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Formulation and physico-chemical standardization of *Viburnum coriaceum* arista

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ABSTRACT

The leaves,stems and roots of Viburnum coriaceum ,belonging to the family Adoxaceae ,were collected from Nilgiri hills of Tamil Nadu and authentificated. Following a successive extraction and a primary organic analysis, it has been known to contain phenolic compounds as its principal phyto-constituents in their hydroalcoholic fraction. . The crude drug (Patha) was formulated in to an asava using conventional anaerobic fermentation process for about 90 days. Apart from some traditional methods of standardization of asava, a new approach was made to select about thirteen numbers of physical, physic-chemical including organoleptic and primary organic analysis and were attempted with the asava to obtain a reproducible and consistent results and the same were recorded.This study can help performing quality control of ayurvedic formulations such as asava and arista excluding only some conventional methods of analysis any more.

Keywords: *Viburnum*, arista, sugar content, Alcohol content, viscosity.

INTRODUCTION

India has a rich heritage of using ayurvedic system of medicine which dates back to 5000 years or more and hosting several thousands of medicinally valuable plants belonging to the hundreds of families. One can not assure that all of these plants possess a long recorded scientific history, although they have been reported to contain medicinally valuable phyto-pharmaceuticals. For many of them, an authentic protocol that has been derived from multidisciplinary approach is

very scant. In particular, the plants, which are growing at an elevated altitude ascending more than 2000 ft and forest dominated hilly areas, which are not easily exposed to plant vendors, botanists, plant collectors and pharmacognosists due to an inaccessibility and climatic conditions of the locations.

The genus *Viburnum* Linn. is a typical example of such a kind, which is dwelling at a high altitude, belonging to the family Adoxaceae. The genus *Viburnum* Linn. includes about 17 species in India and about 200 species distributed throughout the world[1,2]. *Viburnum* Linn. Species have been reported to contain sesquiterpenes[3], triterpenes and phyto-sterols; phenolic compounds and their derivatives such as tannins, flavonoids and anthocyanins and iridoid glycosides in their stems, roots and leaves, and investigated to posses uterine sedative, diuretic, cardiovascular stimulant, antimicrobial, anti-inflammatory, anti-nociceptive, antispasmodic, anti-asthmatic and astringent activities[4,5]. In the late 1960s and early 1980s, the scientific studies on the genus *Viburnum* Linn. were voluminous[6-8]. However, the number of species subjected for the studies and the areas of investigations were markably narrow. After a couple of decades, a few *Viburnum species* re-emerged to be involved for some extensive phytochemical and pharmacological investigations. The typical examples are: iridoid aldehydes and their glycosides in *Viburnum luzonicum*[9] and their cytotoxic effect; vibsane type diterpenes from *Viburnum awabuki*[10]; iridoid glycosides from *Viburnum tinos;* antinociceptive and anti-inflammatory activities of *Viburnum*[11] *lanata*and *Viburnum opulus*[12]; iridoid glycoside from *Viburnum rhytidophyllum*[13].

MATERIALS AND METHODS

Plant Material

The leaves,stems and roots of *V. coriaceum* Blume, were collected (flowering season, June – August) from Nilgiri hills, Tamilnadu, India and authentificated by Dr.V.Chelladurai, Ex. Professor, (Botany), Medicinal plant survey for Siddha, Government of India, as *Viburnum coriaceum* Blume (VC)). Herbarium of the specimen (labeled VC131 forVC) was submitted to the museum of the department of Pharmacognosy, Nandini Nagar Mahavidyalaya College of Pharmacy.

Preparation of *Viburnum* arista by anaerobic fermentation method (An Ayurvedic formulation)

Approximately 1.5 seers (60 g) of the leaves,stems and roots (each 20g,1:1:1 ratio)(patha) were coarsely powdered and added with 32 seers (1024 ml of water) and boiled for about 3 – 5 h to prepare a decoction (Kashaya). The whole mixture was cooled at room temperature and filtered through a cotton cloth to obtain a decoction[14]. The decoction was taken in wooden vats of 2 litre capacity, to which dissolved were 12½ seers (400 g) of jaggery and boiled for half an hour.

