



## Physico-chemical Standardization of Asava and Arista Formulations: An Overview

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Received: 17-01-2017; Revised: 02-03-2017; Accepted: 18-03-2017.

### ABSTRACT

Asava and Arista, two important Ayurvedic formulations, are used since more than 3000 years for the treatment of various diseases. Due to accelerated commercialization, the assurances on safety and efficacy of Ayurvedic products have become an important issue. Determination of qualitative as well as quantitative evaluation parameters of various Asava and Arista formulations are warranted to ensure the quality and safety of these preparations. Therefore, to achieve this, the world health organization advocates to undertake various standardization parameters such as organoleptic evaluation, microscopical evaluation, physico-chemical evaluations (determination of ash values, extractive values, pH, elemental analysis, heavy metals, total solid contents and phytochemical analysis), analytical evaluation, biological evaluation and biotechnological evaluation. Out of all these parameters physico-chemical analysis is significant as it assists in determination of genuinity of drug components and analyses the presence of phyto-components, which represent the therapeutic potential of the drugs. In present review a discussion is presented on the Asava and Arista marketed as well as in-house formulations of which standardization studies have been conducted by various workers based on different physico-chemical parameters as described in the Ayurvedic Pharmacopoeia of India.

**Keywords:** Ayurvedic formulations, Asava, Arista, standardization, physico-chemical parameters.

### INTRODUCTION

Ayurveda considered as one of the world's oldest traditional system of medicine with sound philosophical and experimental basis, which is believed to be over 3000 years old, and is still being practiced today. It is known to be a complete medical system that comprised of physical, psychological, philosophical, ethical, and spiritual health.<sup>1</sup> It also became extremely popular by making the mutual relationship between mankind and nature.<sup>2</sup> Rig Veda, Sam Veda, Yajur Veda and Atharva Veda have made sound impact on evolutionary change of living a healthy life in India.<sup>3</sup> Today, there is a growing interest in accepting treatment with alternative system of medicines, is mainly due to more side effects, less curative values, high cost and enhanced microbial resistance developed by synthetic drugs, as well as surfacing of many complicated life threatening diseases.<sup>4</sup>

Traditional herbal formulations have importance due to their natural alternatives, affordability, safety and harmlessness as compared to modern synthetic drugs. Medicinal plants and their formulations are being used by majority of people all over world for their primary health care.<sup>5</sup> Thus, the vigorous use of traditional herbal medicines has prompted the evaluation of these bioactive compounds by following proper standard methods.<sup>6</sup> According to some reports the ingredients of these herbal formulations are being adulterated with inferior quality drugs/ nondrug adulterants.<sup>7</sup> Therefore, these formulations are not officially recognized in many countries even though these have been used in this

country of origin over many years. The reason may be due to lack of proper standardization, unavailability of research data and inadequate research methodology pertaining to authentication of these herbal formulations.<sup>8</sup> Thus, it has become inevitable to standardize all Ayurvedic formulations according to the evaluation guidelines laid by world health organization, if these formulations are targeted to be consumed worldwide. However, various researchers have undertaken the physico-chemical parameters to standardize some of the marketed and in-house prepared Asava and Arista formulations to establish their genuinity and quality. Based on their reports a compiled discussion is presented in this review, which may further assist other researchers interested in related studies.

### Physico-Chemical Standardization of Some Asava and Arista Formulations

To undertake the standardization study basing on physico-chemical parameters, some workers have considered only the marketed Asava and Arista formulations, some have taken only in-house prepared formulations and for having a comparative assessment some have included both marketed and in-house formulations in their study. Their various study findings are summarized below:

#### Abhayarista

The study of physico-chemical parameters of Abhayarista has been carried out according to the WHO (world health organization) guidelines. The pH and specific gravity of the formulation were found to be 3.83 and 1.242 g/ml



respectively. Phytochemical screening of the formulation revealed the presence of anthraquinone glycoside, tannin, sugar & reducing sugar like ketose, fructose, sucrose and glycogen. The pesticidal residues such as organochloro, organophosphate and carbamate were not detected in the formulation. The formulation does not contain nitrogen, and any of the halogens (chlorine, bromine, iodine). Heavy metals such as cadmium, arsenic or mercury were not detected, however, lead was found to be within permissible limit.<sup>9</sup>

#### Amrtarista

The phytochemical screening of Amrtarista detected the presence of phenols and terpenoids. The alcohol content, specific gravity and pH of the formulation were measured, which found to be 3.6% v/v, 1.2251 and 4.8 respectively.<sup>10</sup>

