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Der Pharmacia Sinica, 2011, 2 (4):87-92

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Isolation of chlorogenic acid from the stems of *Viburnum coriaceum* Blume

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ABSTRACT

The genus Viburnum Linn. belonging to the family Caprifoliaceae, consists of about 20 species throughout the world. Many of these species have been reported to possess phenolic compounds and their glycosides as their major chemical constituents in their stem and root barks, which may be attributed to their remarkable biological activities against smooth muscle related troubles. The main objective of the present study is to detect, isolate and characterize some phenolic compounds from aqueous ethanolic stem extract of two Viburnum Linn. Species collected from Nilgiri hills, Tamilnadu, India. The dried stems of Viburnum coriaceum Blume. were powdered and extracted separately and successively with petroleum ether (60 – 80°C), chloroform and 75% aqueous ethanol. The ethanolic extracts were screened by Thin Layer Chromatographic techniques for the presence of different classes of phenolic compounds. The TLC studies revealed the presence of some flavonoid which was later confirmed to be chlorogenic acid in V.coriaceum by co-TLC technique against the reference substances. The ethanolic residues were adsorbed on the activated neutral alumina and packed in a glass column containing slurry of alumina in acetone. Then, the column was eluted firstly with some non-polar and moderately polar solvents to clear out impurities which remain present in the column followed by a gradient elution with methanol-water mixture. The fractions yielded 35 mg of chlorogenic acid from V.coriaceum. The samples were subjected IR, NMR spectral analysis to evident their molecular structures.

Keywords: *Viburnum*, Caprifoliaceae, gradient elution, alumina, chlorogenic acid.

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*; irdoid glycosides[9] 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[13,14]

MATERIALS AND METHODS [15-17]

Stem and stem branches of *V.coriaceum* were collected from Nilgiri hills, Tamilnadu and authentificated by Dr.V.Chelladurai, Ex. Professor, (Botany), Medicinal plant survey for Siddha, Government of India, as *Viburnum coriaceum* Blume (VC). The materials were dried in the sun for about 15 days and then in the shade. About 1.25 kg of stems of VC were powdered separately using a mechanical grinder in to a moderately coarse powder. The powdered specimens were extracted, in a soxhletor for about 15 – 18 h, by continuous hot percolation method successively with petroleum ether (60 – 80°C), chloroform and 75% aqueous ethanol.

In order to predict the chemical nature of phenolic constituents, which plays a crucial roles in selection of reselection of suitable solvent system to elute phenolic compound using a column, the conventional procedure, a preliminary detection of different classes of phenolic compounds were carried out using appropriate adsorbent, developing phase and locating agents.

A preliminary organic screening [18-20] of alcoholic fraction assisted with paper chromatography and TLC gave a positive test for phenolic compounds such as: tannins (Gold beater's test), flavonoids (Shinoda's test, Zn-HCl, Fluorescent test under UV-254 – quenching due to conjugate system and UV peak maxima), condensed tannins (using paper chromatography), simple phenolic acids (by TLC aided detection), anthocyanins (pH dependent colour change and red colouration of the alcoholic extracts) and glycosides of a few or all of the phenolic compounds (Hydrolysis test).

The extracts were concentrated under reduced pressure to obtain $1/5^{th}$ of its original volume. Petroleum ether extracts and chloroform fractions were tested positive for phytosterols and triterpenoids. The alcoholic extract was tested positive for phenolic compounds, free sugars and glycosides.

The alcoholic extract was stored at room temperature for 7 days to find out sediments or deposits, if any, on the bottom of the container. The extract was filtered to separate a pale brown solids deposited from the dark brown liquid labeled to be Vc2 (solid 3.5g) and Vc1 (brownish ethanolic layer). The Vc2 was concentrated to get 26 g of residue. About 3g of the residue of was adsorbed on 15 g of Brachman's neutral alumina and stirred vigorously on a boiling water bath and then was packed in to the glass column containing a slurry of neutral alumina in petroleum ether (1:2). The column was 3.5×50 cm in dimension; the rate of elution was maintained to be 2 ml/min and 50 ml/fraction. The fractions which were eluted by the individual solvent or solvent combinations were added together and evaporated to observe residue, if any.

