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Quantitative analysis of organic volatile compounds in two ayurveda pharmaceuticals widely used in Sri Lanka

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Abstract
Ashwagandharishta and *Arawindasava* during the fermentation period yields considerable amount of alcohol and organic volatiles. Quantification of these constituents is utmost significant in quality controlling and standardization of products to confirm the consumer safety. 20 brands of *Ashwagandharishta* and 15 commercial brands of *Arawindasava* were tested for ethanol, other alcohols, ethyl acetate and acetaldehyde using standard distillation method and gas chromatography. Percentage of the maximum level of ethanol detected was 8.23v/v, the minimum was 5.1v/v and average was 6.54 ± 0.90 in *Ashvagandharishta* and these values were 8.6 v/v, 5.67 v/v and 7.68 ± 0.80 respectively for *Aravindasava*. Average values of other alcohol, methanol (107.2 ± 73.3), n-propanol (11.9 ± 7.6), isobutanol (33.1 ± 23.0), isoamyl alcohol (117.9 ± 51.4) and isopropyl alcohol (7.3 ± 2.6) were present in mg/L in 20 brands of *Ashvagandharishta*. Similarly, methanol (99.6 ± 66), n-propanol (6.8 ± 4.4), isobutanol (22.3 ± 5.9), iso amyl alcohol (86.4 ± 13.7) and isopropyl alcohol (5.2 ± 2.7) were present in mg/L in 15 brands of *Aravindasava*. The average concentration of ethylacetate in different brands of *Ashvagandarishta* was 104.2 ± 68.00 in mg/L. In *Aravindasava*, the corresponding values were 135.5 ± 50.2 in mg/L. The average value of acetaldehyde in the tested brands of *Ashvagandharishta* was 57.6 ± 30.9 in mg/L. These values were 55.8 ± 15.4 in mg/L respectively for *Aravindasava*.

Keywords: *Arishta*, *Asava*, fermentation, alcohols, ethyl acetate, acetaldehyde

1. Introduction
Arishta and *Asava* are legalized two types of Ayurveda Pharmaceuticals available in Sri Lanka [1]. There are about two hundred registered manufactures for these products and 35 varieties of *Arishta* and 25 varieties of *Asava* is manufactured island wide [2]. These preparations have been used as medicines over 3000 years [3, 4] and are taken as appetizers and stimulants. These preparations could be purchased without medical prescriptions in Sri Lanka. To ensure the quality and safety of *Asava* and *Arishta* to the consumer, they should be brought up to a certain standard. Determination of constituent, trace components and contaminants of these products highlights the importance of ensuring the quality and safety of them to the consumer [5].
In the manufacturing process of *Asava* and *Arishta* following steps are being taken. Aqueous extraction of prescribed medicinal plants concentrated with sweeten maters are subjected to natural fermentation [6] in manufacturing process of Asava and Arishta.Water extraction of a given herbal formula that is fermented in a tightly closed vessel is known as *"Arishta".* Liquor obtained from fermenting mixture of sweetened water with prescribed herbal formula in a tightly closed vessel is known as "*Asava*". *Arishta and Asava* are prepared using natural heterogeneous fermentation process in fermenting vessels. During this process, a wide range of compounds, including alcohols, carbonic acids, aldehydes, and other flavor compounds could be generated like in wine manufacturing process [7].
Ethanol content serves as a quality index and taxation factor for alcoholic beverages [8]. Fibrous plant materials, dried grapes with skin and citrus fruits which are used as raw materials are rich in pectin and pectin esterase and contamination of pots with fungus may generate methanol in the product.
In addition to ethanol and methanol, other organic volatiles that give different odor and taste may be generated in the fermentation process [9]. Some by-products of the alcoholic fermentation, such as acetaldehyde, ethyl acetate and amyl alcohols are mainly responsible for the flavor of fermented alcoholic beverages and their amounts specify the quality of the product [10]. These intermediate products can remain in the final product. Information of the compounds presented in Ayurveda preparations are lacking. This may lead to a negative impression on these products among the quality concern consumers in the modern world. Hence scientific quantitative assessment of the products of *Asava* and *Arishta* could contribute to overcome this problem and could also be contributed to identify and minimize the presence of constituents lead to health risks by giving side effects. Findings of the study lead to initiate legislative actions to maintain the quality and standards of these products. Hence a quantitative assessment of products could be contributed to identify the parameters needed to upgrade the quality and standards of *Asava* and *Arishta* and would be useful in export of these preparations to the international market without legal hesitation. Hence determination of ethanol, methanol and other alcohol, acetaldehyde, ethyl acetate of these ayurvedic preparations is the objectives of the study.