#### Aravindasava

In a comparative study, Aravindasava was prepared using traditional container (earthen pot) and modified method in a glass vessel by using yeast. Yeast-I and yeast-II were isolated from Dasamularista and pippali yeast from Pippaliasava. Formulations prepared in earthen pot showed an increase in pH value (5.51) after fermentation in comparison to formulations prepared in glass vessels (5.39). Depending on type of yeast used, the pH of formulations prepared in glass vessels found marginal variation, dasamula yeast-II (5.38) and pippali yeast (5.41). Further, the formulation prepared using earthen pot showed very slight reduction in specific gravity & reducing sugar (1.006 & 1.3) in comparison to the glass vessels (1.007 & 1.45). Moreover, formulation inoculated with pippali yeast showed decrease in reducing sugar (0.8). The alcohol generation in formulation prepared by glass vessels was more (10.9% v/v) than earthen pot (10.05% v/v). Formulation inoculated with yeast-II showed more alcohol content (10.52% v/v) than yeast-I (8.05% v/v) and pippali yeast (5.9% v/v). Formulation prepared in an earthen pot showed the presence of *Bacillus* sp, *Aspergillus niger*, *Aspergillus wentii* and yeast after fermentation whereas the formulation of glass container showed the presence of rod shaped bacteria, yeast and *Aspergillus niger*.<sup>11</sup>

Phytochemical screening of Aravindasava showed the presence of phenols, glycosides, steroids and alkaloids. The alcohol content, specific gravity and pH of the formulation were found to be 9.6% v/v, 1.0177 and 3.5 respectively.<sup>10</sup> Twenty brands of commercially available Aravindasava were subjected to the determination of total ethanol content, pH and acid value which were stored in sealed bottles at room temperature. All brands showed weak acidic properties whereas alcohol content varied from 13.00% v/v to 7.7% v/v. Acids produced during preparation (especially in the fermentation process) and on storage (oxidation of alcohols) were responsible for the sour taste of the formulation.<sup>12</sup>

#### Arjunarishta

On comparison of Arjunarista prepared in earthen pot and porcelain pot with and without addition of Dhatakibuspa as fermenter found that the yield in earthen pot (24.45% v/v, 35.05% v/v) was less than porcelain pot (62.49% v/v, 63.29% v/v). This might have happened due to greater porosity of earthen pot which caused evaporation and non-occurrence of the same in case of porcelain pot. Similarly the content of Arjunarista prepared with the addition of Dhatakibuspa was found to be less (24.45% v/v, 62.49% v/v) than prepared without Dhatakibuspa (35.05% v/v, 63.29% v/v). The reason may be due to soaking of some quantity of formulation by Dhatakibuspa.<sup>13</sup>

The physico-chemical parameters of in-house prepared Arjunarista were evaluated and found that the pH, specific gravity, alcohol content, total solid content, acid value and refractive index were 4.3, 1.092, 6.42% v/v, 9.94 % w/v, 1.35 and 1.62 respectively.<sup>14</sup>

#### Ashokarista

Various marketed and in-house prepared Ashokaristas were standardized basing on physico-chemical parameters. The total alcohol content was found to be more in Baidhyanath Ashokarista (BA)(8.7% v/v) whereas it was less in Dabur Ashokarista(DA)(5.1% v/v). The pH of BA (4.13) was found to be little higher than the other marketed formulations and in-house preparation. Amongst all Ashokarista preparations, Zandu Ashokarista (ZA) was found to have highest water soluble extractive value (11.5% w/w) and DA was having highest alcohol soluble extractive value (10.7% w/w). There was little difference in total solid content between the BA (21.76% w/v) and in-house preparation (20.3% w/v), but these values were comparable with DA (15.39% w/v) and ZA (15.40% w/v). Maximum surface tension was observed in ZA (80.484 dynes/cm) and minimum in BA (60.485 dynes/cm) which was comparable with DA (60.555 dynes/cm) and in-house preparation (72.4 dynes/cm). Maximum viscosity (3.531 cps) was found in BA and minimum in ZA (1.067 cps), whereas this value was very close between DA (2.335 cps) and in-house preparation (2.56 cps). Phytochemical screening showed the presence of carbohydrates, alkaloids, glycosides, tannins and flavonoids in each of marketed formulations and in-house formulation.<sup>15</sup>

