

Formulation of *Viburnum Punctatum* Arista and its biological potentials

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ABSTRACT

The leaves, stem and the roots of V.punctatum 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. The formulation was screened for its anti diarrhoeal and anthelmintic potentials. Since the Arista contained phenolic compounds as principal constituents, it showed a markable and significant anti diarrhoeal and anthelmintic activities against castor oil induced diarrhoea, $p < 0.01$ and piperazine citrate induced paralysis $p < 0.01$.

Keywords: *Viburnum*, Arista, Patha, anti-dirrhoeal, anti-helmintic.

INTRODUCTION

Viburnum Linn. Species contain sterols, sesqui and triterpenoids, phenolic compounds and their glycosides as their common chemical constituents[1-5]. A few species among 17 in India, namely: *Viburnum punctatum* Buch.-Ham.ex D.Don, *Viburnum coriaceum* Blume and *Viburnum erubescens* Wall.ex DC; have been reported in literature to posses uterine sedative, anti-asthmatic, astringent, anti-inflammatory and anti-microbial activities[6,7]. A verbal enquiry to the local community and plant vendors of Ooty and Coimbatore, Tamilnadu, also supported that the above listed pharmacological activities were traditional and were promising with roots, stem barks and leaves of these species[8].

Among the above listed chemical constituents, phenolic compounds, terpenoids and their glycosides may be the cause for biological responses. In addition to this, a qualitative chemical screening and spectrophotometric analysis of extracts were performed to reveal that the stem part of these three species contains an appreciable amount and a wide range of phenolic compounds[9,10].

Radical scavenging activities of phenolic compounds play a key role in ameliorating healing and even preventing several ailments in living being. It is a well known fact that the plants synthesis phenolic compounds for diverse purposes, which may be of protective, functional or as metabolic end products in nature[11]. But, human exploit them as valuable medicines/ phyto-pharmaceuticals by focusing on their anti-oxidant potential with or without modification.

A quest for a search of herbal phenolic compounds is still a renewed interest in the science of natural products as a source of valuable medicines. The herbal phenolic molecules such as flavonoids, anthocyanins, bioflavones and other phenolic glycosides have, already, been explored and known for their applications against several human ailments-cardiovascular disorders, chronic inflammation and GIT related troubles[12-14].

MATERIALS AND METHODS

Collection of Specimens

The studies were undertaken on some three parts of the species of the genus *Viburnum* namely: *Viburnum punctatum* Buch.-Ham.ex D.Don. The choices of plant parts were the leaves, stems and the roots of these species. The plant specimens for the study were collected from Nilgiri Hills, Tamil Nadu, India, and authenticated by Dr V Chelladurai, former Professor of Botany, Medicinal Plant Survey for Siddha, Government of India, as *Viburnum punctatum* Buch.-Ham.ex D.Don., (V181) and deposited in the department of pharmacognosy at Nandini Nagar Mahavidyalaya College of Pharmacy, Uttar Pradesh. A care was taken to select healthy plants and the plant parts for the study were collected fresh and dried for a couple of weeks to be involved for further studies.

Preparation of *V.coriaceum* root arista by anaerobic fermentation method (An Ayurvedic formulation)

Approximately 1¼ seers (45 g) of the roots of *V.coriaceum* (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[15]. 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 fruticosa*) 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)[16,17].

After the stipulated meriod (60 days), the vessel was withdrawn to examine the preparation which showed a brownish black fluid with a frothing andaromatic 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.

Anti-diarrhoeal activity of Arista[18]**Experimental animals**

Wistar rats (200-250 g) of either sex bred in Central Animal House facility of the Institute were used. The animals were housed under standard conditions, maintained on a 12 h light/dark cycle and had free access to food and water up to the time of experimentation. The animals were acclimatized to the laboratory environment 1 h before the experiments. Animals were randomly distributed into four groups of 6 animals each. Each animal was used only once. All experiments were conducted during the light period (08.00-16.00 h). The protocol was approved by the Institutional Animal Ethical Committee (IAEC) of DOABA College of Pharmacy, Mohali, Punjab, India, and conducted in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Drugs

Castor oil (Paras chemicals Ltd.), and Tween-80 (Merck Ltd.) were used as positive control and as a vehicle respectively. Loperamide and Piperazine citrate were used as a standard drug for anti-diarrhoeal and anthelmintic studies respectively.

Castor oil-induced diarrhoea [19]

The rats were divided into four groups of six animals each, diarrhoea was induced by administering 10 ml/kg of castor oil orally to rats. Group 1 served as control (2ml/kg, p.o.), group 2 received Loperamide (1 mg/kg, p.o.) served as standard and group 3 and 5 received the asava (200 and 400 mg/kg, p.o.), 1 h before castor oil administration. Each rat was then housed in the cages, each provided with a clean filter paper at the bottom. These animals were observed for the characteristic stool and time of onset of diarrhoeal episodes. The observations were recorded every hour up to four hours.

In vitro Anthelmintic screening[20-22]

The earthworms of 3-5cm in length and 0.1-0.2cm in width were used for all experimental protocol due to their anatomical and physiological resemblance with the intestinal round worm parasites of human being. The earthworms for the study were authenticated by Dr.P.N.Tripathi, professor, Department of zoology, K.S.Saket P.Gcollege, Faizabad, U.P, India as *Pheretima posthuma*.

The assay was carried out on adult Indian earth worms (*Pheretima posthuma*). About 5 groups of each six worms were subjected for the current study. The group-I and II were released in to double distilled water and piperazine citrate 10 mg/ml in distilled water respectively as the solvent control and the reference.