Dravyas and Dhataki pushpa (*Woodfordia fructicosa*) were then added to the mixture kept in the wooden vats. The vessel was closed with a clean lid followed by wrapping around the lid with seven consecutive layers of clay smeared cloth. The vessel was buried in cellar (basement) for about a couple of months towards the completion of fermentation process (sandhana)[15,16].

After the stipulated period (60 days), the vessel was withdrawn to examine the preparation which showed a brownish black fluid with a frothing and aromatic odour and alcoholic taste. The final fluid decanted and filtered through a cotton cloth to obtain a clean transparent arista. Then the

arista was bottled and labelled and subjected to some modern methods of standardization and biological screening.

Standardization of arista[17-20]

Determination of total solids

A shallow, flat bottomed flanged dish, about 75 mm in diameter and about 25 mm deep, made of nickel was used for this analysis. Accurately 5 ml of arista was pipetted out and placed in the dish and evaporated at as low temperature as possible on a water bath until the solvent was removed and the residue is apparently dry. Then the dish was placed in an oven and dried to constant weight at 105° C. After the dish was provided with well-fitting cover, it was cooled in a desiccators.

Determination of boiling range (Distilling range)

A distillation unit fit with a thermometer was employed to determine the boiling range of the arista. The apparatus consisted of a distilling flask of 200 ml capacity; a condenser of 60 cm long; a receiver of 100 ml capacity which was graduated with 1 ml division; and a thermometer showing 0°C - 240° C.

The thermometer was positioned in the centre of the neck and the entire assembly was shield after dropping about 100 ml of arista to the distilling flask. With the aid of metallic stand and clamps, the entire assembly was placed on an electric heater having a thermostat, so that adjustment in temperature could be done conveniently. Distillation was switched on and the recorded was the temperature of first drop of the distillate. Then the temperature was increased in such a way the receiver could collect 4 – 5 ml per min. The process was continued until 25% (25 ml) of the distillate reached the receiver and the temperature of the last drop of the distillate to the receiver was also noted.

Necessary correction was employed observing the temperature readings from any variation in the parametric pressure from the normal (101.3 kPa) using following expression.

$t_1 = t_2 + K\,(a - b)$

t_1 – corrected temperature; t_2 – the observed temperature; a= 101.3; b – the barometric pressure of the time of the determination; K – the correction factor.

Determination of congealing range or temperature

The congealing temperature is that point at which there exists a mixture of the liquid phase of a substance and a larger proportion of the solid phase. This experimentation required 1 litre beaker in which two test tubes were placed in such a way one was inserted in to another test tube. The inner test tube contained 15 ml of arista and stopperd with a cork attached with a stirrer and a thermometer with 0.2° C graduation.

The beaker was filled with water and the test tubes were clamped in such a way they were immersed in water and distance of 18 mm be maintained between the bottoms of the beaker and test tube. The temperature at which a substance solidifies upon cooling is a useful index of purity.

Preparation of reference substance
Since arista is a liquid, the process of determination of congealing point was carried out in the same way of raising temperature, while stirring, about the room temperature using the apparatus for congealing point determination and noted down as a reference value.

Preparation of test substance of arista
The temperature of the bath was maintained near 15° C using addition of ice cubes and placed on a heating mantle which was kept turned off. Then the sample was stirred constantly to a rate of 20 cycles per min with simultaneous observation of rise in temperature with the thermometer. The congealing point was still hidden up to the room temperature. Hence, a slow rise of temperature was aided to the bath using the heating mantle until the congealing point appeared which was comparable to that of the standard. The process was repeated three times and the average was tabulated.