Value of pH and sugar percentage of in-house preparation was found to be more (4.63 & 32.6% w/v) than the two marketed formulations (4.3 & 20.7% w/v, 4.14 & 21.3% w/v). The alcohol content was varied from 5.5-11.03% v/v in two marketed formulations while it was 7.27% v/v in case of in-house preparations. The specific gravity was found to be very close between two marketed formulations (1.054, 1.062) and it was higher in in-house preparation (1.132).<sup>16</sup>

Phytochemical studies of Ashokarista showed the presence of phenols, alkaloids and steroids. pH value,



specific gravity and alcohol content were found to be 4.07, 1.1445 and 10.5% v/v respectively.<sup>10</sup>

### Ashwagandharista

Various studies on physico-chemical parameters were conducted by different researchers on marketed and in-house formulated Ashwagandharista.

In one study the physico-chemical parameters of Ashwagandharista were found to be within the standard limit of Ayurvedic pharmacopoeia. The pH, alcohol content, specific gravity and total reducing sugar were found to be 3.77, 7.75% v/v, 0.9991 and 17.5% w/v respectively. The phytochemical screening of formulations revealed the presence of carbohydrates, proteins, steroids, flavonoids, alkaloids and tannins.<sup>17</sup>

In a separate study the total alcohol content, pH and acid value of twenty brands of commercially available Ashwagandharista were determined which were stored in sealed bottles at room temperature. Acid value indicates the total acids present in the product. The acids produced during the fermentation process and storage (oxidation of alcohols) was responsible for the sour taste of preparations. The mean acid value was found to be 0.09. The pH values were found to be increased on the first day, 7<sup>th</sup> day and 14<sup>th</sup> day of opening the bottle. The values were found to be 3.86, 3.88 and 3.93 respectively. Alcohol percentage was varied from 7.27-13.13% v/v in each of the tested brands which were lower than in fortified wines (18-21% v/v) and distilled spirits (40-50%). From the measured pH and acid values it was indicated that Ashwagandharista possessed weak acidic properties.<sup>12</sup>

In another study Ashwagandharista formulations were prepared following traditional (using flowers of *Woodfordia fruticosa*) and non-traditional method (using yeast isolated from *Woodfordia fruticosa*) as fermenter. Traditionally prepared formulations were named as ASG-WFS (with drug Ashwagandha) and ASG-WFB (without drug). Other Ashwagandharista formulations, prepared with different strains of *Saccharomyces*, were named as ASG-20 (*S. cerevisiae*Jm.20), ASG-8 (*S. fibuligera*Jm.8), ASG-10 (*S. fibuligera*Jm.10), ASG-16 (*S. fibuligera*Jm.16), ASG-mix (mixture of *S. cerevisiae*Jm.20, *S. fibuligera*Jm.8, *S. fibuligera*Jm.10, *S. fibuligera*Jm.16), ASG-SC1011 (*S.cerevisiae*SC1011), and ASG-20z (*S.cerevisiae* Jm.20). During the fermentation process kinetic of alcohol generation, consumption of sugar, changes in pH and withanolides extraction were studied. Alcohol generation was proportionately increased with the consumption of residual sugar (substrate). End of fermentation was indicated by the stoppage of alcohol generation and no further consumption of residual sugar. Production of alcohol was found to be in the recommended range of 8-12% v/v in formulations. Thus, it was concluded that the strain *S.cerevisiae* was essential for the alcohol production in the required range. However, it was found that the formulations prepared using strains of *S.*

*fibuligera* did not produce alcohol in the desired range. In this study it was also found that extraction of withanolides was continued even after the stoppage of alcohol generation. The content of withanolides and extraction rate was low in formulations prepared using strains of *S. fibuligera* and was high in case of *S.cerevisiae*. At least one month period was required for complete extraction of withanolides which was observed in formulation ASG-SC1011.<sup>18</sup>

The total ash, acid insoluble ash, water insoluble ash, sulphated ash, alcohol soluble extractive, water soluble extractive and ether soluble extractive values of Ashwagandharista were determined in another study and found to be 0.54% v/v, 0.4% v/v, 0.46 w/w, 0.37% v/v, 31.5% v/v, 27.5% v/v and 15.4% v/v respectively. Phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, tannins and saponins.<sup>19</sup>