2. Materials and Methods

Commercially available 20 brands of *Ashwagandharishta* and 15 brands of *Arawindasava* distributed island wide by registered manufacturers in the Department of Ayurveda were selected for the study. Estimation of ethanol was done by the standard distillation method and, Gas chromatographic (GC) methods were followed for the determination of different levels of methanol, iso propyl alcohol, isoamyl alcohol acetaldehyde and ethyl acetate. Agilent gas chromatogram with flame ionized detector (FID) with capillary column model No - DB -624 Part No - 122-1364, 60.0m (Length) x 250µm (internal diameter) x 1.40µm (Film thickness) and Agilent GC syringe, was used in this study. Alcohol beverage standards were used as reference standard.

2.1 Determination of ethanol in *Ashwagandharishta* and *Arawindasava*

100.00 $cm^3$of each brand of Arishta were subjected to distillation using standard distillation apparatus and electric heating mantle and obtained distillate was measured.

2.2 Preparation of *Arishta and Asava* samples for GC analysis

30.00 cm^3 of Arishta were pipetted out from the bottles and distilled using British standard glassed distil apparatus and total distillation was collected and refrigerated. Same procedure was applied to the Asava sample.

2.3 Analysis of sample for alcohols and volatiles

1.00 µl of distillate were injected to the injection block of gas chromatogram. The injection blocks and flame ionization detector temperatures were kept constant at 250 °C and 300 °C, respectively; nitrogen was used as the carrier gas. The oven temperature was kept at 40 °C held for 6 min and then increased to 140 °C at 10 °C min^{-1}. Inlets Pressure - 29.79 psi Total flow - 99.4 ml/min Split ratio - 48'6:1 Split Flow - 95.1ml/min Column Pressure - 29.79 psi Flow - 2.0ml/min Average velocity - 33cm/sec H_2 Flow rate 40 ml/min Air flow 450 ml/min Make up flow was kept at 45 ml/min. Results were recorded using the system software.

3. Results and Discussion

Percentage of the maximum level of ethanol detected was 8.23v/v and the minimum was 5.1v/v and average was 6.54 ± 0.90 in *Ashvagandharishta* and these values were 8.6v/v and 5.67v/v and 7.68 ± 0.80 respectively for *Aravindasava*. Methanol (max 316.1, min 31.1; mean 107.2 ± 73.3), n-propanol (max 28.1, min 3.2; mean 11.9 ± 7.6), isobutanol (max 89.2, min 7.6; mean 33.1 ± 23.0), isoamyl alcohol (max 241.1, min 45.0; mean 117.9 ± 51.4) and isopropyl alcohol (max 13.7, min 4.6; mean 7.3 ± 2.6) were present in mg/L in 20 brands of *Ashvagandharishta*. Similarly, methanol (max 241.3, min 28.3; mean 99.6 ± 66.0), n-propanol (max 13.5, min 1.6; mean 6.8 ± 4.4), iso butanol (max 31.4, min 8.2; mean 22.3 ± 5.9), iso amyl alcohol (max 109.5, min 69.1; mean 86.4 ± 13.7) and isopropyl alcohol (max 11.6, min 0.0; mean 5.2 ± 2.7) were present in mg/L in 15 brands of *Aravindasava*. The concentration of ethyl acetate in different brands of *Ashvagandarisht*a varied from 26.4 to 221.4, with an average of 104.2 ± 68.00 in mg/L. In *Aravindasava*, the corresponding values were 50.2, 224.0 and 135.5 ± 50.2 in mg/L. Levels of acetaldehyde in the tested brands of *Ashvagandharishta* varied with a maximum of 147.2 and a minimum of 31.1 and the mean value was 57.6 ± 30.9 in mg/L. These values were 73.5, 27.6 and 55.8 ± 15.4 in mg/L respectively for *Aravindasava*.

