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FORMULATION AND PHYSICO-CHEMICAL STANDARDIZATION OF TRIPLE *VIBURNUM* ROOT ARISTA

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ABSTRACT

The roots of *V.punctatum, V.coriaceum* and *V.erubescens* were collected (flowering season, June – August) from Nilgiri hills, Tamilnadu, 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 90 days.The formulation was standardized by some 13 methods of physico-chemical analysis to obtain a cosistant and reproducible parameters. This study can help in reevaluating the formulation as well as in conducting further scientific investigations on it.

INTRODUCTION

The genus *Viburnum* Linn. species under the family Caprifoliaceae (formerly) and Adoxaceae (recently) includes about 200 species distributed throughout the world, and about 17 of them have been reported in India; their growth is favoured at an altitude from 1500 – 2500 ft, and are frequently seen in Himalayan tracts, Nilgiri hills and Coimbatore[1,2].

Viburnum Linn. Species have been reported to contain sesquiterpenes[3], triterpenes and phytosterols; phenolic compounds and their glycosides such as: tannins, flavonoids and anthocyanins, irridoid glycosides on their stem, root and leaves, and investigated to posses uterine sedative, diuretic, cardiovascular stimulant, antimicrobial, anti-inflammatory, anti-nociceptive, antispasmodic, anti-asthmatic and astringent activities[4]. In the late 1960s and early 1980s, the magnitude of scientific investigations on the genus *Viburnum* Linn. were voluminous in regard to some phytochemical aspects of constituents from the stems, root barks and leaves of these species[5-7]. However, the number of species exploited for studies and areas of investigations were very limited. After a couple of decades, some more *Viburnum* species appeared for having been investigated of their phytochemical and pharmacological characteristics. The typical examples are: iridoid aldehydes and their glycosides in *Viburnum luzonicum*[8], and their cytotoxic effect; vibsane type diterpene from *Viburnum awabuki*[9]; irdoid glycosides from *Viburnum tinos;* antinociceptive and anti-inflammatory activities of *Viburnum lanata*[10], and *Viburnum opulus*[11], and an iridoid glucoside from *Viburnum rhytidophyllum*[12]. And a detailed pharmacognostical studies have, recently, been carried out on a few of the species which deserves a noteworthy in this section, since the same species have been screened for their antibacterial spectrum also[13,14].

In addition to the above, a questionnaire and a verbal enquiry have been recently conducted to the local dwellers, tribal and the herbalists of Nilgiri hills and Coimbatore hills, Tamilnadu, India, about the ethno-pharmacological status of some *Viburnum* species, has also revealed that the leaves, stem bark and root barks of mature plants had been reliably in usage to the non-pregnant uterus[15], the GIT related ailments, and are also in application as an ideal healing aid against inflammation[16].Accounting the above information, it was decided to formulate an arista from three *Viburnum* leaves and the same was screened for anti ulcer effect after its standardization employing some physico-chemical methods.

MATERIALS AND METHODS

Plant Material

The roots of *V.punctatum, V.coriaceum* and *V.erubescens* 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 punctatum* Buch.-Ham.ex D.Don (VP), *Viburnum coriaceum* Blume (VC) and *Viburnum erubescens* Wall.ex DC (VE). Herbarium of the specimens (labeled V181, VC131 and VE131 for VP, VC and VE respectively) was submitted to the museum of the department of Pharmacognosy, Nandini Nagar Mahavidyalaya College of Pharmacy.

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

Approximately 1.5 seers (60 g) of the 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[17]. 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) [18,19].

After the stipulated period (90 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

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[20].

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[20].

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[20].

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 content

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[20].

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[20].

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[20].

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[20].

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[21].

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 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[21].

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[21].

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[21].

Primary organic analysis

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 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[22].

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[23].

RESULTS AND DISCUSSION

The results of physical and physic-chemical analysis of 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).

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

S.No.	Parameters	Report/Values
1.	Total solids	39.5±0.25% w/ml
2.	Boiling range	72±0.04 – 102±0.04° C
3.	Congealing point	57±0.02 – 64±0.04° C
4.	Content of ethanol	22.5% v/v at 32° C
5.	Freezing point	8±0.06° C
6.	Loss on drying	17.52±0.60% w/w
7.	Loss on Ignition	3.1±0.55% w/v
8.	pH	4.9
9.	Refractive Index against water (1.332)	1.315
10.	Viscosity against water (0.9982)	1.9664 poise at 32° C
11.	Weight per ml	1.100 g/ml
12.	Total free sugar content	23.5 g % w/ml
13.	Fluorescence analysis (Long UV) a. Arista	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

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

Table 3. Organoleptic analysis of arista

S.No.	Parameters/Characters	Results
1.	Colour	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

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[29,30] . 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 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 39.5±0.25% 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 72±0.04° C to 102±0.04° 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 57±0.02° C to 64±0.04° C as the congealing point. Making no modification in the setting of apparatus the freezing point of the arista was determined to be 8±0.06° 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 22.5% 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 17.52±0.60% 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 3.1±0.55% w/v. To determine the acidity or alkalinity of the arista, pH value was determined to be 4.9 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 1.315.

By employing an Oswald - type viscometer, viscosity was determined against water to be 1.9664 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 1.100 g/ml at room temperature. The total free sugar content using Benedict's reagent for quantitative analysis was determined to be 23.5 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 through which further phyto-chemical and biological studies can be progressed on this formulation.

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