

FORMULATION AND PHYSICO-CHEMICAL STANDARDIZATION OF *VIBURNUM CORIACEUM* BARK ARISTAK Prabhu^{1*}, P K Karar², S Hemalatha³ and K Ponnudurai⁴¹Department of Pharmacognosy, Nandini Nagar Mahavidyalaya College of Pharmacy, Nawabganj, Gonda, Uttar Pradesh, India²Department of Pharmaceutical Chemistry, DOABA College of Pharmacy, Kharar, Mohali, Punjab, India³Department of Pharmaceutics, Pharmacognosy Division, IT-Banaras Hindu University, Varanasi, Uttar Pradesh, India⁴Department of Pharmacology, Nandini Nagar Mahavidyalaya College of Pharmacy, Nawabganj, Gonda, Uttar Pradesh, India

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ABSTRACT

The barks of *V. coriaceum* were collected from Nilgiri hills, Tamil Nadu, India. A primary organic analysis conducted on the species revealed that the presence of bio-active molecules such as tannins, saponins, phenolic compounds (flavonoids) and other phenolic glycosides as their principal phyto-constituents. The crude drug (Patha) was formulated in to an arista using conventional anaerobic fermentation process for about 60 days. Apart from some traditional methods of standardization of arista, a new approach was made to select about thirteen numbers of physical, physico-chemical including organoleptic and primary organic analysis were attempted with the arista to obtain a reproducible and consistent results and the same were recorded. The current study may act as a referential tool for how to find out novel methods of standardization of ayurvedic liquid formulations such as Arista and Asava.

KEYWORDS: *Viburnum*, Arista, Patha, Congealing point, Alcohol content***Corresponding author**K.Prabhu, Department of Pharmacognosy, Nandini Nagar Mahavidyalaya College of Pharmacy, Nawabganj – 271303, Gonda, Uttar Pradesh, India E-mail: prabhu.cognosy@gmail.com, prabhu.cognosy@rediffmail.com**INTRODUCTION**

Caprifoliaceae is the family of 12 genera and contains about 450 species; the genus *Viburnum* is one of the genera (formerly under Caprifoliaceae; but recently under the family Adoxaceae) having about 200 species throughout the world. A few of *Viburnum* species are noteworthy of their popularity as medicinally potential plants, for example, *Viburnum prunifolium*, which is official in the BHP (1983), and has a mention in pharmacognosy text¹ as Black Haw bark for its morphology and medicinal properties; and also for its phytochemistry².

The *Viburnum* species grow in India (Himalaya, Khasi hills, Arunachal Pradesh, Tamil Nadu (Nilgiri and Coimbatore hills), Nepal, South America and Java, Korea, China and Japan; Flavonoids were isolated of from the barks of *Viburnum prunifolium*³; β -sitosterol (C₂₉H₅₀O), m.p. 136 – 137°C was isolated from *Viburnum prunifolium* and *Viburnum opulus*⁴.

Procyanidins such as: B₁₋₅ and C₁ were isolated from the stem barks of *Viburnum burkwoodii*; Catechin, m.p. 177 °C, was isolated from *Viburnum burkwoodii* Leaves; (-) Epicatechin, m.p. 240 - 242 °C, was isolated from the stem barks of *Viburnum burkwoodii*⁵; Bergenin, a sesquiterpenoid was isolated from roots of *Viburnum nervosum* Hook⁶; Pharmacognostical studies were carried out on the roots of *Viburnum nervosum* Hook⁷; Amentoflavone and apigenin were isolated from the leaf extracts of *Viburnum coriaceum* in pure form and their structures were elucidated by spectral techniques⁸; Fatty oils extracted from seed of *Viburnum coriaceum* Blume, were tested *in vitro* against five species of bacteria, i.e., *Bacillus pumilus*, *B. subtilis*, *Salmonella typhosa*, *S. typhi*, *S. paratyphi*, *Micrococcus pyogenes albus* and *Staphylococcus albus* and the investigations revealed that the fatty oils have potent antibacterial activity against *B. subtilis*, *S. typhosa* and *S. paratyphi*⁹; Deoiled meal of *Viburnum coriaceum* seeds contained 25.9% protein. Meal contained all essential amino acids except

