TÜBİTAK

Research Article

Turk J Bot
35 (2011) 663-670

doi:10.3906/bot-1006-7

Comparative micromorphological and phytochemical studies on the roots of three *Viburnum* (Caprifoliaceae) species

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Received: 04.06.2010
Accepted: 29.03.2011

Abstract: In this study, the roots of *Viburnum punctatum* Buch.-Ham. ex D.Don, *Viburnum coriaceum* Blume, and *Viburnum erubescens* Wall. ex DC. were collected from the Nilgiri and Coimbatore hills in Tamil Nadu, India. Transverse sections of plant roots were prepared with the aid of a rotary microtome. The sections, each at a thickness of 10 µm, were stained and fixed in Canada balsam and the morphoanatomical features of each specimen were noted. The specimens were powdered using a mechanical grinder and were mounted in suitable media for starch grains, sclereids, druses, fibres, and vessels. Morphoanatomical features of diagnostic importance were recorded under different magnifications, followed by the determination of dimension and histological features of root components using a calibrated eyepiece micrometer and a polariser. The starch grains averaged 12, 17, and 20 µm in diameter in *V. erubescens*, *V. punctatum*, and *V. coriaceum*, respectively. The fibres were about 1 mm long in *V. punctatum* and *V. coriaceum*, whereas those found in *V. erubescens* specimens were around 700 µm. Qualitative chemical screening indicated that phytosterols, triterpenoids, and phenolic compounds and their glycosides were among the commonly found phytoconstituents.

Key words: *Viburnum*, starch grains, sclereids, druses, sieve elements

Introduction

The plant kingdom still holds many species of plants containing chemical substances of medicinal value that have yet to be explored. A typical example of such a category is *Viburnum* L., a genus belonging to the family Caprifoliaceae (Gamble, 1935; CSIR, 2003) under the order Dipsacales or, alternatively, Adoxaceae sensu (Angiosperm Phylogeny Group, 2009). *Viburnum* contains about 200 species distributed mainly in the temperate zone of the northern hemisphere, and approximately 17 species have been reported from India, particularly in the Himalayan, Nilgiri, and Coimbatore hills (CSIR, 2003). The leaves, stem barks, and roots of many of these species have been investigated recently and have been shown to possess various cytotoxic (Tomassini et al., 1997), antimicrobial (CSIR, 2003), antinociceptive (Altun et al., 2009), antispasmodic,

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and uterine-relaxant activities (Nadkarni, 2002). These effects may be attributed to the presence of simple, polyphenolic compounds (Hoerhammer et al., 1965) and the glycosides, sesquiterpenes (Khosa et al., 1979), diterpenes (Fukuyama et al., 2005), and iridoid glycosides (Tomassini et al., 2006) that make up their chemical constituents.

Viburnum is an extremely variable genus of shrubs or medium-sized trees found in India, commonly 3-5 m high with an evergreen canopy. Identification of the individual species of *Viburnum* is still a difficult task for a taxonomist, botanist, or pharmacognostic expert because the morphological features of these species often appear to be identical and because of common polyploidy (Scott, 1950), especially when more than one species exists together at a single locality. This is why the taxonomical/botanical recognition and the wild collection of the *Viburnum* species are usually undertaken by native plant vendors and herbalists during the flowering season (May-July) in India.

The objective of the current research was to discover parameters that will be useful and sufficiently constant to identify and differentiate the roots of 3 *Viburnum* species, both whole as well as in the powdered form, based on their microscopic features, the micromeasurement of histological components, and preliminary phytochemical screening.

