance to the regulatory authorities, scientific organizations and manufacturers for developing standards.

Key words: Ashokarishta, ayurved, balarishta, dashmoolaarishta, spectrophotometer

Ayurveda, 'the science of life' is a traditional system of medicine in India which dates back to ancient times. This system of medicine is based on the principle of balance and counter balance. It uses extensively plant derived compound formulation to treat various ailments. Plants are complex mixtures of compounds and no single compound is thought to provide the desired activity. Some compounds produce the desired therapeutic actions, while others reinforce the same, and yet others are considered to neutralize and counteract any possible side effects[1]. *Ashavas* and *arishtas* are very popular in India, probably due to their taste and alcoholic content in addition to their medicinal uses and physiological importance[2]. The formulation is prepared by making a decoction of plants in specified amounts as listed in Ayurvedic Formulary of India (AFI). Crushed jaggery and the flowers of *Woodfordia fruticosa* are then added and preparation is kept for a specified period of time during which it undergoes fermentation generating alcohol that helps extraction of active principles and also serves as preservative for these formulations. The official Ayurvedic Formulary of India lists thirty-seven *ashavas* and *arishtas*[2].

Standardization is an important aspect for establishing the quality and/or efficacy of *asav-arishta*. Generally, two approaches being used for standardization are fingerprint analysis by HPLC/HPTLC and quantitation of individual chemical markers. These approaches are costly and require skilled expertise. Spectrophotometer is a simple instrument and a versatile tool to develop and standardized various Ayurvedic formulations. Many papers have been reported which mentions the use of spectrophotometer in quality control of herbal plants. These methods do not require costly markers, skilled expertise and can be used in conjunction with other methods of analysis. We at Ayurchem, continuously strive to develop spectrophotometric methods to standardize and implement as quality control parameters to maintain batch to batch consistency.

The aim of the present study was to develop fast, simple and cheapest spectrophotometric methods for standardization of *asav-arishta* by determining the amount of functional group as whole (total phenolics, total alkaloids, total flavonoids and total saponins). These are the major functional groups which can be used on regular basis apart from total bitters and glycosides. Quantification of functional groups also follows the ancient principle of Ayurved which uses the mixture of different plants and not the isolated molecule.

Folin-Ciocalteau reagent, sodium bicarbonate, gallic acid, aluminum chloride, quercetin, bismuth nitrate pentahydrate, nitric acid, thiourea, methanol, sodium disulfide, saponin standard, anisealdehyde, ethyl acetate, sulphuric acid all the chemicals were of analytical grade. UV-spectrophotometer shimadzu 1800 and cyclomixer (Remi) were used in the study.

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Ashokarishta (F1, F2 and F3), *Dashmoolaarishta* (F4 F5 and F6) and *Balarishta* (F7, F8 and F9) were collected from market as well as from in-house production. Samples were analyzed for pH, Sugar percentage, Alcohol content and specific gravity.

Total phenolics were determined using Folin-Ciocalteau reagent[3]. The sample (200 µl) was mixed with 200 µl of Folin-Ciocalteau reagent (previously diluted 1:1 with distilled water) and allowed to stand at room temperature for 5 min. A 2000 µl sodium bicarbonate solution (7% w/v) was added to the mixture. After 90 min at room temperature, absorbance was measured at 700 nm using a UV/Vis spectrophotometer. Total phenolics were quantified by calibration curve obtained from measuring the absorbance of a known concentration of gallic acid standard. The concentrations are expressed as milligrams of gallic acid equivalents (GAE) per ml of sample.

Total flavonoids were estimated using $AlCl_3$ method. Sample solutions were prepared in 80% methanol. To prepare $AlCl_3$ reagent, 133 mg crystalline aluminium chloride and 400 mg crystalline sodium acetate was dissolved in 100 ml of 80% methanol. For flavonoid estimation, to 2 ml of sample, 400 µl of water and 1 ml of $AlCl_3$ reagent was added. Absorbance was recorded at 430 nm against blank containing no $AlCl_3$ reagent. Stock solution of quercetin (1 mg/ml) was prepared in 80% methanol. Various dilutions of quercetin (5-25 µg/ml) were prepared in methanol and a standard curve was plotted. The amount of flavonoids was calculated as quercetin equivalent from the calibration curve of quercetin (5-25 µg/ml)[4].

Total saponin determination was done using anisaldehyde reagent. Sample solution was prepared in water. For total saponins estimation 500 µl of sample, 500 µl of 0.5% anisealdehyde reagent, were mixed and kept aside for 10 min. Later, 2 ml of 50% sulphuric acid reagent was added and tubes were mixed. Tubes were then kept in water bath with constant temperature of 60º. After 10 min tubes were cooled and absorbance was taken at 435 nm. The amount of saponins was calculated as saponin equivalent from the calibration curve of standard saponin (100-1000 µg/ml)[5].

