

**ISOLATION AND SPECTRAL IDENTIFICATION OF ARBUTIN FROM THE ROOTS OF  
*VIBURNUM ERUBESCENS* WALL. EX DC**K Prabhu<sup>1\*</sup>, P K Karar<sup>2</sup>, S Hemalatha<sup>3</sup>, K Ponnudurai<sup>4</sup><sup>1</sup>Department of Pharmacognosy, Nandini Nagar Mahavidyalaya College of Pharmacy, Nawabganj, Gonda, Uttar Pradesh, India<sup>2</sup>Department of Pharmaceutical Chemistry, DOABA College of Pharmacy, Kharar, Mohali, Punjab, India<sup>3</sup>Department of Pharmaceutics, Pharmacognosy Division, IT-Banaras Hindu University, Varanasi, Uttar Pradesh, India<sup>4</sup>Department of Pharmacology, Nandini Nagar Mahavidyalaya College of Pharmacy, Nawabganj, Gonda, Uttar Pradesh, India

Received on: 12/03/2011 Revised on: 02/05/2011 Accepted on: 20/05/2011

**ABSTRACT**

The roots of *Viburnum erubescens* belonging to the family Adoxaceae is collected from Nilgiri hills of Tamil Nadu and authenticated. 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. From 1.25 kg of the root extract, approximately 40 mg of Arbutin was isolated using a column chromatography and characterised by spectroscopy. The current study may be useful to progress further investigation on the isolation of other phenolic compounds and their biological potential for the treatment of human ailments.

**KEYWORDS:** *Viburnum erubescens*, Column, Arbutin, Alumina, Methanol**\*Corresponding author**E-mail: [prabhu.cognosy@gmail.com](mailto:prabhu.cognosy@gmail.com); [prabhu.cognosy@rediffmail.com](mailto:prabhu.cognosy@rediffmail.com)**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<sup>1,2</sup>.

*Viburnum* Linn. Species have been reported to contain sesquiterpenes<sup>3</sup>, 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 possess uterine sedative, diuretic, cardiovascular stimulant, antimicrobial, anti-inflammatory, anti-nociceptive, antispasmodic, anti-asthmatic and astringent activities<sup>4</sup>. 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<sup>5-7</sup>. 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: irridoid aldehydes and their glycosides in *Viburnum luzonicum*<sup>8</sup>, and their cytotoxic effect; vibsane type diterpene from *Viburnum awabuki*<sup>9</sup>; irridoid glycosides from *Viburnum tinus*; antinociceptive and anti-inflammatory activities of *Viburnum lanata*<sup>10</sup>, and *Viburnum opulus*<sup>11</sup>, and an irridoid glucoside from *Viburnum rhytidophyllum*<sup>12</sup>. 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<sup>13,14</sup>.

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, which has also revealed that the leaves, stem bark and root barks of mature plants had been reliably in usage to the non-pregnant uterus<sup>15</sup>, the GIT related ailments, and are

also in application as an ideal healing aid against inflammation<sup>16</sup>, infections by protozoal and bacterial origin, as well as one of the best home remedies; some of the above have also been scientifically proven.

The phenolic compounds of plant origin are versatile in biological activities. Their presence in plants, probably may be due to one or all of the following purposes: feed deterrents against cattle; (pathogenic) defence against microbial attack; as precursors for biosynthesis or as metabolic end products of plant metabolism; pH-dependent colouring agents, especially in floral organs and leaves; as the building blocks of polymeric phenolic molecules of heavy molecular weight such as tannins, procyanidins and lignans; and as antioxidants.

Isolation of phenolic compound by virtual solvent extraction process is supposed to be a highly tedious process, because of its high magnitude of reactivity with other co-molecules of the plants such as proteins (astringent effect) and carboxylic acids to form esters during extraction, in addition to their delicate nature of decomposition in presence of heat, acids, alkali and inorganic elements such as heavy metals. Phenols, cresols, xylenols and halogenated phenolic derivatives are most powerful antimicrobials (often referred to be "Disinfectants" which are unsuitable for oral administration in the living beings). In this context, the phenols of plant origin are remarkably suiting for application in living system besides an advantage that the desired activity is achieved at a very low concentrations, being parasitotrophic rather than organotrophic.

## MATERIALS AND METHODS

### Collection of Research Material

The roots of *Viburnum erubescens* were collected from Nilgiri hills, Tamil Nadu, India. In the month of August-2010 and dried well in the sun and shade for near about a couple of weeks. The specimens of the study was authenticated by Dr.V.Chelladurai, Ex. Professor, (Botany), Medicinal plant survey for Siddha, Government of India, as *Viburnum erubescens* Wall.ex DC, a herbarium (labelled VE131) was submitted to the museum of the department, Nandini Nagar Mahavidyalaya College of Pharmacy, Gonda Uttar Pradesh, India.

