Original Article

Determination of alcoholic content of Asava and Arishta by distillation method and specific gravity method

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ABSTRACT

Background: Ayurveda is a traditional Indian medicinal system being practiced for thousands of years. More than 1,200 species of plants, nearly 100 minerals and over 100 animal products comprise the Ayurvedic Pharmacopoeia Asava and Arishta are unique dosage form discovered by Ayurveda having indefinite shelf life and it was said that the "older the better it is". Because this dosage form has an inherent attribute of continuous hydro-alcoholic extraction and probably formation of natural analogues of the chemical compounds present in the medicinal plants. **Materials and Methods:** Marketed formulation of Asava and analytical grade ethanol were used. The alcoholic content was determined by the Specific gravity method. Initially, the distillation of Asava formulation was carried out and then in the second step, from the distillate, the specific gravity was calculated. **Results:** The apparent specific gravity calculated was 0.9948, which was found to be less than that of the marketed formulation. **Conclusion:** Ethanol content is a key factor for bio-absorption/efficacy of formulations. However, the conc. of alcohol in the formulation should be in the range of 3-10% and therefore, it is essential to authenticate the marketed formulations.

Keywords: Arishta, Asava, distillation, specific gravity

INTRODUCTION

Ayurvedic fermented formulations

Asavas and arishtas

Researchers consider Ayurveda as the oldest healing science. Ayurveda in Sanskrit refers to "the science of life." The knowledge and concept of Ayurveda came in India more than 5000 years ago. The Ayurvedic formulations do not focus on treating the disease, instead they help in promoting the health of an individual.[1]

Asava-arishta has been traditionally used in Ayurveda since long time but its novel hydroalcoholic extraction method is not much explored yet. This advanced dosage form probably results into transformation of several phytochemical compounds present in the herbs used to prepare it and thereby either rendering them less toxic or more potent, besides helping in their faster absorption. Asavas and arishtas are alcoholic formulations which are synthesized by the fermentation of herbal juices or their decoctions, along with the addition of sugars. Arishtas are prepared when the herbs undergo the process of decoction in boiling water while asavas are prepared by directly using fresh herbal juices. Both of these formulations then undergo the fermentation process, after the addition of sugar source with dhataki (*Woodfordia fruticosa* Kurz) flowers. Both of these formulations contain up to 12% by volume of alcohol, these formulations are sweet, slightly acidic and they have agreeable aroma. Since alcohol is present in these formulations, it leads to the following merits, such as higher keeping quality, enhanced therapeutic potential, improvement in the efficiency of extraction of drug molecules from the herbs and improvement in drug delivery into the human body sites.[1]

As already discussed, these formulations contain up to 12% by volume of alcohol, to determine the alcoholic content in these formulations; following methods are used.

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ALCOHOLIC CONTENT DETERMINATION METHODS

Gas chromatography

The variation in ethanol content in *asava* and *arista* preparations stands a major problem in fixing up their limits as a quality control parameter. Therefore, to establish its limit over a period of time, Selvan *et al.*; 2015, carried out a study to determine the ethanol content of the formulations *Kumaryasava* and *Mustakarista* on a storage period of 30 days by specific gravity as well as chromatographic (gas chromatography [GC]) methods. A simple, rapid, precise, and accurate GC method was used for the determination of ethanol in the marketed preparations of *Kumaryasava* and *Mustakarista*. GC analysis was performed on Chemito GC7610 with flame ionization detector using Carbowax 20M (stationary phase) packed with steel column with inner diameter of 2 mm. Nitrogen, the carrier gas was used at a flow rate of 1 kg/cm^2/min. The oven temperature was maintained at 20–50° and the detector at 280°, injector at 140–250°. A range of standard solutions of ethanol was prepared containing 1, 2, 3, 4, and 5% v/v of ethanol using ethanol 98% and HPLC grade water. From the standard solution, 1 ml was diluted to 10 ml with HPLC grade water. Then, 1 µl of solution was injected and chromatogram was recorded. The area was plotted against concentration of ethanol to obtain a calibration graph. For sample analysis, 25 ml of syrup was taken in a 500 ml distillation flask. To this, 5 ml of 0.1 N sodium hydroxide solution, 10 mg of phenolphthalein powder, and 150 ml of HPLC grade water were added. Resulting mixture was heated to 110° and 100 ml of distillate was collected. Distillate (1 ml) was diluted to 10 ml with HPLC grade water and an aliquot of this solution was analyzed as described earlier and chromatogram was noted. The ethanol content of the formulations was determined by gas chromatography method at two levels (i) at the opening of the container and (ii) after 30 days of opening of the container. The ethanol content determined on day 0 varied from 0.1% to 1.6% v/v and on 30th day it reduced to 0.011–0.025% v/v.[2]