Materials and methods

The plant specimens used in the present study were collected from the Nilgiri hills of Tamil Nadu, India, and identified by Dr. V. Chelladurai, an expert in medicinal plant survey for Siddha, Government of India. Dr. Chelladurai determined that the samples represented *Viburnum punctatum* Buch.-Ham. ex D.Don, *Viburnum coriaceum* Blume, and *Viburnum erubescens* Wall. ex DC. Care was taken to ensure the selection of healthy plants and their normal organs. Specimens were removed from the plant and fixed in formalin aceto-alcohol (FAA; 5 mL formalin + 5 mL acetic acid + 90 mL 70% ethyl alcohol). After 24 h of fixation, the specimens were dehydrated with a graded series of tertiary-butyl alcohol (TBA) and infiltrated by a gradual addition of paraffin wax (melting point: 58-60 °C) until the TBA solution attained supersaturation. At that point, the specimens were cast into paraffin blocks.

The paraffin-embedded specimens were sectioned to a thickness of 10-12 µm with a rotary microtome. Dewaxing of the sections was performed according to the customary procedure (Johansen, 1940: 523). Since toluidine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were obtained. The dye rendered the cellulosic walls pink, the lignified cells blue, the suberin dark green, the mucilage violet, and the protein bodies blue. The sections were also stained with safranin, fast-green, and IKI (for starch). Glycerine-mounted temporary preparations were prepared for maceration and cleared using Jeffery's maceration fluid (Sass, 1940: 222; Easu, 1964: 767).

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with a Nikon Labophot-2 microscope. For normal observations, bright field was used; for the study of crystals, starch grains, and lignified cells, polarised light was employed. Since these structures have a birefringent property, they appear bright against a dark background when subjected to polarised light. The magnifications adapted were indicated by scale bars (Güvenç & Duman, 2010).

The dried roots of *V. coriaceum*, *V. punctatum*, and *V. erubescens* were ground in a mechanical grinder to obtain fine and very fine powders. The specimens were then mounted using suitable reagents to trace out starch grains, sclereids, druses, fibres, and vessels. Aided by a calibrated eyepiece micrometer, the dimensions of the above components were measured and reported (Mutlu, 2010). To ensure reproducibility of the parameters, a minimum of at least 40 characters of each histological component were measured, and the report was presented in terms of mean size ranges in micrometres (µm).

Sclereids and starch grains, abundant in all 3 of the species studied, were subjected to the lycopodium spore method (Lala, 1981; Wallis, 2005) in order to enumerate their number per milligram of powered root.

The successive extraction with solvents of increasing polarity was performed with the objective of evaluating a primary organic analysis. About 150 g of sun- and shade-dried (for 15 days) roots of all species were separately powdered into a moderately

coarse powder and then successively soxhleted for about 15-18 h. When this was complete, the percentage extracts were determined (Babu et al., 2010) and subjected to the following primary organic analysis: the Liebermann-Burchard test (for steroids and triterpenes), Mayer and Dragendorff's test (for alkaloids), Fehling's and Benedict's test (for reducing sugar), the xanthoprotein test (for protein and amino acids), the ferric chloride test (for phenolic compounds), the Shinoda test (for flavonoids); the paper chromatographic test with alkali (for condensed tannins), the haemolytic test (for saponins), the hydrolytic test (for glycosides in general); and blood colouration of the hydroalcoholic extract (for anthocyanins) (Prabhu et al., 2009).

Results and discussion

Root of *Viburnum punctatum*

A thick root of more than 3 mm in diameter was selected for the study. The periderm is 250 µm thick, deeply fissured and made of dark, tabular, narrow phellem cells; the cortex consists of radially compressed oblong parenchyma cells (Figure 1). The secondary phloem is represented by funnel-shaped, dilated rays; the ray cells are large and rectangular in shape; the sieve elements and phloem parenchyma occur in conical bands; the sieve elements are polygonal in outline, while the companion cells are small and occur in a corner. The secondary xylem includes angular, wide, diffuse, thin-walled vessels that are solitary and up to 130 µm wide; the xylem rays are prominent, thick-walled, and lignified. Starch grains are abundant within ray cells whereas the thick masses of sclereids between the secondary phloem and the cortex are scattered. The calcium oxalate crystals are less frequent, druses in structure, and are up to 30 µm in diameter (Figure 2).