Table 1. Isolation of Chlorogenic acid from ethanolic extract of *V. Coriaceum*

Fractions	Mobile phase solvent(s)	Residue (+/ –)	TLC Profile	No. of Spots	R_f Value
1 – 3	Acetone	+		-	-
4 – 7	Benzene	+		-	Tailing
8 – 12*	**Ethylacetate**	**+++** (VE3)	Absolute ethanol as developing agent, 0.2% alcoholic $FeCl_3$ as locating agent	**1**	**0.46**
13 – 17*	**Ethylacetate : Methanol (1:1)**	**+++** (VE4)		**2**	**0.41; 0.44**
18 – 21	Methanol (absolute)	++		3	0.81; 0.79; 0.54
22 – 25	Water	++		2	0.40; 0.49

50 ml/fraction; 2 ml/min; 50×3.5 cm column; Silicagel-G for column chromatography grade (slurry in acetone), ¯– residue absent; $^{+}$ – negligible quantity; $^{++}$ – moderate quantity; $^{+++}$ – considerable quantity, * – fraction gave a positive for phenolics.

RESULTS AND DISCUSSION

The alcoholic stem extract was dark brownish and turbid which on filteration, yielded about 1.5 g solid residue (yellowish brown) which was insufficient for handling. So, the filterate was selected and then concentrated under reduced pressure to get a brownish residue (VC1) (28 g). The residue was adsorbed on a paper bulb and placed in a small scale soxhletor, and was fractionated successively with diethyl ether, ethyl acetate and absolute ethanol for about 3 h. The last two fractions gave a positive test for phenolic compounds. The ester fraction was selected and labelled to be VC2 (Table 4).

Ethyl acetate soluble constituents (VC2)

Silicagel-G for column chromatography was mixed with three of its volume of acetone and triturated well to result an uniformly dispersed and pourable slurry. The slurry was poured in to a column (3.5×50 cm) in such a way that no air bubbles be trapped.

The residue of ethyl acetate fraction was adsorbed on silicagel-G (column chromatography grade) at a ratio of 1:5 and stirred well placing on a boiling water bath. The sample-adsorbent mixture was then placed in to the column containing slurry of silicagel in acetone. The column was eluted with acetone (1 – 3 fractions), benzene (4 – 7), ethyl acetate (8 – 12), ethyl acetate: absolute methanol (1:1) (13 – 17), absolute methanol (18 – 21) and finally with distilled water (22 – 25).

Isolation of Chlorogenic acid and spectral characterization [21]
Fractions 1 to 3 were added together and evaporated showing a pinkish residue which was, by volume, insufficient to handle. Fractions 4 to 7 yielded no any residue. Fractions 8 to 12 and 13 to 17 yielded yellowish brown and pale brown residues respectively. Fractions 13 to 17 were added together and evaporated to get a pale brown residue (thin layer), labelled VC4 (phenolic in nature). Fractions 8 – 12 were added together and evaporated and labelled to be VC3 [On complete evaporation, VC3 and VC4 transformed in to a thin layer (flakes and shining)].

Small quantity of VC3 was dissolved in ethyl acetate and run on a TLC plate using absolute ethanol as developing phase, silicagel-G as stationary phase. A distinct yellowish to orange spot resulted on the chromatogram against a spray of 3% methanolic H_2SO_4 as locating agent (R_f-0.46).

Both VC3 and VC4 flakes were pale brown in colour, sparingly soluble in acetone absolute ethanol, methanol and water; soluble in ethyl acetate and ether; insoluble in benzene and chloroform. The flakes melted between 196 – 200 °C.