Thin-layer chromatography (TLC)

Singh *et al.*; 2010, in their research work presented TLC method for the standardization of the Ayurvedic preparation, Arjunarishta and alcohol content was determined in this formulation by the procedure given ahead; for performing TLC, 2 g of powdered (Terminalia Arjuna) drug was taken in tarred silica crucible. The powdered drug was incinerated by heating until free carbon got released. Residue was cooled and kept in desiccator. The ash was weighed and calculated as the percentage of total ash.[3]

Specific gravity method

Sayyad *et al.*; 2012, determined the alcoholic content in Arishtas by specific gravity method using the following procedure. To the distillation flask, 25 ml of preparation was added and its temperature was noted. It was diluted with equal volume of water. Afterward, it was distilled and distillate about 2 ml less than the total volume was collected. Water was added to measure exactly the same volume of original test liquid and adjusted to temperature which was already noted before. Specific gravity of this liquid was determined and alcohol content was analyzed using relative density table, which is prescribed by United States Pharmacopoeia and the alcohol content was found out to be 6.42% w/w.[4]

In addition, Tamboli *et al.*; 2018, determined the alcoholic content in Asavas by specific gravity method using the following procedure. 25 ml of preparation was mixed separately with about 100 ml of water and saturated with sodium chloride. Then, 100 ml of hexane was added and the mixtures were vigorously shaken for 2–3 min and were allowed to stand for 15–20 min. Then, the lower layers were run into the distillation flask, the hexane layer was washed by shaking vigorously with about 25 ml of sodium chloride solution, allowed to separate and washed liquors were run into the first saline solution. Mixed solutions were made alkaline with 1 M sodium hydroxide using solid phenolphthalein as indicator. Little pumice powder and 100 mL of water were added. Mixture was then distilled and not <90 ml of distillates were collected into 100 ml volumetric flasks and made up the volume with distilled water. Specific gravities of both the mixtures were determined and alcohol contents were calculated and found out to be 7.2081±0.003 %v/v.[5]

MATERIALS AND METHODS

Only two reagents were used; marketed formulation of Asava and analytical grade ethanol. The alcoholic content was determined from Asava by the specific gravity method because this method provides an approximation of the alcohol content only; also this method assumes that the difference in specific gravity, before and after fermentation is due solely to the conversion of sugars before fermentation into alcohol. The experiment was performed in triplicate.

Step 1: Distillation of sample of Ayurvedic formulation.[6]

For liquids presumed to be containing <30% v/v of alcohol:

a. 25 mL sample was taken in a suitable distilling apparatus and the temperature was noted, at which the volume was measured
b. Equal volume of water was added and it was distilled
c. Distillate was collected 2 mL less than original volume of the test liquid (i.e., 23 mL)
d. The temperature was adjusted at which the original test liquid was to be measured (room temperature)
e. Water was added to make up the volume up to 25 mL and mixed.

The distillate must be clear (and not more than slightly cloudy and should not contain more traces of volatile substances other than alcohol and water).

Afterward, we carried out the next step;

Step 2-Determination of specific gravity of sample (distillate).[6]

a. Specific gravity method is easiest and simplest method to determine the concentration of ethanol volume/volume in the given sample
b. The specific gravity of 100% alcohol (ethanol) at 25°C is 0.7899

Table 1: AOAC chart[7]