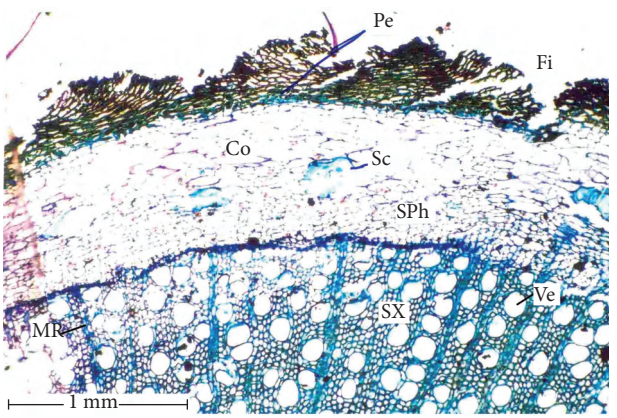

Figure 1. A transverse section of the *Viburnum punctatum* root. Pe: periderm, Co: cortex, Sc: sclereids, Fi: fissure, SPh: secondary phloem, SX: secondary xylem, Ve: vessel, MR: medullary rays.

Root of *Viburnum coriaceum*

A thick lateral root of 3.5 mm diameter was selected. The transverse section shows a wide, dark periderm and solid, dense secondary xylem cylinders. A narrow zone of cortex and the secondary phloem fall between the periderm and the secondary xylem cylinders (Figure 3). The periderm is up to 200 µm wide, the entire zone is dark and tanniniferous; the periderm develops narrow, irregular fissures; beneath this is found a narrow zone of cortex. The secondary phloem rays are narrow and straight with large tannin-filled cells; the sieve tubes are polygonal, thin-walled, and clustered.

The secondary xylem consists of radially oblong cells that are thin and straight; the vessels are angular, thin-walled, wide, and diffuse in a distribution that is mostly solitary and up to 100 µm wide. However, multiples of 2 vessels are also rarely seen. The xylem

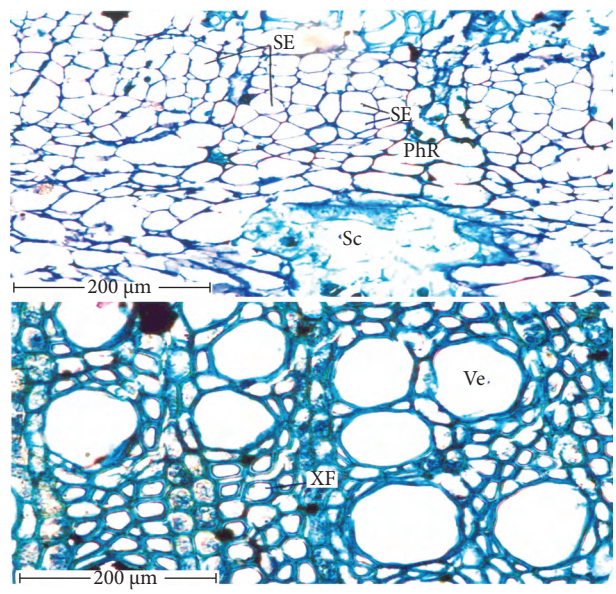

Figure 2. The secondary phloem and xylem in the root of *Viburnum punctatum*. PhR: phloem ray, SE: sieve element, Sc: sclereids, Ve: vessel, XF: xylem fibres.