Physico-chemical parameters of three marketed Ashwagandharista formulation ASA-DAB (Ashwagandharista by Dabur), ASA-AVP (Ashwagandharista by AryaVaidy Pharmacy) and ASA-BDN (Ashwagandharista by Baidyanath) were evaluated in another study. The total solid content of ASA-DAB (14.19% v/v) and ASA-AVP (14.38% v/v) found to be less than the limits described in the API (18.5 % v/v). The content of reducing sugar in ASA-DAB (16.6% v/v) and ASA-AVP (16.5% v/v) were found to be more than the specified limit (16% v/v) and it was less in ASA-BDN (15.2% v/v), which may be due to excessive fermentation of jaggery and more generation of alcohol (1.73% v/v). Alcohol content of ASA-DAB (8.67% v/v) and ASA-AVP (8.40% v/v) were found to be within the limit.<sup>20</sup>

Phytochemical screening of the Aswagandharista showed the presence of major phytoconstituents such as phenol, terpenoids, steroids and alkaloids. The alcohol content, specific gravity and pH of the formulations were found to be 9.8% v/v, 1.1833 and 3.9 respectively.<sup>10</sup>

### Ayaskrti

Different batches of Ayaskrti formulation were procured from market and their physico-chemical parameters were evaluated. Total solid content of three batches (17.5% v/v, 17.6% v/v and 17.9% v/v) were found to be more than standard value (9-17% v/v). Specific gravity of first and second batch (1.0663 and 1.0356) was within the standard limit (1.01-1.07). Three batches of formulation showed presence of higher percentage of reducing sugar (4.67% v/v, 4.68% v/v and 4.91% v/v) than the prescribed limit (2-4% v/v) and the percentage of non reducing sugar (0.79% v/v, 0.89% v/v and 0.68% v/v) were found to be within limit (not more than 1.0). The pH values of all three batches (3.78, 3.79 and 3.80) were found to be within the limit (3.5-5). Alcohol content of all the batches (6.65% v/v, 6.56% v/v and 6.66% v/v) showed slightly higher than the standard limit (5.3-6.5% v/v).<sup>21</sup>



## Balarista

The physico-chemical parameters of marketed and in-house prepared Balarista were evaluated. The pH value of in-house prepared formulation (4.83) was found to be less acidic than the two marketed formulation (3.92, 3.27). Sugar percentage in in-house preparation (31.042% v/v) was found comparatively higher than the two marketed formulations (28.81 % w/v, 24.82% v/v). The alcohol content of in-house preparation (8.55% v/v) was found to be nearly equal to one marketed formulation (8.47% v/v). Another marketed formulation showed lower alcohol content (5.5% v/v) than these two formulations. The specific gravity of two marketed formulations (1.089 and 1.083) was found to be less than the in-house preparation (1.113).<sup>16</sup>

In a separate study Balarista was prepared and evaluated for organoleptic and biochemical changes. Color of the formulation was changed from light to dark brown indicating the low extraction of phytoconstituents from the ingredients used in the preparation and change in fragrance also gave the indication of extraction of phytochemicals. Initially taste of Balarista was sweet due to presence of bulk volume jaggery in the formulation, subsequently the taste changed to sour due to utilization of sugar by the microorganisms during fermentation and also due to extraction of phytoconstituents. Free amino acids were continuously decreased during the fermentation process. It was observed that during initial 5 days of fermentation pH level was changed from 5.5 to 3.5 and it was maintained till the end of fermentation, indicating the major biochemical changes occurred during these 5 days. Total solid content in the formulation was gradually reduced from 0 days to 15<sup>th</sup> day. Total sugar content was gradually decreased from 0 day to 15 days with simultaneous increase in level of ethanol. Concentration of starch gradually decreased in successive stages of fermentation. These changes might be due to the growth and metabolic activity of microbes present in the formulation. Ethanol concentration of the final product was found to be 6.5% v/v.<sup>22</sup>

In another study, phytochemical screening of Balarista showed the presence of phenols, terpenoids, and alkaloids. Alcohol content, specific gravity and pH of the formulation were found to be 6% v/v, 1.1782% v/v and 3.74 respectively.<sup>10</sup>

## Candanasava

The effect of time on the fermentation and storage of Candanasava was studied and evaluated by physico-chemical parameters. Candanasava was prepared and the content was analysed at 15 days interval. It was observed that the yield was reduced to 44.4% v/v in 90 days in the earthen pot. A major loss was noticed in the first 15 days and then after it was gradually decreased. The pH value changed from 4.7 to 3.3 within 45 days, which might be due to the production of acid metabolic product by the surface fungus or fermenting Bacillus and the acidity was

decreased in the 60<sup>th</sup> and 75<sup>th</sup> day, which might be due to neutralization of acid by an active principle released from the drug interaction. On 90<sup>th</sup> day again the acidity was increased to 3.3 which might be due to the metabolic action of the organism. There was a decrease in specific gravity from 1.08 to 1.008 and also solid content from 22.66%w/v to 4.95%w/v.

