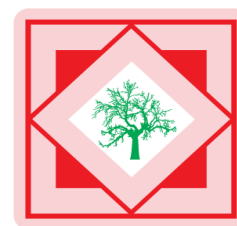




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Formulation of *viburnum coriaceum* arista and determination of its anticonvulsant activity in mice

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ABSTRACT

The leaves, stems and roots of *Viburnum coriaceum* were collected from Nilgiri hills, Tamil Nadu, India. A primary organic analysis on the species revealed 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 prevention of seizures induced by MES in laboratory animals is the most commonly used preliminary screening test for characterizing potential anticonvulsant drugs. The MES test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures. The present study revealed that the *Viburnum* arista blocked tonic seizures induced by MES significantly ($p < 0.001$) at the dose 200, 400 and 600mg/kg. b.wt. (p.o).

Key words: *Viburnum coriaceum*, Patha, MES, arista, anticonvulsant.

INTRODUCTION

Ayurveda is accepted to be the oldest treatise on medical system which In Indian system of traditional medicine, it is presumed that the knowledge of Ayurveda is given by God of a different world. It is accepted as the oldest written medical system that is also supposed to be more effective in certain cases than modern therapies. We need not to go in any controversy regarding its origine, as Ayurveda is an independent and self sufficient medical system, which has stood the test of science. The origin of Ayurveda has been lost in prehistoric antiquity, but their characteristic concepts appear to have been nurtured between 2500 and 500 BC in India The following are the very recently carried out pharmacognostical, biological and phyto-chemical investigations on *viburnum* species: Pharmacognostic investigations on the leaves of *Viburnum coriaceum* Blume [1] ;Pharmacognostic and Preliminary Phytochemical Investigations on the Leaves of *Viburnum punctatum* Buch.-Ham.ex D.Don [2]; Pharmacognostical Investigations on

the Stem of Two *Viburnum* Linn. Species – A Comparative Study [3]; Pharmacognostic Investigation of the Leaves and Stems of *Viburnum erubescens* Wall.ex DC [4]; Total Phenolic Content and *in vitro* Antioxidant Potentials of Ethanolic Stem Extracts of Three *Viburnum* Linn. Species [5]. Anti-inflammatory, analgesic and anti-spasmodic activities of three *viburnum* linn. species [6]. Antiulcer Activity of Ethanolic Stem Extracts of three *Viburnum* Linn. Species – A Comparative Evaluation [7]; Morphological, Microscopical and Physico-chemical Investigations on the roots of *Viburnum punctatum* Buch.-Ham.ex D.Don [8]; Histo-chemical analysis on the Leaves, Stem and Roots of three *Viburnum* Linn. species [9]; Pharmacognostical Evaluation of a Triple *Viburnum* churna having anti-inflammatory potentials [10]; Formulation and physico-chemical standardization of *Viburnum coriaceum* bark arista [11]; Total Phenolic Content and *In vitro* Anti-oxidant Potentials of Ethanolic Root Extracts of Three *Viburnum* Linn. species – A Comparative Study [12]; Formulation and standardization of a triple *Viburnum* churna Having Anti diarrhoeal Activity [13]; Pharmacognostical and biological investigations on three *Viburnum* Linn. species – An overview [14]; A study on physico-chemical standardization of a formulated Triple *Viburnum* root Asava possessing Anti-helmintic activity [15]; Isolation of astragalin and amentoflavone from the roots of *Viburnum erubescens* Wall.ex DC and their effect against human pathogenic bacteria [16]; A study on physical and physico-chemical standardization of a formulated asava possessing anti-diarrhoeal potential [17].

MATERIALS AND METHODS

The leaves, stems and roots 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, *Viburnum coriaceum* Blume. Herbarium of the specimens (labelled VC131) was submitted at 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 week and subjected to research studies.

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

Approximately 1.35 seers (45 g) of *V.coriaceum* (patha)(leaves, stems and roots, 1:1:1 ratio) 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^[20]. 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) [18-21].

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. Apart from this conducted was a primary organic analysis [22] on both the arista and patha (Table 1) [23-27].

Table 1. Primary organic analysis of aristaa against patha

S.No.	Phytoconstituents	Report	75% ethanolic extract of patha
1.	Carbohydrate	++	++
2.	Free reducing 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, ⁺⁺, ⁺⁺⁺ -Test well pronounced

Evaluation of Anti-seizure Activity

MES induced seizures:

Corneal electrodes were used for bilateral delivery of electrical stimulus (maximal electroshock seizures, MES-50mA ; 50 Hz ; 0.2 sec) convulsive shock including Hind Limb Tonic Extension (HLTE) in 99% of animals , was previously determined [28-31]. The electrical stimulus was applied using a stimulator apparatus for five groups of 5 mice each. In which one control pretreated with 2% tween 80 solution (10 ml/kg), one standard with phenytoin as positive control (25 mg/kg i.p.) and three groups pretreated with 400 mg/kg. p.o. of ethanolic extract (Table 2) .

Table 2. Effect of *Viburnum arista* on M.E.S. induced seizures in mice

S.No.	Treatment	Duration of HLTE	Mortality (%)	Recovery (%)
1.	Vehicle	13.27 ± 0.61	78	18
2.	arista -200	9.54 ± 0.25	20	80 *
3.	arista -400	6.37 ± 0.42	0	100 *
4.	arista -600	5.70 ± 0.81	0	100 *
5.	Phenytoin	3.51 ± 0.13	0	100 *

Values are mean ± SEM mice were pre-treated with vehicle and ME extracts orally 60 minutes before the electroconvulsive shock, * $p < 0.001$, (n=5), HLTE-hind limb tonic extension.

The time of peak effect of phenytoin as 30 min after administration was previously established. The time for the extract to reach its maximum effect was determined as 60 min after oral administration. The orientation for the anticonvulsant effects was abolition of HLTE with in 10 sec after delivery of the electroshock.

The data were statistically evaluated using one way ANOVA followed by Dunnett's test using the Graph pad instant Demo (DATA SET 1.ISD) version, p values of 0.001 were considered to be significant.

RESULTS AND DISCUSSION

The prevention of seizures induced by MES in laboratory animals is the most commonly used preliminary screening test for characterizing potential anticonvulsant drugs. The MES test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures.

The present study revealed that the *Viburnum arista* blocked tonic seizures induced by MES significantly ($P < 0.001$) at the dose 400 mg/kg. b. wt.p. o. The ED₅₀ (200, 400 and 600 mg/kg b. wt.) values obtained from the extracts indicate that it has enough potency against MES induced seizures. MES induced tonic seizures can be prevented by drugs that inhibits voltage dependant Na⁺ channels, such as Phenytoin, Valproate, Felbamate and Lamotrigine or by drugs that block glutamatergic excitation mediated by the N-methyl-D-aspartate (NMDA) receptor such as felbamate. The study showed that the *Viburnum arista* can inhibit voltage dependant Na⁺ channels as Phenytoin in MES induces tonic seizures.

CONCLUSION

The MES test is considered to be a predicator of likely therapeutic efficacy against generalized tonic-clonic seizures. The present study revealed that the *Viburnum arista* blocked tonic seizures induced by MES significantly ($p < 0.001$) at the dose 200, 400 and 600 mg/kg. b. wt. (p.o). This study will make awareness that a test by increasing the dose of the drug may result in an increase in therapeutic efficacy and thereby to prove the test drug may be a very efficient anti convulsant formulation.

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