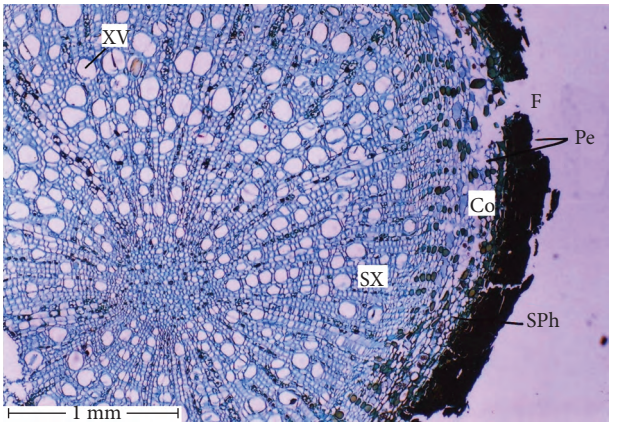

Figure 3. A transverse section of thin root from *Viburnum coriaceum*. Co: cortex, F: fissure, Pe: periderm, SPh: secondary phloem, SX: secondary xylem, XV: xylem vessels.

fibres are either thin-walled with a wide lumen or thick-walled with a narrow lumen. The vessels and fibres are lignified.

Calcium oxalate crystals are abundant in the root bark in the form of rosettes and druses; the starch grains are abundant in the secondary xylem. The crystals as rosettes are circular and discoid with a central brownish spot and serrate margin; druses in the bark are up to 40 µm wide, whereas rosettes are 50 µm wide. The starch grains are circular, concentric, and seen in the thin-walled xylem parenchyma; the grains are simple and solitary and are about 10-20 µm in diameter (Figure 4).

Root of *Viburnum erubescens*

A lateral root of 3.5 mm was subject to investigation. The periderm is superficial in position and up to 250 µm thick; it consists of phellem; phellodermis is not distinct. The phellem represents about 20 layers of narrow, tangential oblong tabular cells, which are homogeneous in appearance and suberised.

The cortical zone is fairly wide (250 µm) and parenchymatous. The cortical cells are radially compressed and tangentially oblong. Along the inner boundary of the cortex is a band of isolated small groups of brachysclereids. These sclereids are isodiametric or tangentially oblong with thick lignified walls and wide lumen. The isometric sclereids are about 60 µm thick, whereas the elongated cells are about 140 × 40 µm (Figure 5).

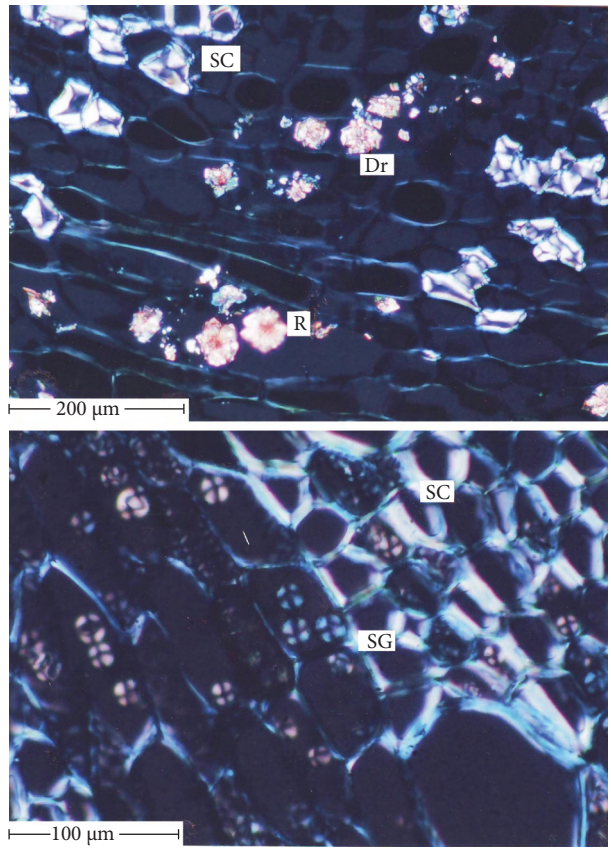

Figure 4. Crystal and starch grain distribution in the root of *Viburnum coriaceum*, as seen under polarised light microscope. Dr: druses, SC: sclereids, SG: starch grains, R: rosette.