tryptophan. Percentage of leucine, lysine, threonine and valine were higher in *V.coriaceum* than in soybean¹⁰; Iridoid glycosides were isolated from *Viburnum rhytidophyllum*¹¹; Decoction of leaves of *Viburnum foetidum* Wall was used in various uterine disorders¹²; Iridoid glycosides and p-Coumaryl iridoids were isolated from methanolic extract of dried leaves of *Viburnum luzonicum* and their cytotoxic effect were also studied¹³; Change on storage of biological activity of *Viburnum opulus* seed components was studied¹⁴; Rearranged vibsane type diterpenes were isolated from *Viburnum awabuki* and photochemical reaction of vibsane-B was also carried out¹⁵; Epicotyl Dormancy in *Viburnum acerifolium* was studied¹⁶; Iridoid glycosides were isolated from *Viburnum chinshanense*¹⁷; Antinociceptive and anti-inflammatory activities were studied on *Viburnum lanata*¹⁸; Antinociceptive and anti-inflammatory activities were studied on *Viburnum opulus*¹⁹.

From the above observation, it may be stated that *Viburnum* Linn species have biologically potential phyto-constituents and their exploitation in scientific studies necessitates either isolation of phyto-constituents or formulation in crude form to explore the biological values.

From the above, before concluding that the arista formulated might possess phenolic compounds of therapeutic potential (confirmed by a primary organic analysis on the various solvent extracts of crude drug (Patha) as well as with the arista itself in the current study), and establishing the therapeutically potential of this arista, it is essential to find out how the formulation can be standardized to obtain reproducible parameters for its identity and recognition. Hence, the current study centres mainly on the standardization of the formulated arista using some physical and physico-chemical methods, which have ensured reproducibility during the experimentation.

MATERIALS AND METHODS

The barks of *Viburnum coriaceum* were collected from Nilgiri hills, Tamil Nadu, India and authenticated by Dr.V.Chelladurai, Ex. Professor, (Botany), Medicinal plant survey for Siddha, Government of India as *Viburnum coriaceum* Blume. Herbarium of the specimens (labelled VC131) was submitted to the museum of the department of Pharmacognosy, Nandini Nagar Mahavidyalaya College of Pharmacy. The crude drugs were dried in the sun for a couple of weeks and subjected to research studies.

Preparation of *V.coriaceum* bark arista by anaerobic fermentation method (an ayurvedic formulation)

Approximately 0.612 seers (20 g) of the barks of *V.coriaceum* (patha) were coarsely powdered and added with 16 seers (512 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 decoction²⁰. The decoction was taken in wooden vats of 1 litre capacity, to which dissolved were 6.125 seers (200 g) of jaggery and boiled for half an hour.

Dravyas and Dhataki pushpa (*Woodfordia fruticosa*) were then added to the mixture which was 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)^{21,22}.

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 was 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.

Standardization of arista

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 low temperature as possible on a water bath until the solvent was removed and the residue was 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 desiccator.

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 of the flask 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 have

been carried out 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 collected 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 recorded.

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 a 1 litre beaker in which two test tubes were placed in such a way one was inserted in to other test tube. The inner test tube contained 15 ml of arista and was stoppered 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 at a 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 a change 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 recorded.

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 a relative density was determined to calculate the percentage v/v alcohol of the arista²³.

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 at least 3 times to get the average freezing point of arista.

Loss on Drying

About 10 ml (10.36 g) of the arista under study were accurately pipetted out and transferred to a tarred china dish which was already known for its weight and kept in a hot air oven at 100 – 105° C for an hour. Then, the sample was weighed along with china dish to deduct the actual weight of empty tarred china dish. The weight of the content was noted to calculate the percentage loss on drying with reference to the arista²⁴.

Determination of loss of ignition

Though determination of loss on ignition is best suiting some solid formulation like churna, the principle behind it is to convert all metallic oxalate, chloride, sulphate, phosphate, silicate etc., 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 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 – 105° C for 1 h and ignited to constant weight in a muffle furnace at 600 - 625° 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 a consistency and then tabulated²⁵.

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 between 1.7 – 10.12.