Percentages by volume at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravity at various temperatures												
Apparent specific gravity	**15.56/15.56**	**20/20**	**22/22**	**24/24**	**25/25**	**26/26**	**28/28**	**30/30**	**32/32**	**34/34**	**35/35**	**36/36**
1.0000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.9999	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
98	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
97	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
96	0.27	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.24
95	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
94	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
93	0.47	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
92	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53
91	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
90	0.67	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66
89	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73
88	0.80	0.80	0.80	0.80	0.80	0.80	0.79	0.79	0.79	0.79	0.79	0.79
87	0.87	0.87	0.87	0.87	0.87	0.87	0.86	0.86	0.86	0.86	0.86	0.86
86	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93
85	1.00	1.00	1.00	1.00	1.00	1.00	0.99	0.99	0.99	0.99	0.99	0.99
84	0.07	0.07	0.07	0.07	0.07	0.07	1.06	1.06	1.06	1.06	1.06	1.06
83	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
82	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.19	0.19	0.19	0.19	0.19
81	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.26	0.26	0.26
80	0.34	0.34	0.34	0.34	0.34	0.33	0.33	0.32	0.32	0.32	0.32	0.32
79	0.41	0.41	0.41	0.40	0.40	0.40	0.40	0.39	0.39	0.39	0.39	0.39
78	0.48	0.48	0.48	0.47	0.47	0.47	0.47	0.46	0.46	0.45	0.46	0.46
77	0.54	0.54	0.54	0.54	0.54	0.53	0.53	0.53	0.53	0.53	0.52	0.52
76	0.61	0.61	0.61	0.60	0.60	0.60	0.60	0.59	0.59	0.59	0.59	0.59
75	0.68	0.68	0.68	0.67	0.67	0.67	0.67	0.66	0.66	0.66	0.66	0.66
74	0.75	0.75	0.75	0.74	0.74	0.73	0.73	0.73	0.73	0.72	0.72	0.72
73	0.82	0.81	0.81	0.81	0.81	0.80	0.80	0.80	0.80	0.79	0.79	0.79
72	0.88	0.88	0.88	0.87	0.87	0.87	0.86	0.86	0.86	0.85	0.85	0.85
71	0.95	0.95	0.95	0.94	0.94	0.94	0.93	0.93	0.93	0.92	0.92	0.92
70	2.02	2.02	2.02	2.01	2.01	2.01	2.00	2.00	2.00	0.99	0.99	0.99
69	0.09	0.09	0.09	0.08	0.08	0.08	0.07	0.07	0.06	2.05	2.05	2.05
68	0.16	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.13	0.12	0.12	0.12
67	0.23	0.22	0.22	0.21	0.21	0.21	0.20	0.20	0.20	0.19	0.19	0.19
66	0.30	0.29	0.29	0.28	0.28	0.28	0.27	0.27	0.27	0.26	0.26	0.26
65	0.37	0.36	0.36	0.35	0.35	0.35	0.34	0.34	0.33	0.32	0.32	0.32
64	0.43	0.43	0.43	0.42	0.42	0.42	0.41	0.41	0.40	0.39	0.39	0.39
63	0.50	0.50	0.50	0.49	0.49	0.49	0.48	0.48	0.47	0.46	0.46	0.46
62	0.57	0.57	0.57	0.56	0.56	0.56	0.55	0.54	0.54	0.53	0.53	0.53
61	0.64	0.64	0.64	0.63	0.63	0.63	0.62	0.61	0.60	0.60	0.59	0.59
60	0.71	0.70	0.70	0.70	0.70	0.70	0.69	0.68	0.67	0.67	0.66	0.66
59	0.78	0.77	0.77	0.77	0.77	0.77	0.76	0.75	0.74	0.74	0.73	0.73
58	0.85	0.84	0.84	0.83	0.83	0.83	0.82	0.82	0.81	0.81	0.80	0.80
57	0.92	0.91	0.91	0.90	0.90	0.90	0.89	0.88	0.87	0.87	0.86	0.86
56	0.99	0.98	0.98	0.97	0.97	0.97	0.96	0.95	0.94	0.94	0.93	0.93
55	30.06	3.05	3.05	3.04	3.04	3.04	3.03	3.02	3.01	3.01	3.00	3.00
54	0.13	0.12	0.12	0.11	0.11	0.11	0.10	0.09	0.08	0.08	0.07	0.07
53	0.20	0.19	0.19	0.18	0.18	0.18	0.17	0.16	0.15	0.15	0.14	0.14
52	0.27	0.26	0.26	0.25	0.25	0.25	0.24	0.23	0.22	0.22	0.21	0.21
51	0.34	0.33	0.33	0.32	0.32	0.32	0.31	0.30	0.29	0.28	0.27	0.27
50	0.41	0.40	0.40	0.39	0.39	0.39	0.38	0.37	0.36	0.35	0.34	0.34
49	0.49	0.47	0.47	0.46	0.46	0.46	0.45	0.44	0.43	0.42	0.41	0.41
48	0.56	0.54	0.54	0.53	0.53	0.53	0.52	0.51	0.50	0.49	0.48	0.48
47	0.63	0.61	0.61	0.60	0.60	0.60	0.59	0.58	0.57	0.56	0.55	0.55
46	0.70	0.68	0.68	0.67	0.67	0.67	0.66	0.65	0.64	0.61	0.62	0.62
45	0.77	0.76	0.75	0.74	0.74	0.74	0.73	0.72	0.70	0.69	0.68	0.68
44	0.84	0.83	0.82	0.81	0.81	0.81	0.79	0.78	0.77	0.76	0.75	0.75
43	0.91	0.90	0.89	0.88	0.88	0.88	0.86	0.85	0.84	0.81	0.82	0.82
42	0.99	0.97	0.96	0.95	0.95	0.95	0.93	0.92	0.91	0.90	0.89	0.89