The secondary phloem, 200 µm wide, is differentiated into a wider outer portion of collapsed sieve elements and dilated parenchymatous cells of phloem rays; the rays are in layers of 2 or more and show tangentially oblong and brick-shaped ray cells. The inner phloem includes intact sieve elements and narrow rays that are not dilated; the phloem elements lie in parallel radial rows.

The secondary xylem is dense and differentiated into vessels, fibres, parenchyma, and rays. The rays are radially rectangular, thick-walled, and lignified cells. The vessels are diffuse, predominantly solitary, angular or elliptical, thin-walled, and wide (60 µm in diameter). The xylem fibres are thick and lignified; their lumen is wide; the cells are polygonal in cut view and are 20-30 µm wide. The xylem parenchyma is scanty and sporadic; only 1 or 2 parenchyma cells occur adjacent to the vessels (Figure 6).

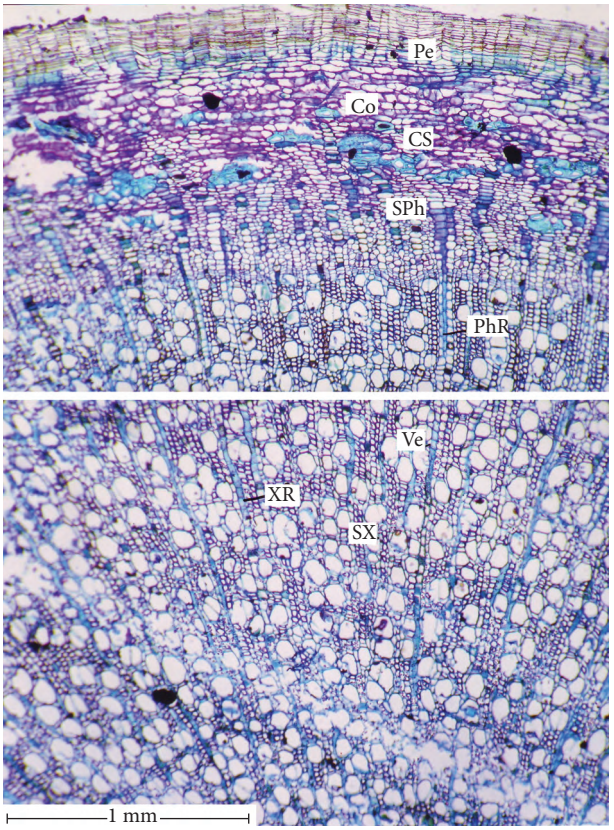

Figure 5. A transverse section of the *Viburnum erubescens* root. Co: cortex, CS: cortical sclereids, Pe: periderm, PhR: phloem ray, SPh: secondary phloem, SX: secondary xylem, XR: xylem ray.

The number of sclereids and starch grains per milligram of powdered root of *V. punctatum* was 125 ± 15 and 1300 ± 30, respectively. For *V. coriaceum*, these numbers were 420 ± 30 and 1200 ± 40, respectively. Our findings for *V. erubescens* indicated 340 ± 15 sclereids and 1700 ± 15 starch grains.

The typical characters of an angiosperm dicotyledonous root were observed with all 3 species. However, the individual histological zones showed wide variations in their dimension, shape, composition, and histochemical characteristics. The starch grains of *V. erubescens* were as large as 12 µm in diameter, while those found in *V. punctatum* and *V. coriaceum* reached 17 and 20 µm, respectively. The size ranges of sclereids in *V. punctatum*, *V. coriaceum*, and *V. erubescens* were proximal. Therefore, the number of sclereids per milligram of root powder was determined by the lycopodium spore method. The fibres were as long as 1 mm in *V. punctatum* and