Determination of Refractive index

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 25° C employing the wavelength of the D line of sodium ($\lambda=589.3$ nm), after calibrating the apparatus against distilled water whose n_D^{20} at 25° 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 minimise wetting the tube above the mark. The tube was placed vertically in a water bath maintained a temperature of 35° 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 revealed²⁶.

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 (water)

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.

Fluorescence analysis of arista

The arista as it was and then added with water; methanol and ethyl-acetate were shaken well and kept under a long UV light aided chamber to note down the colour change²⁷.

Primary organic analysis

About 100 g of the barks of *V.coriaceum* (Patha) were powdered in a mechanical grinder, after a screening for the presence of foreign bodies, in to a moderately coarse powder and then 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 a primary organic analysis^{28,29}.

Primary organic analysis of the both the extracts and the arista were carried out with suitable chemical reagents of research grade which led to a observation 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 reference³⁰.

RESULT AND DISCUSSION

The results of physical and physico-chemical analysis of *V.coriaceum* bark arista were tabulated and discussed in detail under this section (Table 1). 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 *V.coriaceum* bark has been proven to possess a remarkable antioxidant, anti-inflammatory and

antiulcer activities. However, this drug, so far, has not been formulated in to any form and standardized for its value³¹⁻³³. The *V.coriaceum* bark arista itself and the arista added with water, 80% methanol and ethylacetate were observed under UV radiation showing dark brown, yellowish 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 brownish black 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 showed a darkening 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 was determined to be $46.5 \pm 0.15\%$ 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 evaporate from the lowest point in the distillation flask, as far as distilling range of the arista is concerned. In this event, the arista showed $70 \pm 0.09^\circ\text{C}$ to $105 \pm 0.06^\circ\text{C}$ as its boiling range.

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 $62 \pm 0.08^\circ\text{C}$ to $65 \pm 0.06^\circ\text{C}$ as the congealing point. Making no modification in the setting of apparatus the freezing point of the arista was determined to be $8 \pm 0.08^\circ\text{C}$.

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 21% 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 $18.12 \pm 0.50\%$ 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 have been 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 $3.1 \pm 0.33\%$ w/v. To determine the acidity or alkalinity of the arista, pH value was determined to be 4.55 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 an Abbe refractometer against water was measured to be 1.440.

By employing an Oswald - type viscometer, viscosity was determined against water to be 1.9096 poise at 32°C . Since arista is a liquid formulation, by using a calibrated Pygrometer, the weight per ml of the arista was determined to be 1.036 g/ml at room temperature. The total free sugar content using Benedict's reagent for quantitative analysis was determined to be 20 g %.

CONCLUSION

The current study of formulation and its standardization of arista by physic-chemical methods is a new approach and an attempt to add on the number of analytical methods by which an ayurvedic formulation such as arista can be conveniently subjected for its quality control, unlike adopting a few processes such as "determination of alcohol and sugar content" alone. Hence, we conclude, herewith, that this study will be a referential tool for attempting various methods of analysis to standardize an ayurvedic formulation like arista, asava etc., in future.

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Table 1: Standardization of arista by physico-chemical methods

S.No.	Parameters	Report/Values
1.	Total solids	46.5±0.15% w/ml
2.	Boiling range	70±0.04 – 105±0.06° C
3.	Congeaing point	62±0.008 – 65±0.06° C
4.	Content of ethanol	21% v/v at 32° C
5.	Freezing point	8±0.08° C
6.	Loss on drying	18.12±0.50% w/w
7.	Loss on Ignition	3.1±0.33% w/v
8.	pH	4.5
9.	Refractive Index against water (1.332)	1.440
10.	Viscosity against water (0.9982)	1.9096 poise at 32° C
11.	Weight per ml	1.036 g/ml
12.	Total free sugar content	20 g % w/ml
13.	Fluorescence analysis (Long UV)	
	a. Arista	Dark brown
	b. Arista in water	Yellowish brown
	c. Arista with methanol	Yellowish brown
	d. Arista with ethylacetate	Pale brown

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

Table 2. Primary organic analysis of arista against patha

S.No.	Phytoconstituents	Report	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

Table 3. Organoleptic analysis of arista

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