### Successive solvent extraction and primary organic analysis

About 1.25 kg of the roots of *V.erubescens* 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 was subjected for the determination of percentage extractives<sup>17,18</sup> and a primary organic analysis.

Primary organic analysis was carried out with suitable chemical reagents of research grade which led to a conclusion that the phenolic compounds were well pronounced with hydroalcoholic extract that may be suitable to be chosen for further phytochemical analysis<sup>19</sup>.

## RESULT AND DISCUSSION

### Sample preparation and column packing

75% ethanolic stem extract of *V.erubescens* yielded on storage about 6.5 g of reddish orange deposits (labelled VE1) and a brownish ethanolic layer. The residue of the later on evaporation yielded about 38.5 g of dark brown residue (labeled-VE2).

### Isolation of Arbutin from VE2 fractions of VEEE

#### Sample preparation and column packing

VE2 (the filtrate labelled, after separating of VE1-a sediment) was evaporated under reduced pressure to yield 35.5 g of residue. About 20 g of the residue was blended with 100 g of neutral alumina on a water bath to obtain a uniformly dispersed mixture. A slurry of alumina in diethyl ether was prepared and poured into a glass column (3.5 × 50 cm dimension) in such a way that no cracking of alumina and trapping of air bubbles occurred, followed by addition of the sample-adsorbent mixture at a ratio of 1:5.

#### Solvent elution (50 ml/fraction)

The content of the column was fractionated with diethyl ether (1 – 3); acetone (4 – 6); acetone-ethyl acetate (1:1 ratio) 7 - 9; ethyl acetate (10 – 13); ethyl acetate-chloroform (1:1) 14 – 16; chloroform (17 – 19) and finally with methanol-water gradient elution, with mixtures of decreasing polarity such as methanol-water (30:70 volumes); (40:60), (60:40), (80:20); and (90:10) each of five fractions viz., 20 – 24, 25 – 29, 30 – 34, 35 – 39 and 40 – 44 respectively. The yield of the fractions, 25 – 34, and 30 – 40 was appreciable and so were evaporated under reduced pressure to collect the respective residues i.e., (25 – 29) and (30 – 34) separately (Table 1).

#### Physico-chemical features of the residue from fraction (25 – 34)

A pale yellowish fraction, upon evaporation yielded a transparent yellowish, glassy residue. The residue was freely soluble in aqueous ethanol and methanol (50%) and hot water; and soluble in absolute methanol, ethanol and in water at room temperature; insoluble in ethyl acetate, acetone, benzene, ether and carbon tetrachloride.

A dilute solution in methanol showed  $\lambda_{\max}$  at 225 nm [characteristic of a long chromophore of Ph-C=C-].

The crude compound from 25 – 29 fractions was labelled to be VE2a and the compound from 30 – 34 being VE2b. Both the compounds (crude residues) melted between 142 – 147°C.

#### Homogeneity of the compound

Crude compound from fractions 25 – 29 and 30 – 34 were subjected to ascending TLC using 50% aqueous ethanol as developing phase, and 0.2% alcoholic ferric chloride as locating agent.

Both VE2a and VE2b assumed 0.42 and 0.44 as  $R_f$  values which were comparable, besides showing a green colour with a spray of 0.2% alcoholic ferric chloride solution on the chromatograms.

#### Purification and spectral characterization

The crude samples (VE2a and VE2b) mixed together and dissolved in 50 ml of 50% aqueous methanol and added was 1 g of activated charcoal. The whole mixture was stirred with a glass rod thoroughly and filtered. The filtrate then was evaporated to get colourless crystals whose homogeneity was screened by TLC, as done before ( $R_f$  – 0.44). The crystals were repeatedly crystallized from hot water to yield about 40 mg of pure crystals.

The isolated compound was subjected to spectral analysis to confirm the compound to be Arbutin ( $C_{12}H_{16}O_7$ , m.p. 145°C). The spectral data of the isolated compound as follows:

**IR (KBr)  $\nu$   $cm^{-1}$ :** 3429.5 (–OH stretching aromatic alcohol); 3022.3 (C–H aromatic-H stretching); 1522.0 (–C–C– aromatic ring); 1026.1 – 1216.6 (–C–O stretching) (Figure 1).  **$^1H$ -NMR ( $CDCl_3$ ) 300 MHz, TMS,  $\delta$  ppm:** 6.827, 6.624 (multiplets, Ar–H aromatic proton); 5.589 (singlet, carbon attached to –O – of glycoside); 5.101 (singlet, Ar–OH aromatic hydroxyl proton); 3.910, 3.79, 3.761 (multiplet, glycosidic carbon); 2.950.2 (singlet, C–H alkane); 2.011 (singlet, –OH alcoholic hydroxyl proton) (Figure 2).