(*Contd…*)

Table 1: (*Continued*)

Percentages by volume at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravity at various temperatures												
Apparent specific gravity	**15.56/15.56**	**20/20**	**22/22**	**24/24**	**25/25**	**26/26**	**28/28**	**30/30**	**32/32**	**34/34**	**35/35**	**36/36**
41	4.06	4.04	4.03	4.02	4.02	4.02	4.00	0.99	0.98	0.97	0.96	0.96
40	0.13	0.11	0.10	0.10	0.09	0.09	0.07	4.06	4.05	4.04	4.03	4.03
39	0.20	0.18	0.17	0.17	0.16	0.16	0.14	0.13	0.12	0.11	0.10	0.10
38	0.28	0.26	0.25	0.25	0.24	0.23	0.21	0.20	0.19	0.18	0.17	0.17
37	0.35	0.33	0.32	0.32	0.31	0.30	0.28	0.27	0.26	0.25	0.24	0.24
36	0.42	0.40	0.39	0.39	0.38	30.7	0.36	0.35	0.33	0.32	0.31	0.30
35	0.50	0.48	0.47	0.46	0.45	0.44	0.43	40.2	0.40	0.39	0.38	0.37
34	0.57	0.55	0.54	0.53	0.52	0.51	0.50	0.49	0.47	0.46	0.45	0.44
33	0.64	0.62	0.61	0.60	0.59	0.58	0.57	0.55	0.54	0.53	0.52	0.51
32	0.71	0.69	0.68	0.67	0.66	0.65	0.64	0.63	0.61	0.60	0.59	0.58
31	0.79	0.77	0.76	0.75	0.74	0.73	0.72	0.70	0.68	0.67	0.66	0.65

c. While specific gravity of distilled water at 25°C is 1
d. The mixture of water and alcohol (ethanol) will have specific gravity between these two limits, i.e., 0.7899 and 1
e. The more the water content in the given sample the specific gravity is near to 1
f. When the concentration of alcohol (ethanol) in the sample is higher, the specific gravity falls near to 0.7899
g. Hence, the specific gravity of mixture of water and ethanol cannot be more than 1 and cannot be <0.7899
h. To find out concentration of alcohol (ethanol) practically the requirement is specific gravity bottle
i. The weight of empty specific gravity bottle (SGB) along with stopper requires to be measured (W_1)=.....
j. Then, the specific gravity bottle requires to be filled with pure distilled water up to its rim and put the stopper
k. The excess amount of water comes out of the stopper, it must be wiped out

RESULTS

The below mentioned calculations were carried out to find the alcoholic content.

Weight of empty specific gravity bottle (SGB), W_1 = 17.32 g

Weight of SGB + distilled water, W_2 = 42.55 g

Weight of water (W_2-W_1) = 25.23 g

Weight of SGB + distillate, W_3 = 42.42 g

Weight of distillate (W_3- W_1) = 25.10 g

Apparent specific gravity = Weight of distillate/Weight of water

= 25.10/25.23

= 0.9948.

DISCUSSION

By referring to AOAC chart (at temperature 25°C) as shown in Table 1,[7] the alcohol content was found to be 3.51 % v/v, which was less than the marketed formulation of Asava.

CONCLUSION

Asava and arishta are widely used Ayurvedic formulations available in a range of brands in the market. Therefore, it is essential that authentic and quality formulations reach the consumer. The ethanol content being a key factor for bio-absorption/efficacy of formulations is of utmost importance. When alcohol is present in the formulation, it adds to several advantages, such as better keeping quality, enhanced therapeutic properties, improvement in the efficiency of extraction of drug molecules from the herbs, and improvement in drug delivery into the human body sites. However, the conc. of alcohol in the formulation should be in the range of 3–10% and it was estimated by specific gravity method.

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