Alkaloids were precipitated in sample using drangendorff reagent[6] and was allowed to stand for 10 min. Later on samples were centrifuged and precipitate was dissolved in concentrated nitric acid. Yellow colour was developed to above solution using 3% thiourea solution and absorbance was read at 435 nm. The amount of alkaloids was calculated as µg/ml.

There are around 6000 herbal manufacturers in India. More than 4000 units are producing Ayurvedic medicines, out of which 95% are small scale industries which lacks expertise in standardization and quality control methods mainly due to insufficient funds. There should be simple methods available for the small scale industries which can maintain batch to batch consistency. HPTLC/HPLC involve lot of cost and high level of skilled expertise is required. Spectrophotmetric methods developed in our laboratory involves simple and cost effective method for quality control and to maintain batch to batch consistency of *asav-arishta* being manufactured.

Samples of *Ashokarishta, Dashmularishta* and *Balarishta* were selected because they are most selling product in market. Samples of different manufacturer

TABLE 1: ESTIMATION OF PH, SUGAR PERCENTAGE, ALCOHOL CONTENT AND SPECIFIC GRAVITY

Formulations	pH	Sugar percentage	Alcohol content	Specific gravity
Ashokarishtam				
F1 (Marketed)	4.3±0.01	20.70±0.01	11.03±0.15	1.054±0.01
F2 (Marketed)	4.14±0.01	21.302±0.01	5.5±0.21	1.062±0.01
F3 (In house)	4.63±0.01	32.606±0.01	7.27±0.15	1.132±0.01
Dashmularishtam				
F4 (Marketed)	4.02±0.01	33.771±0.01	7.12±0.20	1.124±0.01
F5 (Marketed)	4.6±0.01	20.106±0.01	7.12±0.20	1.062±0.01
F6 (In house)	4.48±0.01	33.23±0.01	7.43±0.20	1.134±0.01
Balarishta				
F7 (Marketed)	3.92±0.01	28.815±0.01	8.47±0.15	1.089±0.01
F8 (Marketed)	3.27±0.01	24.825±0.01	5.5±0.2	1.083±0.01
F9 (In house)	4.83±0.01	31.042±0.01	8.55±0. 15	1.113±0.01

Each value is the mean±SD, n=3

TABLE 2: QUANTIFICATION OF FUNCTIONAL GROUPS

Formulations	Total phenolics (mg/ml)	Total flavonoids (μg/ml)	Total alkaloids (μg/ml)	Total saponins (mg/ml)
Ashokarishtam				
F1 (Marketed)	20.21±0.44	236.67±10.97	913±37.86	50.38±1.18
F2 (Marketed)	13.75±0.35	107.67±1.53	566.67±23.09	43.33±0.55
F3 (In house)	16.07±0.20	213.00±8.89	336.67±11.55	336.67±11.55
Dashmularishtam				
F4 (Marketed)	11.18±0.52	293.67±1.15	644.67±0.58	91.60±1.12
F5 (Marketed)	7.25±1.82	185.00±4.58	142.33±9.50	40.55±1.04
F6 (In house)	23.78±0.61	204.67±12.86	244.33±12.90	82.05±1.18
Balarishta				
F7 (Marketed)	6.39±0.41	227.00±1.00	480.33±2.89	58.61±0.74
F8 (Marketed)	16.06±0.89	74.00±4.36	112.67±9.24	54.17±3.05
F9 (In house)	5.68±0.38	306.67±25.17	75.33±5.69	101.28±1.37

Each value is the mean±SD, n=3

and in-house were collected, care was taken to match the manufacture date of the product. As per the Ayurvedic formulary of India pH, sugar %, specific gravity and alcohol content were estimated and shown in Table 1. Apart from regular analysis one can also estimate various other phytoconstituent using simple methods. The spectrophotometric methods developed at our end showed good linearity with the standards. Various phytoconstituents (total phenolics, total flavonoids, total saponins and total alkloids) estimated in different samples of different *asav-aristha* are been shown in Table 2. The difference in the values of phytoconstituents may arise due to variation in geographical conditions of raw materials, different methods of processing. Thus by analyzing different batches of *asav-arishta* one can finalize the limits of different phytoconstituents and can implement in regular quality control methods to maintain batch-to-batch consistency.

The spectrophotometric methods for the quantification of various phytoconstituents developed in the laboratory of Ayurchem Products are simple and can be implemented as quality control methods in conjunction with other analytical methods. These quantitative analytical methods can not only be applied to *asav-aristha* but also to various other formulations.

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