The content of sugar was gradually decreased from 26.65% v/v to 1.62% v/v with the passage of time, which might be due to utilization by non alcohol fermenting organism. The alcohol content was found to be decreased by the passage of time. The alcohol content was 9.8% v/v during first 30 days, which was reduced to 7.84%v/v during 90 days of fermentation indicating evaporation or breaking down. However, growth of fungus was observed in all pots after prolong incubation. In contrast, no growth of fungus and no alteration in physico-chemical parameter were observed in formulations stored in glass bottles.<sup>23</sup>

In another experiment the value of pH (3.78), reducing sugar (3.88% v/v) and non reducing sugar (0.98% v/v) were found, which were in standard range, whereas total solid content (10.656% v/v) and specific gravity (1.0456) were found slightly above the standard value. This might be due to the presence of more content of fine components in the formulation.<sup>24</sup>

The marketed Candanasava formulation was evaluated for organoleptic and physico-chemical parameters. The color of the formulation changed from brown to dark brown due to low extraction of phytoconstituents from the ingredients used in the preparation. Change in fragrance indicated the extraction of phytochemicals. Initially taste was sweet due to the presence of bulk volume of jaggery but subsequently it changed to astringent due to utilization of sugar by the microorganisms. Amount of free amino acids were continuously decreased during the fermentation process. The pH was gradually changed from 4.7 to 3.7 during the initial 5 days of formulation and the value remained constant till the completion of fermentation. The total solid content was reduced from 219.21 mg/ml to 97.9 mg/ml in the 5 days of fermentation and then gradually reduced to 6.5 mg/ml; this may be due to the presence of coarse herbal materials which were settled in the successive stages of fermentation.

During initial 5 days of formulation total sugar content was suddenly reduced, which may be correlated with the increased ethanol content. These changes might be due to the growth and metabolic activity of microbes present in the preparation. Ethanol concentration was gradually increased up to 15 days of fermentation, slightly reduced in the middle and finally maintained at 9.3% v/v. The specific gravity was found to be 1.0 which indicated the watery nature of preparation. The content of free amino acid was gradually decreased after 10 days of fermentation and then slowly reduced till the end of preparation.<sup>22</sup>



Phytochemical screening of the Chandanasava showed the presence of phenols, terpenoids and steroids. The alcohol content, specific gravity and pH of the formulation were found to be 6.6 % v/v, 1.0265 and 3.43 respectively.<sup>10</sup>

### Dasamularista

In an in-house study, Dasamularista was prepared using different containers (earthen pot, stainless steel and porcelain jar) of equal shape and size in which the effect of fermentation with different volumes of solution was studied. The ingredients were filled up to 1/2, 2/3, 3/4 and almost full capacity of the earthen pots. Analytical values such as sugar content (21.66% v/v, 22.83% v/v and 21.66% v/v), solid content (27.47% v/v, 27.57% v/v and 26.43% v/v) and specific gravity (1.102, 1.106 and 1.097) were found to be similar in pots filled up to 1/2, 2/3, and full capacity of the container. However, the pot filled with 3/4<sup>th</sup> of its capacity showed pH (4.35) whereas sugar content (20.61% v/v) and solid content (24.34% v/v) were found to be lower than the other pots. It was found that alcohol production was increased (7.44% v/v) with increased uptake of the drug during fermentation where 3/4<sup>th</sup> volume of the pot was filled with ingredients, but further increase in the volume led to decrease in the production of alcohol (5.64% v/v). Formulation prepared in porcelain jar and steel vessel did not show appreciable changes in pH (3.77, 3.78), specific gravity (1.10, 1.098) and solid content (26.48% v/v, 26.36% v/v), but alcohol production was comparable to the findings of earthen pot containing drug up to 3/4<sup>th</sup> of its volume (7.16% v/v, 7.44% v/v and 7.44% v/v). From this study, it was inferred that the pot filled up to 3/4<sup>th</sup> capacity was most suitable for fermentation of Dasamularista.<sup>25</sup>