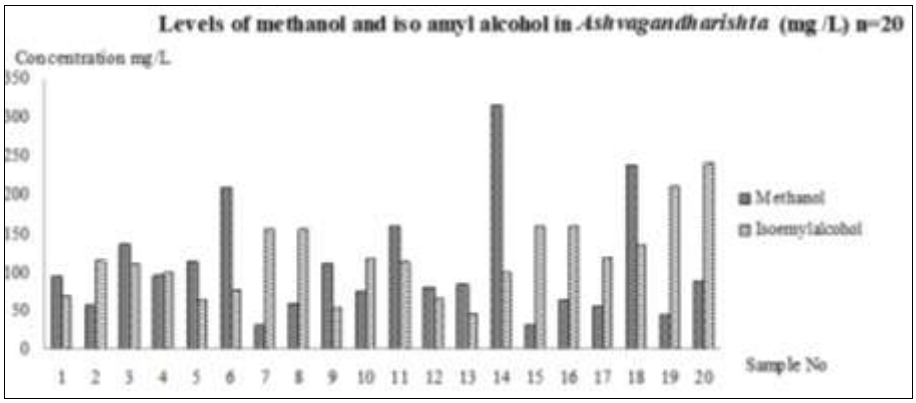

Fig 1: Levels of methanol and Iso amyl alcohol in Ashvagandharishta

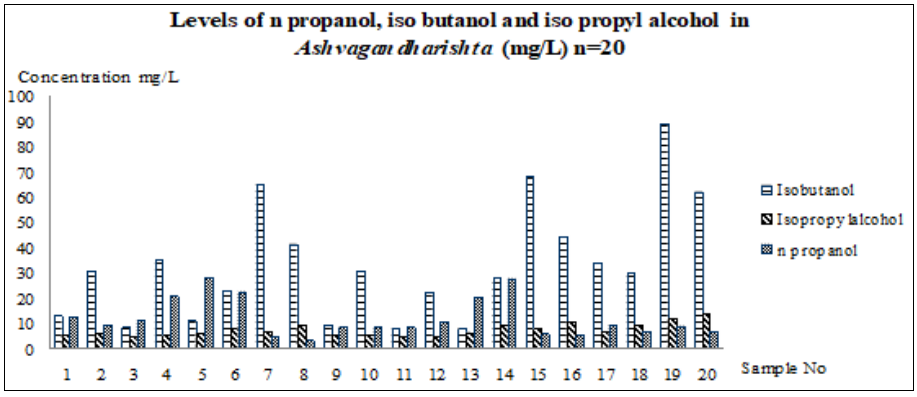

Fig 2: Levels of n propanol, iso butanol and iso propyl alcohol in *Ashvagandharishta*