The ethyl acetate fractions 8 – 12 and ethyl acetate-alcohol (1:1) fractions 13 – 17 gave greenish brown colour with alcoholic ferric chloride solution.VC4 was also run on a TLC plate as that of VC3 to obtain two distinct spots (R_f-0.41; 0.44; using 0.2% ethanolic $FeCl_3$ solution as locating agent). Accounting the proximity among R_f values, both VC3 and VC4 were dissolved in 5 ml of ethyl acetate. The residue of ethyl acetate fraction then was adsorbed on 10 g of silicagel-G (column grade), and stirred well on a boiling water bath and packed in to an analytical column containing slurry of silicagel-G (column grade) in acetone, at 1:2 ratio. The column was of 25 cm long and 1 cm wide.

Figure 1. IR spectrum of Chlorogenic acid

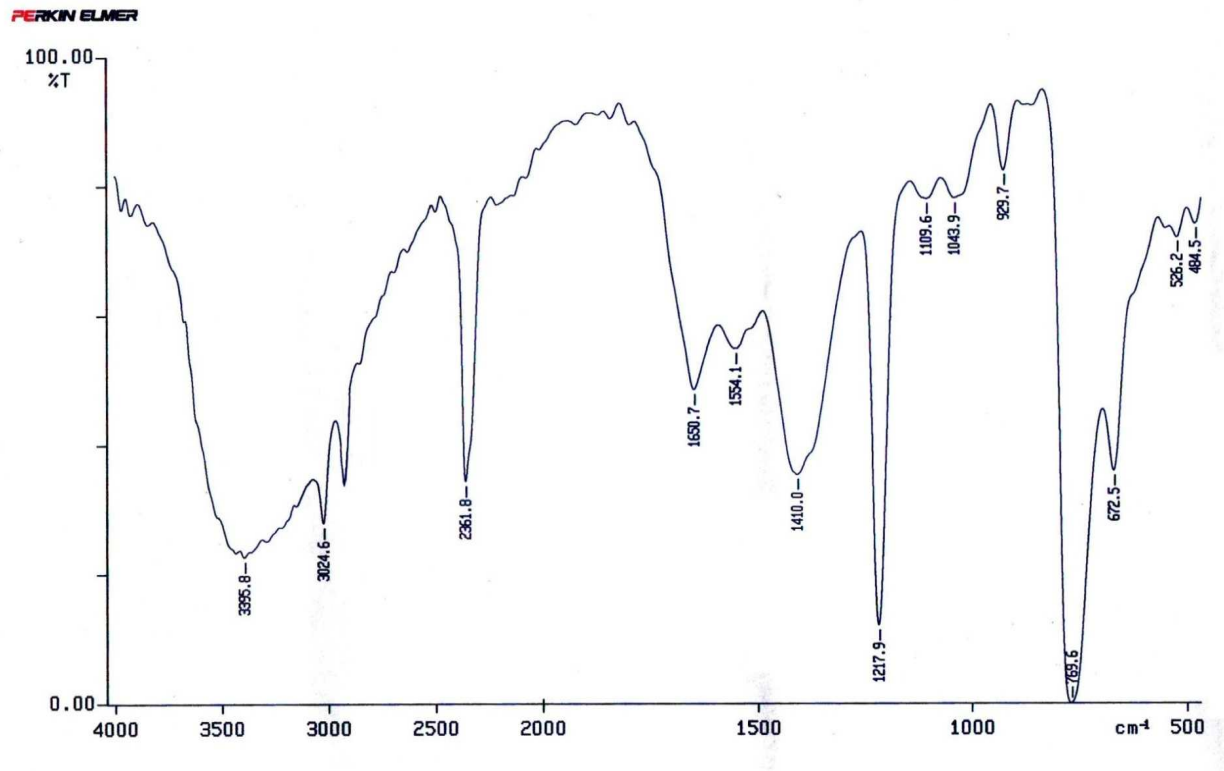
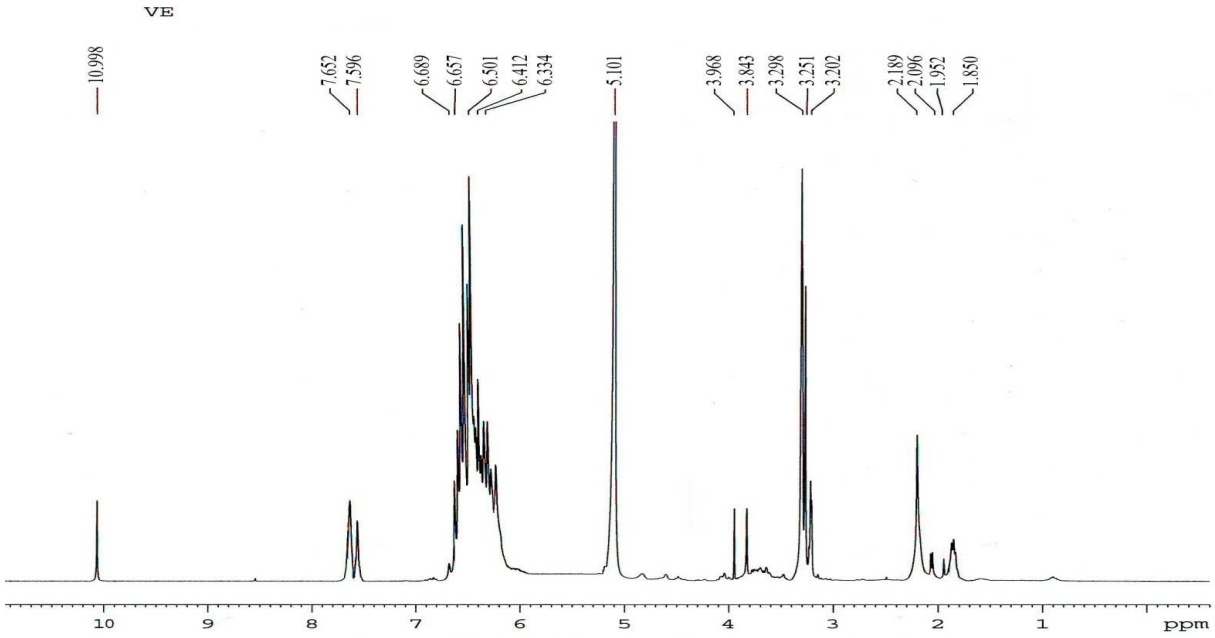