Determination of ethanol
25 ml of arista were accurately measured and mixed with 100 ml of double distilled water and poured in to a separating funnel. The mixture was saturated with sodium chloride and added was 100 ml of hexane, shaken vigorously 2 – 3 min. The mixture was allowed to stand for half an hour. The lower layer was run in to a distillation flask. The hexane layer was washed with 25 ml of concentrated sodium chloride solution in a separating funnel then the NaCl layer was added to the distillation flask. The whole mixture was made alkaline with 1 M sodium hydroxide solution using solid phenolphthalein as indicator. To this added were a little pumice powder and 100 ml of water.

The whole mixture was distilled to obtain 90 ml of distillate. The distillate was poured in to a 100 ml volumetric flask and made the volume to 100 ml with double distilled water. Using this mixture relative density was determined to calculate the percentage v/v alcohol of the arista.

Determination of freezing point of arista
Freezing point is the maximum temperature occurring during the solidification of a super-cooled liquid. The apparatus for its determination was designed as that of the apparatus used in the determination of congealing point of arista.

About 5 ml of arista was placed in the inner test tube, which was immersed in a 500 ml capacited beaker containing water, fitted with a thermometer and a stirrer. The stirring was carried out at a rate of 25 cycles per min with simultaneous reduction in temperature by keep on adding ice cubes. When the temperature of the arista was observed to be 5° C or below, the beaker was filled with saturated NaCl solution to stabilize or maintain temperature. The process was continued until some seed crystals of arista were present. The process was repeated 3 times at least to get the average freezing point of arista.

Loss on Drying
About 10 ml (11.02 g) of the arista under study were accurately pipetted out and transferred to a tarred china dish which was known for its weight and kept in a hot air oven at 100 – 105º C for an hour. Then, the sample was weighed along with china dish to deduct the actual weight of tarred china dish. The weight of the content was noted to calculate the percentage loss on drying with reference to the arista.

Determination of loss of ignition

Though determination of loss on ignition is best suiting solid formulation like churna and the principle behind it is to convert all metallic oxalate, chloride, sulphate, phosphate, silicate et., in to their concerned oxide form.

Arista is a liquid formulation containing active principle in alcohol along with minerals in its aqueous layer or unfiltered fine crude drug particle during the preparatory moments. Hence, this method of standardization was tried with 10 ml arista also using a silica crucible, after allowing arista be auto-evaporated at room temperature for about 1 h.

Loss on Ignition

A silica crucible was heated for about 30 min to red hot and cooled in a desiccator to note down its weight. About 10 ml of the arista was pipette out and then dried at 100 – 105º C for 1 h and ignited to constant weight in a muffle furnace at 600 - 625º C, until a carbon free ash formed. The crucible was allowed to cool in a desiccator after each ignition and care was taken to avoid catching fire. The weight of the carbon free ash was determined. The procedure was repeated to obtain a standard deviation to ensure consistency and then tabulated.

Determination of pH of arista

To determine the acidity or alkalinity of the arista at room temperature, potentiometric method was employed. The buffer solutions A – H were prepared using carbon dioxide free water as solvent as given in Indian Pharmacopoeia-1996 (A-95) which helped to detect the pH of arista whose range may be from 1.7 – 10.12.

Determination of Refractive index [21]

The refractive index (n) of a substance with reference to air is the ratio of the sine of angle of incidence to the sine of the angle of refraction of beam of passing from air in to the substance. The refractive index was conveniently measured using the Abbe refractometer at 25º C employing the wavelength of the D line of sodium (λ=589.3 nm), after calibrating the apparatus against distilled water whose nD^{20} at 25º C was 1.3225.

Determination of viscosity of arista

The determination of viscosity of arista was carried out by means of capillary viscometer at room temperature. The viscometer was washed and dried completely. Then the viscometer was filled and examined through L tube to slightly above the mark G using a long pipette to minimize wetting the tube above the mark. The tube was placed vertically in a water bath maintained a temperature of 35º C and allowed to stand for half an hour to reach equilibrium. The volume of arista was adjusted so that the bottom of the meniscus settled at the mark G. The liquid was sucked to the point about 5 mm above the mark E and the pressure was revealed.