#### CONCLUSION

From 1.25 kg of the *V. erubescens* root extract, approximately 40 mg of Arbutin was isolated using a column chromatography and characterised by spectroscopy. The current study may be useful to progress further investigation on the isolation of other phenolic compounds and their biological potential for the treatment of human ailments.

#### REFERENCES

1. Trease GE and Evans WC. Pharmacognosy. 10<sup>th</sup> ed. Berilliee, Tindal, London, 2002, p. 519 -547.

- The Wealth of India. A Dictionary of Indian Raw materials and Industrial Products – Raw Material Series, Publication and Information Directorate, CSIR, New Delhi, 2003, 10, p. 437 – 446.
- Khosa RL, Wahi AK, Mohan Y and Ray AB. Isolation of Bergenin from roots of *Viburnum nervosum* Hook, Indian J Pharm, 1979; 41(3): 120.
- Nadkarni KM. Indian Materia Medica, 2<sup>nd</sup> ed, Popular Prakashan, Bombay, India, 2002, 1, p. 1271 – 1272.
- Hoerhammer L, Wagner H and Reinhardt H. Isolation of flavonoids from the barks of *Viburnum prunifolium* Dent. Apothekerzer, 1965; 105(40): 1371.
- Yunusova SG, Karimova AR, Tsyrlina EM, Yunusova MS and Denisenko ON. Change on storage of biological activity of *Viburnum opulus* seed components. Chem Nat Comp, 2004, 40(5): 423 – 426.
- Wahi AK, Khosa RL and Mohan Y. Pharmacognostical studies on the roots of *Viburnum nervosum* Hook., Bot Res, 1981; 3: 205.
- Tomassini L, Gao J, Foddai S, Serafini M, Ventrone A and Nicoletti. Iridoid glycosides from *Viburnum chinshanense*, Nat Prod Res, 2006; 20(8): 697 – 700.
- Fukuyama Y, Kubo M, Minami H, Yuasa H, Matsuo A, Fujii T, Morisaki M and Harada K. Rearranged vibsane type diterpenes from *Viburnum awabuki* and photochemical reaction of vibsantin-B. Chem Pharm Bull, 2005; 53(1):72 – 80.
- Sever YB, Saltan CG, Altun ML and Ozbek H. Antinociceptive and Anti-inflammatory Activities of *Viburnum lantana*, Pharm Biol, 2007; 45(3): 241-245.
- Altun ML, Saltan CG, Sever YB and Ozbek H. Antinociceptive and anti-inflammatory activities of *Viburnum opulus*, Pharm Biol, 2009; 47(7): 653-658.
- Tomassini L, Dejan B, Foddai S and Nicoletti M. Iridoid glucosides from *Viburnum rhytidophyllum*, Phytochemistry, 1997; 44(4): 751-753.
- Prabhu K, Karar PK, Hemalatha S and Ponnudurai K. Anti-inflammatory, Analgesic and Antispasmodic activities of three *Viburnum* Linn. Species. International Journal of Current Trends in Science and Technology, 2010; 1(3): 175–186.
- Prabhu K, Karar PK, Hemalatha S and Ponnudurai K. Total Phenolic Content and In vitro Anti-oxidant Potentials of Ethanolic Stem Extracts of Three *Viburnum* Linn. species – A Comparative Study. Asian Journal of Chemistry, 2011; 23(2): 867-870.
- Prabhu K, Karar PK, Hemalatha S and Ponnudurai K. Pharmacognostical Investigations on the Stem of Two *Viburnum* Linn. Species A Comparative Study, International Journal of Pharmaceutical Research, 2009; 1(2): 43-50.
- Prabhu K, Karar PK, Ponnudurai K and Hemalatha S. Pharmacognostic Investigation of the Leaves and Stems of *Viburnum erubescens* Wall.ex DC, Tropical Journal of Pharmaceutical Research, 2009; 8(6): 557-566.
- Pharmacopoeia of India. Ministry of Health and Family Welfare, The Controller of Publications, New Delhi, India, 1996, 2, A47 - A89.
- Harborne JB. Phytochemical methods, 3<sup>rd</sup> ed, Chapman and Hall, London, 2005, p. 49-244.
- Wagner H, Bladt S and Zgainski EM. Plant drug analysis, A thin layer chromatography atlas, Tokyo: Springer Verlag Berlin Heidelberg, New York, 1983, p. 117 – 222