Figure 2. ^{1}H-NMR spectrum of Chlorogenic acid



The column was eluted with 20 ml each of acetone, and then with 20 ml ethyl acetate. The later was evaporated to obtain shining and pale brownish flakes which melted at 198°C.

Small quantity of the substance was dissolved in ethyl acetate and run on a TLC using absolute ethanol as developing phase and silicagel-G as stationary phase. The chromatogram showed a distinct spot against the spray of 3% H_2SO_4 (methanolic) R_f-0.46 (a dark green spot). The crude sample was crystallized repeatedly from distilled water and stored at 4°C to get about 95 mg of crystals. The spectral studies resulted the following: IR (KBr) ν cm^{-1}: 3395.8 (–OH aromatic alcohol stretching); 3024.6 (Ar–H aromatic ring stretching); 2960.1, (CH=CH stretching); 1217.9, (C–O stretching); 1650.7, (>C=O stretching); 1554.1, (–C=C– aromatic ring). ^{1}H-NMR (CD_3OD) 300 MHz, TMS, δ ppm: 10.998 (singlet, –COOH carboxyl proton); 7.652 (doublet, ethylene CH=CH); 6.689, 6.657, 6.501 (multiplet, Ar–H); 5.101 (doublet, Ar–OH); 3.968, 3.843, 3.298, 3.202 (multiplet, cyclohexane proton); 2.189 (singlet, Alcoholic–OH); 1.95, 1.85 (doublet, $–CH_2$ proton of cyclohexane).

CONCLUSION

About 35mg of chlorogenic acid were isolated from 1.25kg of stems of *V. coriaceum.* This study may provide informations on an economic isolation of the same substance using various solvents and methods as well as biological values of this compound and the species.

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