The time taken was measured for the bottom of the meniscus to fall from the top of mark E to the top edge of mark F. Then, the kinematic viscosity (V) in square mm per sec (mm^2s^{-1}) using the expression V=Kt

The constant (K) of the instrument was determined on a liquid of known viscosity (Dextran injection or saline).

Determination of weight per ml of arista

The weight per ml of a liquid is the weight, in g, of 1 ml of the liquid when weighed in air at room temperature. A thoroughly clean and dry Pycnometer was selected and filled with arista and weighed in air at room temperature. The procedure was repeated 3 times and average value of the weight of 1 ml of arista was calculated.

Primary organic analysis [22]

About 100 g of the crude drug (Patha) were powdered in a mechanical grinder, after a screening for the presence of foreign bodies, in to a moderately coarse powder were soxhleted successively with solvents of increasing polarity such as petroleum ether, benzene, chloroform and 75% ethanol (15 – 19 h) and a part of the extracts and the arista were subjected for the determination of and a primary organic analysis.

Primary organic analysis [23] of the both the extracts and the arista were carried out with suitable chemical reagents of research grade which led to a conclusion that the phenolic compounds were well pronounced.

Determination of total free sugar content in arista

The total free sugar content of arista was estimated using Benedict's reagent for quantitative analysis and reported in terms of percentage w/ml as per the reference.

RESULTS AND DISCUSSION

The results of physical and physic-chemical analysis of arista were tabulated and discussed in detail under the section discussion (Table 1).

Table 1. Standardization of arista by physical and physico-chemical methods

S.No.	Parameters	Report/Values
1.	Total solids	42.8±0.15% w/ml
2.	Boiling range	76±0.04 – 107±0.06° C
3.	Congealing point	60±0.008 – 67±0.06° C
4.	Content of ethanol	23% v/v at 32° C
5.	Freezing point	10±0.08° C
6.	Loss on drying	20.82±0.50% w/w
7.	Loss on Ignition	3.2±0.33% w/v
8.	pH	4.5
9.	Refractive Index against water (1.332)	1.477
10.	Viscosity against water (0.9982)	1.9121 poise at 32° C
11.	Weight per ml	1.122 g/ml
12.	Total free sugar content	23 g % w/ml
13.	Fluorescence analysis (Long UV)	
	a. Arista	Greenish brown
	b. Arista in water	brown
	c. Arista with methanol	Yellowish brown
	d. Arista with ethylacetate	Pale brown

Results are presented as mean±Standard Deviation, n =3

The primary organic analysis on the both ethanolic extract of the crude drug (Patha) as well as the arista itself gave a positive test for carbohydrates (Molisch's test); amino acid (Xanthoproteic test); free sugar (Fehling's' and Benedict's test); tannins (Gold beater's test); general phenolic compounds (dilute ferric chloride test); flavonoid (Shinoda's test and pH dependent colour test

by Mg-HCl); saponins (Haemolytic test); general glycosides (by hydrolytic test after exhausting free sugar); phenolic glycoside (by hydrolysis followed by phase separation by non-polar solvent and testing of the same); and the presence of anthocyanins (Blood red colouration of both alcoholic and aqueous extract) (Table 2). An organoleptic analysis was also carried out on the arista and the results were tabulated (Table 3).

It is noteworthy and deserves a mention here that the ethanolic extract of Viburnum secies has been proven to possess a remarkable antioxidant, anti-inflammatotry and antiulcer activities. However, this drug, so far, has not been formulated in to any form and standardized for its value. The arista itself and the arista added with water, 80% methanol and ethylacetate were observed under UV radiation showing greenish brown, brown, yellowish brown and pale brown colouration respectively.A primary organic analysis conducted on the arista itself as well as the ethanolic extract of the patha revealed the presence of carbohydrate, amino acid, free sugar, saponins, tannins, phenolic compounds (general), flavonoids, saponins and glycosides (phenolic glycosides). However, presence of phyto-sterols and triterpenes were in the negative.The arista was greenish brown in colour; aromatic in odour; aromatic and sweet in taste; sticky after minutes in texture between fingers; pourable and non-sticky in nature to view; it turned brownish green after its evaporation, when kept under room temperature; and smelled ethanolic and pleasant while heating on a boiling water bath.