Table 1. Isolation of Arbutin from VE2 fraction of VEEE

Fraction	Mobile Phase	Residue	TLC profile	No. of spots	R <sub>f</sub> Values
1 – 3	Diethyl ether	-	-	-	-
4 – 6	Acetone	+	EtOAc-CH <sub>3</sub> OH-H <sub>2</sub> O (100:13.5:10)	3	0.65, 0.58, 0.72
7 – 9	Acetone- Ethyl acetate (1:1)	-	EtOAc-CH <sub>3</sub> OH-H <sub>2</sub> O (100:13.5:10)	-	-
10 – 13	EtOAc	+	EtOAc-CH <sub>3</sub> OH-H <sub>2</sub> O (100:13.5:10)	3	0.71, 0.57, 0.45
14 – 16	EtOAc -CHCl <sub>3</sub> (1:1)	-	EtOAc-CH <sub>3</sub> OH-H <sub>2</sub> O (100:13.5:10)	-	-
17 – 19	CHCl <sub>3</sub>	-	EtOAc-CH <sub>3</sub> OH-H <sub>2</sub> O (100:13.5:10)	-	-
20 – 24	Methanol-water (30:70)	+	EtOAc-CH <sub>3</sub> OH-H <sub>2</sub> O (100:13.5:10)	2	0.52, 0.74
25 – 29*	CH <sub>3</sub> OH-H <sub>2</sub> O (40:60)	++	Alcoholic FeCl <sub>3</sub> as locating agent, 50% ethanol as developing agent	1	0.42
30 – 34*	CH <sub>3</sub> OH-H <sub>2</sub> O (60: 40)	+++		1	0.44
35 – 39*	CH <sub>3</sub> OH-H <sub>2</sub> O (80: 20)	++	EtOAc-CH <sub>3</sub> OH-H <sub>2</sub> O (100:13.5:10)	2	0.48, 0.38
40 – 44*	CH <sub>3</sub> OH-H <sub>2</sub> O (90: 10)	++	EtOAc-CH <sub>3</sub> OH-H <sub>2</sub> O (100:13.5:10)	3	0.48, 0.45, 0.35

50 ml/fraction, 2 ml/min, 3.5 × 50 cm dimension, Slurry of alumina in diethyl ether, - residue absent; + - negligible quantity; ++ - moderate quantity; +++ - considerable quantity, EtOAc - Ethyl acetate, \* - phenolic test positive.

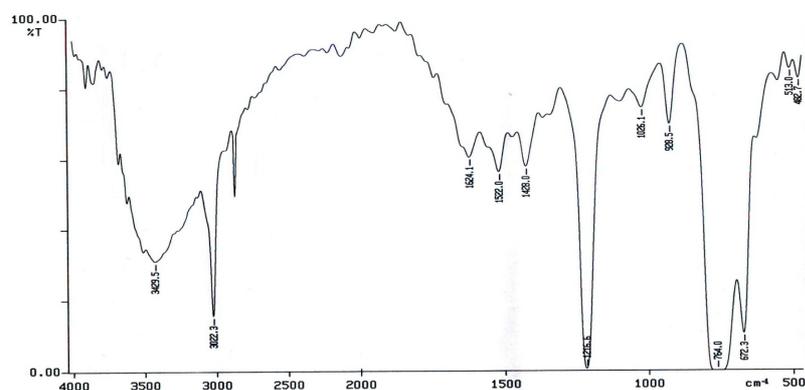
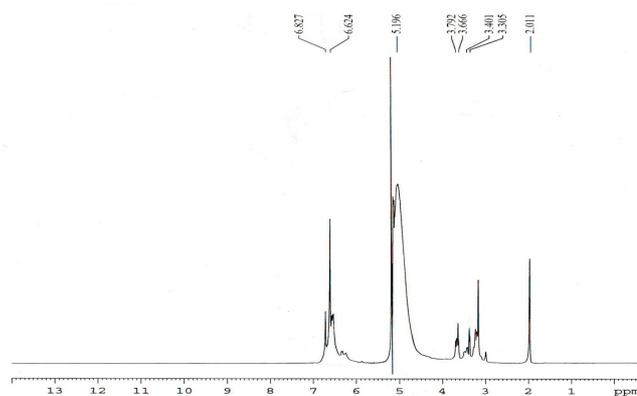


Figure 1. IR spectrum of Arbutin

Figure 2. <sup>1</sup>H-NMR spectrum of Arbutin

Source of support: Nil, Conflict of interest: None Declared