In a comparative study, marketed Dasamularista and in-house preparation were evaluated by physico-chemical parameters. The pH of in-house preparation (4.48) was found to be different from other two marketed formulation (4.02, 4.6). The sugar percentage in in-house preparation (33.23% v/v) was found comparable to marketed formulation (20.10% v/v, 33.77% v/v). The alcohol content and specific gravity of in-house preparation (7.43% v/v & 1.134) was higher than the marketed formulation (7.12% v/v each & 1.124, 1.062).<sup>16</sup>

Five different brands of marketed Dasamularista were standardized. The percentage of alcohol content (4-12% v/v) was found to vary among the different brands of Dasamularista but it was within the acceptable limit.<sup>26</sup>

Phytochemical screening of the Dasamularista showed the presence of phenols, terpenoids, glycosides and alkaloids. The alcohol content, specific gravity and pH of the formulation were found to be 8.2 % v/v, 1.2612 and 4.2 respectively.<sup>10</sup>

### Drakshasava

In the study of Drakshasava, species of *Saccharomyces* were isolated from madhuca flower (*Madhuca longifolia*)

and its effect was studied during the fermentation. The result showed almost identical yield of Drakshasava, which was prepared using both earthen pots and glass vessels. The difference observed in pH value between earthen pot (4.9) and glass pot (4.5) might be due to evaporation of water. The change in pH of the fermentation medium did not have any impact on increase in alcohol production. Isolated *Saccharomyces* species were capable of initiating fermentation and the alcohol produced in earthen pot (6.18% v/v) was higher than glass container (5.9% v/v).<sup>27</sup>

The in-house prepared Drakshasava was evaluated by physico-chemical parameters. The total solid content (17.08% v/v), specific gravity (1.09 gm/mol), reducing sugar (18.20% v/v), non reducing sugar (0.52% v/v) and pH value (4.32) were within the prescribed limit but alcohol content (1.2% v/v) was found to be very less as compared to standard limit (5-10% v/v).<sup>28</sup>

### Draksharista

Two marketed Draksharista formulations (coded as BDH and DTP) were standardized. The result of these two formulations were found to be pH (3.98, 5.17), refractive index (1.39, 1.38), specific gravity (1.072, 1.07), viscosity (2.04, 2.93), acid value (0.092, 0.091), alcohol content (13.2% v/v, 5.2% v/v) and total solid content (6.36% v/v, 10.50% v/v) respectively. Formulation having less alcohol content was having higher pH value. Alcohol content of formulation BDH was higher than standard limit (5-12% v/v). Solid content of DTP formulation was higher than BDH formulation indicated that the increased viscosity was directly proportional with increased solid content.<sup>29</sup>

In an interesting study, Draksharista was prepared by traditional method and under different experimental conditions such as: decoction of main drug only; decoction after mixing with other drugs and sweetening agent in an earthen pot and glass vessels; after autoclave and inoculation; after adjusting pH-8; after adjusting pH-8 autoclaved and inoculated with *Saccharomyces*; etc. to know the effect of container, effect of pH and effect of inoculation with *Saccharomyces cerevisiae* in autoclaved medium. Physico-chemical study of these formulations revealed that formulation prepared in glass container showed less acidic (pH 4.2) and higher specific gravity (1.14) than the earthen container. Alcohol content of glass container (5% v/v) was more than earthen container (4% v/v). Sugar consumption in glass vessel (16.66% v/v) was higher than earthen vessel (13.76% v/v). Draksharista prepared by autoclaving and inoculating with *Saccharomyces cerevisiae* showed pH 4.3, specific gravity 1.17, solid contents 40.7% v/v, sugar consumption 10.3% v/v and alcohol content 5% v/v. Draksharista which was prepared by changing pH-8 before fermentation showed pH-4.5, specific gravity 1.14, solid contents 27% v/v, sugar consumption 16.26% v/v and alcohol content 4% v/v. Draksharista prepared at pH-8, autoclaved and inoculated with *Saccharomyces cerevisiae* showed pH-5.1, specific



gravity 1.17, solid contents 39.6% v/v, sugar consumption 8.85w/v and alcohol content 4% v/v.