The group III, IV and V were dropped in to the *Viburnum* root arista of concentrations 100, 250 and 500 mg equivalent/ml. The total volume of each solution was maintained about 50 ml, so that the worms can be conveniently exposed to the different substances selected.

Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°C.

Statistical analysis

The determination for the significant intergroup difference was analysed separately and Student's *t*-test was used for comparison ($p < 0.01$).

RESULTS AND DISCUSSION

The primary organic analysis [23,24] 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 1). An organoleptic analysis was also carried out on the arista and the results were tabulated (Table 2).

Table 1. 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

It is noteworthy and deserves a mention here that the ethanolic extract of *Viburnum* species 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 value. The arista itself and the arista added with water, 80% methanol and ethylacetate were observed under UV radiation showing brown, brown, yellowish brown and pale brown colouration respectively

Table 2 . 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

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.

Anti-diarrhoeal potentials

There has been a significant reduction in the incident and severity of diarrhoea produced with animals treated with standard (loperamide 1 mg/kg) that is statistically (69.65 ± 2.55) when compared to the control groups treated with arista. In this event, the *Viburnum* root asava (200 mg/kg and 400 mg/kg) have shown a significant reduction in the severity of diarrhoea $p < 0.01$ and $p < 0.001$ (being 19.94 ± 1.31 , 49.29 ± 1.89 respectively) when compared to that of the result witnessed by the control group. The magnitude of anti-diarrhoeal activity shown by the arista treated animals can be comparable or proximal to that of the animals administered with the standard drug. However, the arista cannot be suitably described to be as equally as effective anti-diarrhoeal drug to the standard (Table 3).

Table 3. Effect of *Viburnum* arista on Castor oil induced diarrhoea

S.No.	Treatment/Dose (mg/kg)	Mean weight of defecation after 4 h	Percentage inhibition of defecation
1.	Control (Tween-80, 2 ml/kg)	20.14 ± 0.54	-
2.	<i>Viburnum</i> arista (200 mg/kg)	15.72 ± 0.36	$19.94 \pm 1.31^*$
3.	<i>Viburnum</i> arista (400 mg/kg)	9.81 ± 0.40	$49.29 \pm 1.89^{**}$
4.	Loperamide (1 mg/kg)	5.91 ± 0.51	$69.65 \pm 2.55^{**}$

Values are presented as mean \pm SEM of 6 animals.

Symbols represent statistical significance: * - $p < 0.01$, ** - $p < 0.001$

Anthelmintic potential

The earthworms introduced to distilled water (group-I, control) were very alive and showed neither signs of paralysis nor any death. The group-II, which was treated with reference anthelmintic drug (piperazine citrate 10 mg/ml, showed a significant paralysis of worms, 19.50 ± 1.02), however, no death was evident.

Table 4. Anthelmintic activity of *Viburnum* arista

S. No.	Test Substance	Concentration (mg/ml)	Time taken for Paralysis (P) and Death (D) of worms in min	
			P	D
1.	Distilled water (control)	-	-	-
2.	Piperazine citrate (Standard)	10	19.50 ± 1.02	-
3.	<i>Viburnum</i> arista	100	29.81 ± 0.49	85.01 ± 2.33
4.	<i>Viburnum</i> arista	250	20.54 ± 0.55	56.47 ± 0.94
5.	<i>Viburnum</i> arista	500	17.41 ± 0.37	23.11 ± 0.42

Values are presented as mean \pm SEM, $n=6$

The group III, IV and V, which were introduced to the solution containing the arista equivalent to 100, 250 and 500 mg/ml respectively, resulted both paralysis (28.81 ± 0.49 , 20.54 ± 0.55 and 17.41 ± 0.37) as well as death (85.01 ± 2.33 , 56.47 ± 0.94 and 23.11 ± 0.42) significantly. Hence, the

paralytic capacity of the arista was comparable to that of the reference subjected, but the same time, the formulation is more powerful and effective than the standard in causing a significant mortality of the earth worms $p < 0.01$ (Table 4).

Probable mechanism of anti-diarrhoeal and anthelmintic activities of *Viburnum arista*

The oral administration of castor oil is acid hydrolysed in the gastrointestinal tract in to ricinoleic acid; the later is converted in to its salt form namely: sodium ricinoleate which is a powerful irritant of mucosal membranes of the physiological system. The irritant effect of sodium ricinoleate increases the propulsive or peristaltic movement leading to drastic purgation with watery stools. The *Viburnum arista* has been analysed to contain tannins and other phenolic compounds which may accelerate astringent effect (precipitation of mucosal proteins). Hence, there has been a formation of a barrier which resists the irritant effect of castor oil and its further effect on the GIT inducing diarrhoea.

The phenolic compounds such As tannins and flavonoids bind with intestinal enzymes of earthworms as well as the glycoprotein part of the cuticular layer of the worms thereby, crippling the protein dependent movements of the body (muscles) of the worms leading to an irreversible paralysis and finally to death.

CONCLUSION

Viburnum Linn. species are medicinally valuable population. Hence, an arista from *V.punctatum* was formulated and analysed for its primary organic and sensual features. Considering the nature of the phyto-constituents of the formulation, anti diarrhoeal and anthelmintic activities were screened for the arista to obtain a markable results that the drug possesses a comparable magnitude of such effects, when compared to that of the references subjected in the present study. This study can be useful as a referential tool to progress some advanced research on this formulation.

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