The term total solid is applied to the residue obtained where the prescribed amount of the preparation is dried to constant weight. The total solid of the arista were determined to be 42.8±0.15% w/ml. The lower limit of the range is the temperature indicated by the thermometer when the first drop of condensate leaves the tip of the condenser and the upper limit is the temperature at which the last drop evaporates from the lowest point in the distillation flask, as far as distilling range of the arista is concerned. In this event, the arista showed 76±0.09° C to 107±0.06° C as its boiling range.

Table 2. Primary organic analysis of arista against patha

S.No.	Phytoconstituents	Arista	75% ethanolic extract of patha
1.	Carbohydrate	+++	++
2.	Free sugar	+++	++
3.	Amino acid	++	+
4.	Alkaloid	-	-
5.	Saponins	++	+
6.	Phyto-sterols	-	-
7.	Triterpenoids	-	-
8.	Tannins	+++	++
9.	Flavonoids	+++	++
10.	Glycosides (general)	++	++
11.	Glycoside (specific) (Phenolic glycosides)	+++	+++
12.	Anthocyanins	+++	+++

$^{+}$ *- Test positive,* $^{-}$*- Test negative*

The congealing temperature is that point at which there exists a mixture of the liquid (fused) phase of a substance and increasing proportion of solid phase. The arista, in this case, showed 60±0.08° C to 67±0.06° C as the congealing point. Making no modification in the setting of apparatus the freezing point of the arista was determined to be 10±0.08° C.

Table 3. Organoleptic analysis of arista

S.No.	Parameters/Characters	Results
1.	Colour	Greenish brown
2.	Odour	Aromatic
3.	Taste	Ethanolic and Sweet
4.	Texture	Sticky after minutes
5.	Nature	Pourable, Non-sticky
6.	Colour change at room temperature	darkening when volume reduced
7.	Odour upon heating	Ethanolic and pleasant

Since the principle behind the formulation of arista is that conversion of sugar (jaggery) in to ethanol by anaerobic fermentation process, determination of total alcohol concentration was determined to be 23% v/v at 32° C by distillation cum specific gravity method. Loss on drying is a versatile method of standardization applicable for materials existing in liquid, solid, semisolid state. On the basis of the above principle, loss on drying of the arista was determined to be 20.82±0.50% w/w.

Although loss on ignition is best suiting to standardize formulation such as churna, it cannot be stated that arista may not be standardizable by this method. Because, the principle behind the loss on ignition is to determine the quantity of inorganic elements which could be convertible in to their corresponding oxides, which include both physiological as well as non-physiological ashes.

Hence, the loss on ignition of the arista in percentage w/v as determined to be 3.2±0.33% w/v. To determine the acidity or alkalinity of the arista, pH value was determined to be 4.5 by potentiometric method. Determination of refractive index is one of the best suiting standardizing process for liquid formulation with reference to air; the refractive index of the arista using as Abbe refractometer against water was measured to be 1.477.

By employing an Oswald - type viscometer, viscosity was determined against water to be 1.9121 poise at 32°C. Since arista is a liquid formulation, by using a calibrated Pygnometer, the weight per ml of the arista was determined to be 1.122 g/ml at room temperature. The total free sugar content using Benedict's reagent for quantitative analysis was determined to be 23 g %.

CONCLUSION

Every formulation of Ayurvedic system of medicine has its own modern scientific principle behind its preparation and standardization.The current study is to prove the same and to lay a path that further studies can be progressed on this phenolic rich formulation.

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