It was observed that the species of *Saccharomyces* isolated from madhuca flower was capable of causing fermentation in Draksharista but its performance was less as compared to that of *Bacillus* species. Difference in alcoholic content may be due to the presence of other micro-organisms associated with ingredients. The value of physico-chemical parameters did not differ appreciably in the Arista made in glass and the earthen pot; however, some were affected by the evaporation of water from the earthen pot.<sup>30</sup>

#### Kanakasava

The result of physico-chemical parameter study of formulated Kanakasava showed pH value (3.86), specific gravity (1.046), viscosity (1.52), alcohol content (7.18% v/v) and total solid content (14.64% v/v). The formulation was found to be devoid of microbial growth. Heavy metals (Cadmium, Lead, Mercury and Arsenic) were present less than the European Pharmacopoeial limit and pesticide residue were found to be absent in the formulation.<sup>31</sup>

In another study marketed Kanakasava was standardized by physico-chemical parameters. The result of mean pH (4.153-4.231) and acid value (4.941) indicated that all marketed formulations were weak acidic in nature.<sup>32</sup>

#### Kanakabindvarista

Kanakabindvarista was formulated using yeast as the fermenter and it was evaluated based on physico-chemical parameters. All the batches of Kanakabindvarista were found to be of similar color after completion of fermentation. The end product of formulation was thin in consistency due to break down and conversion of sugar. The pH range of 3-5 was considered as the most favorable condition for growth and fermentation activity of yeast. Final product showed alcohol content (5.04% v/v-9.76% v/v), reducing sugar (22.81% v/v-25.15% v/v), non-reducing sugar (3.44% v/v-4.21%w/v) and total sugar (26.25% v/v-28.86% v/v). Total solid content was found to be less in formulations. At the initial stage specific gravity was more due to addition of jaggery and honey. Subsequently the content of total solid and specific gravity was decreased in the final product indicating utilization of sugar with an increase in alcohol generation. From the result, it was observed that there was less variation in physico-chemical parameters of all batches of Kanakabindvarista. Methanol was found to be absent in all the batches of formulation.<sup>33</sup>

#### Karpurasava

Karpurasava was prepared according to the textual methods and standardized by physico-chemical parameters. Different observed values of total alcohol content (8.7% v/v), specific gravity (0.995) and total solid content (0.62% v/v) were found to be within the prescribed limits.<sup>34</sup>

#### Kumaryasava

Marketed brand of Kumaryasava was evaluated by physico-chemical parameters. The variations in ethanol content of the marketed formulations were determined by both specific gravity and gas chromatographic method on a storage period of 30 days. Specific gravity and gas chromatographic method showed that there was gradual reduction in ethanol content on storage. GC method provided much accurate and precise result as compared to specific gravity method. Variation in alcohol content was observed with different containers used in the manufacturing processes. The reduction of self generated alcohol might be due to vaporization on opening of the container. Therefore, Asava and Arista preparations must have to be consumed within a shorter period of time or formulation could be prepared in smaller volume containers in divided doses. It was observed that the formulations became acidic on storage and the acidity was further enhanced on long storage.<sup>35</sup>

In another study, marketed Kumaryasava was evaluated by physico-chemical parameters and the observed values were pH (3.62), viscosity (1.6738), specific gravity (1.1243), solid content (34.68% v/v), alcohol content (6.18% v/v), reducing sugar (7% v/v) and non-reducing sugar (0.27% v/v).<sup>36</sup>

Phytochemical screening of the Kumaryasava showed the presence of phenols and glycosides. The alcohol content, specific gravity and pH of the formulation were found to be 6.9 % v/v, 1.1246 and 3.42 respectively.<sup>10</sup>

#### Kutajarista

In a study, Kutajarista was evaluated for physico-chemical parameters and the results obtained such as total solids (25.176% v/v), specific gravity (1.1011), reducing sugar (17.2% v/v), non-reducing sugar (0.9% v/v), pH value (4) and alcohol content (5.18% v/v) were found to be within the standard limits.<sup>37</sup> In another study it showed pH (3.96) which was decreased during fermentation. Decrease of the sugar content (22% v/v) and increase in alcohol content (11% v/v) was the indication of active fermentation. Specific gravity was found to be 1.04.<sup>38</sup> Phytochemical screening of the Kutajarista showed the presence of phenols, steroids and terpenoids. Alcohol content, specific gravity and pH of the formulation were found to be 7.2 % v/v, 1.0766 and 3.54 respectively.<sup>10</sup>