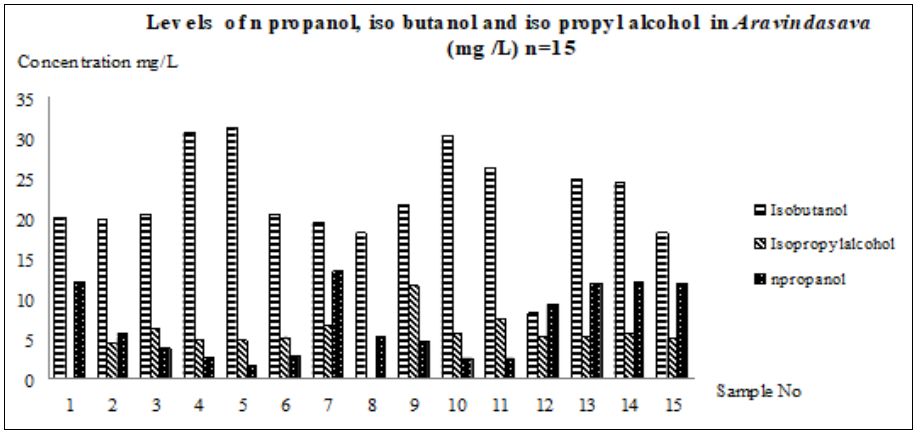

Fig 3: Levels of methanol and iso amyl alcohol in Aravindasava

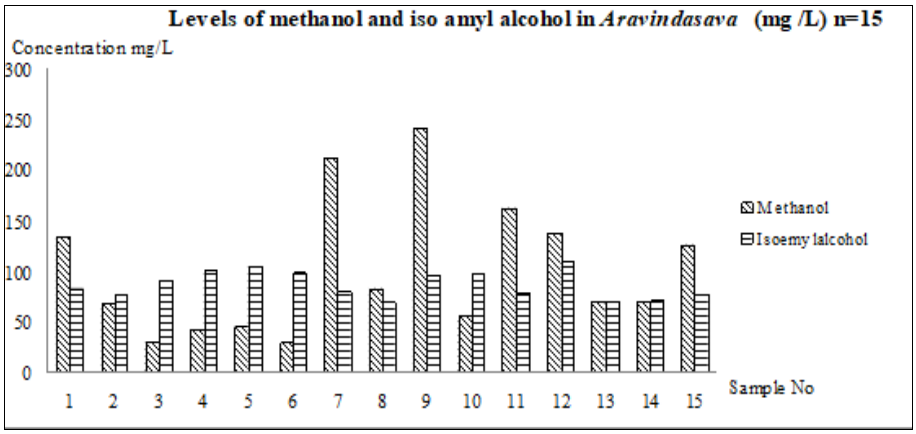

Fig 4: Levels of n propanol, iso butanol and iso propyl alcohol in Aravindasava

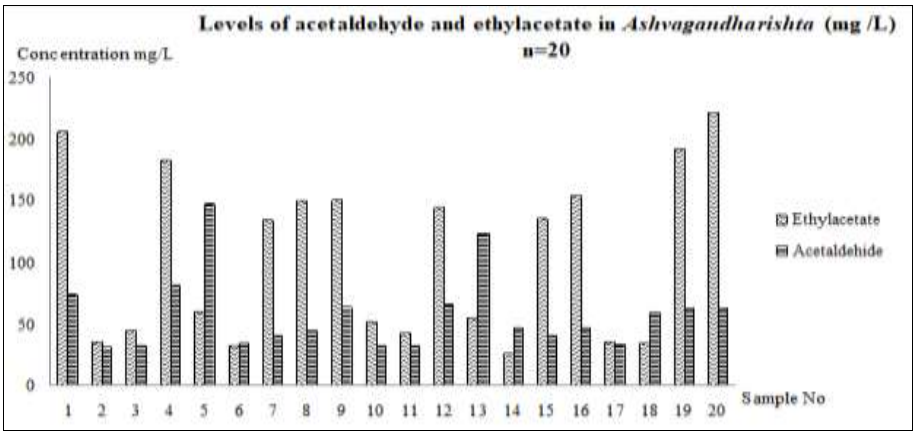

Fig 5: Levels of acetaldehyde and ethyl acetate in *Ashvagandharishta*

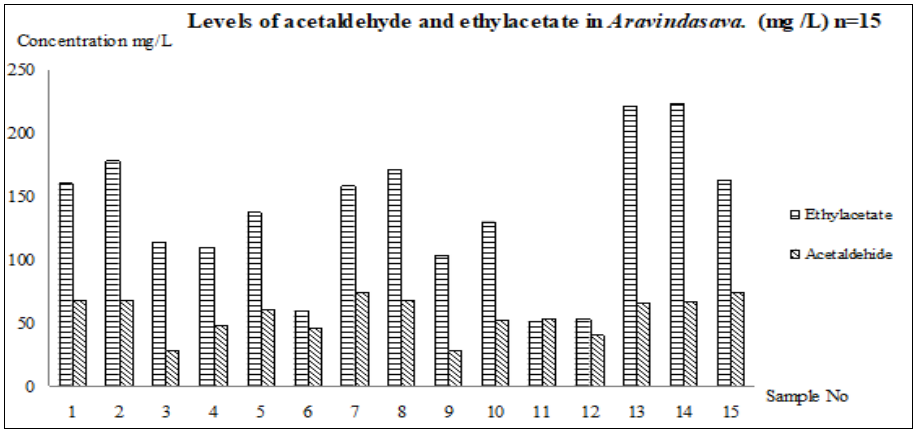

Fig 6: Levels of acetaldehyde and ethyl acetate in *Aravindasava*

Ethanol present in this products serve in different ways as a preservative, solvent for the herbal extraction, stimulant and appetizer for the consumer and could be used as a quality index and taxation factor in herbal alcoholic beverage industry. Presence of toxic methanol in the product could be questioned by the quality concerned society. Methanol is a by Product of the natural fermentation, which may reach concentrations as high as 300 mg/L in wines [11]. Concentration levels of methanol in wine varieties from 30.5 to 121.4 mg/L in white wines 62.5 to 84.6 mg/L in rose wine, 61.0 to 207.0 mg/L in red wines [12] and 18-152 mg/L in African traditional brew [13]. The average intake of methanol from natural sources varies but limited data suggests an average intake of considerably less than 10 mg/day. The average intake of alcohol consumers varies between 0 and 600 mg/day, depending on the source and in some cases the quality of the beverages. The high ethanol/methanol ratio in alcoholic beverages must have a very significant protective effect. Ethanol, which is found in natural food sources, to behave as an antidote for methanol toxicity should be present in the ratio 5: 500,000 times methanol: ethanol. The probability of methanol toxicity of these products could be minimum because of the presence of high ratio of ethanol to methanol and the volume of the prescribed dosage for daily consumption is small.

The role of natural yeast strains has taken an important place in the manufacturing process of *Asana* and *Arista*. During the growth of yeast strains in fermenting process, formation of acetaldehyde has been documented and also acetaldehyde can be formed by acetic acid bacteria (AAB). AAB form acetaldehyde by oxidizing ethanol. On average levels of acetaldehyde in red wines contain 30 mg/L, white wine 80 mg/L and Sherries 300 mg/L of acetaldehyde [14]. Other alcohols could be the intermediate or by-products of the fermentation process.

4. Conclusions

The results showed that the range of detected levels of methanol in tested Sri Lankan brands of *Arishta and Asava* do not exceed the recorded levels of methanol in wine. The level of toxic methanol should be minimized in the product to avoid the health risk to the consumers. At low levels, acetaldehyde can contribute pleasant fruity aromas to these products. However, levels of acetaldehyde in these products can be a risk. Further studies are suggested to understand the influence of acetaldehyde in the human body after consumption of *Asava* and *Arishta.* Results revealed that the condition of manufacturing process of products should be standardized to maintain standards to avoid significant variation of tested parameters in different brands

5. Acknowledgments

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