In a separate study, the role of different types of container and fermenter on preparation of Kutajarista were assessed. Kutajarista was prepared by two methods, traditionally (using Dhatakipuspa) and non-traditionally (using dried bakery yeast) as the fermenter. Different material of containers such as mud, wood, stainless steel and plastic were used for preparation. Following observations were made during the study: Highest percentage of yield of formulation (97% v/v) was found in stainless steel container than other containers. Formulation prepared in stainless steel container using yeast was less acidic at pH value (4.4) than other



containers. Formulation prepared in plastic container using dhatakupuspa showed highest specific gravity (1.05) than others. Formulation prepared in stainless container using yeast showed higher alcohol content (8% v/v) than other containers. Formulation prepared in wooden container using dhatakupuspa showed more solid content (18.7% v/v) than stainless steel (14.6% v/v) container. High sugar content (16.3% v/v) and reducing sugar (9.4% v/v) were found in formulation prepared in mud pot using yeast than dhatakupuspa as the fermenter. Higher percentage of non-reducing sugar (7.6% v/v) was found in formulation prepared in plastic container using yeast as fermenter than dhatakupuspa.

It was observed that the onset and completion of fermentation process were very rapid if the formulation was prepared using yeast, because the fermentation process was started on the next day and completed within a month. But in case of formulation prepared using dhatakupuspa, the fermentation was started on 5<sup>th</sup> day and completed in the second month. In the later case fermentation process was delayed due to slow natural growth and multiplication of yeast cells, which need some time to start their enzymatic action. The taste of the formulation prepared in mud and wooden container was sourer (due to bacterial growth) than the formulation prepared in stainless steel and plastic container. *Mycoderma aceti* causes an acidic type of fermentation and gives much more taste to the preparation. The sample prepared by using yeast was having bitter taste than the sample prepared using dhatakupuspa. This might be due to conversion of the alkaloids into their salts. The sample prepared using dhatakupuspa was found to possess increased alcohol percentage up to 60 days indicating longer duration of the fermentation process.<sup>39</sup>

#### Lohasava

Phytochemical screening of the Lohasava showed the presence of phenols and terpenoids. Alcohol content, specific gravity and pH of the formulation were found to be 9% v/v, 1.1174 and 3.46 respectively.<sup>10</sup>

#### Mustakarista

In this study, Mustakarista was prepared using three different containers made up of earth, steel and wood and their evaluation results were compared with the marketed formulation. Formulation prepared in steel container showed higher alcohol content (14.13% v/v), total solid content (49.73% v/v) and specific gravity (1.1) than the other containers. The pH value (3.32) and viscosity (3.29 cps) were found high for formulation prepared in earthen pot. Phytochemical screening of all formulations showed the presence of carbohydrate, amino acids, flavonoids, alkaloids and tannins. Formulation prepared in steel container was found to be most significant.<sup>40</sup>

In another study, four different brands of marketed Mustakarista were evaluated by physico-chemical parameters. The pH of all formulations (3.89-3.98)

became acidic on storage and became more acidic on further storage. The physico-chemical parameter analysis of different marketed formulations showed varying results; mean acid value (1.72-1.83), boiling range (73<sup>o</sup>-75<sup>o</sup>), total solid (25.96-33.83), loss on drying (79.62-88.48), viscosity (1.81-2.0) and weight per ml (1.06-1.07). Phytochemical study of Mustakarista showed the presence of carbohydrates, glycosides, amino acids, saponins, phenols, tannins and flavonoids.<sup>35</sup>

#### Saraswataristam

Saraswataristam was prepared and evaluated by physical parameters. Total ash, acid insoluble ash, water insoluble ash, sulphated ash, alcohol soluble extractive, water soluble extractive and ether soluble extractive values were found to be 1.1% v/v, 0.54% v/v, 0.4% v/v, 0.35% v/v, 30.2% v/v, 28.5% v/v and 15.8% v/v respectively. It's phytochemical screening showed the presence of alkaloids, flavonoids, steroids, tannins and saponins.<sup>40</sup>

#### CONCLUSION

Medicinal plant formulations in form of Ayurvedic preparations play an important role in the general healthcare system of many developing countries worldwide and are rapidly gaining popularity in many developed countries as well. Therefore, there is a need for maintaining technical standards in manufacturing and development of evaluation procedures to standardize the Ayurvedic formulations so as to enhance their acceptability and commercialize them worldwide. This would contribute to increased reliance and making them more competitive for global markets. The deployment of WHO recommended various standardization procedures in testing various quality parameters to ensure an effective quality control Ayurvedic product cannot be over emphasized. The assurance on the safety and efficacy of a Ayurvedic drug requires monitoring of the quality of the formulation starting from collection through processing to the finished packaged product. This will strengthen the natural drug regulatory process and maintain the desired quality and efficacy.

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**Source of Support: Nil, Conflict of Interest: None.**