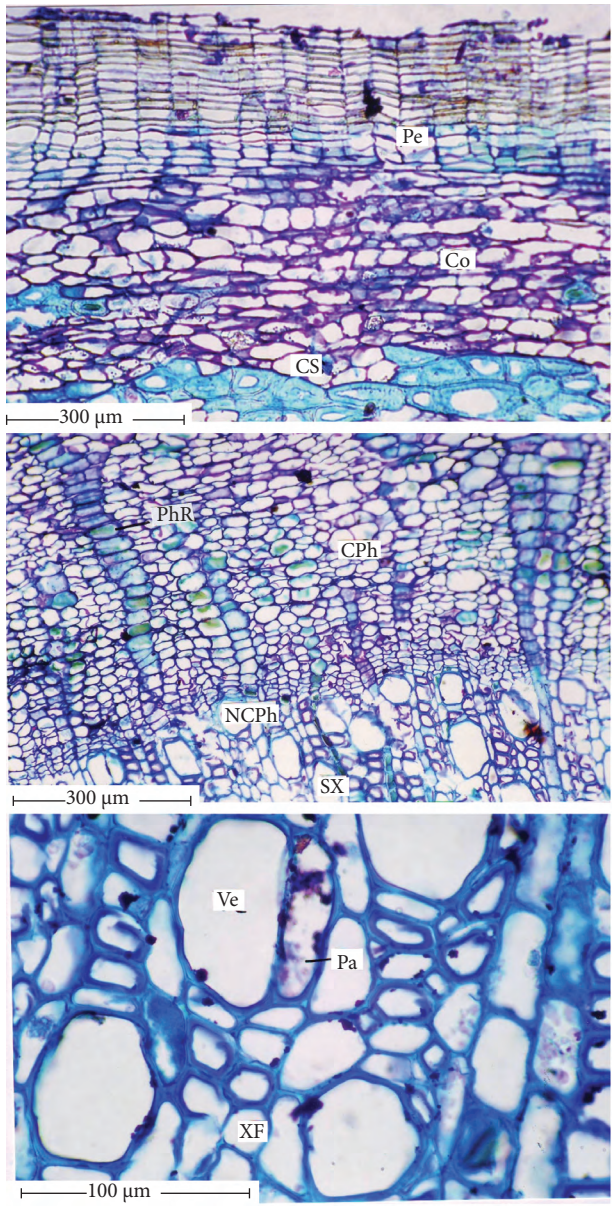

Figure 6. The periderm, cortex, secondary phloem, and xylem of *Viburnum erubescens* root. Co: cortex, CS: cortical sclereids, CPh: collapsed phloem, NCPh: noncollapsed phloem, Pa: parenchyma, PhR: phloem ray, SX: secondary xylem, Ve: vessel, XF: xylem fibre.

V. coriaceum, whereas in *V. erubescens*, the fibres were only found to be up to 700 µm. The dimension of xylem vessels and druses in *V. punctatum* and *V. coriaceum* was significantly larger than that of *V. erubescens* (Table 1).

A qualitative chemical examination performed on *V. punctatum*, *V. coriaceum*, and *V. erubescens* revealed

Table 1. The anatomical similarities, dissimilarities, and micrometrics of roots of the species *Viburnum punctatum* (*V. punc.*), *V. coriaceum* (*V. cori.*), and *V. erubescens* (*V. erub.*). Data indicate the mean of 40 individual characters of each component.

Special features	Species	Anatomy	Similarities & dissimilarities	Micrometrics
Sclereids between cortex and secondary phloem as thin bands	*V. punc.*	Periderm	250 µm thick, fissured, narrow phellem with tabular dark cells; phellodermis absent	Sclereids 30-65 µm; starch grains 3.5-17 µm; fibres 500 µm-1 mm, rarely 1.3 mm; druses 10-30 µm; vessels 60-130 µm)
		Cortex	Radially compressed with oblong parenchyma	
		Secondary phloem	Dilated rays, polygonal sieve elements, companion cells occur in the corner	
		Secondary xylem	Angular, thin-walled, diffuse, lignified, wide, and angular vessels 130 µm in diameter; starch grains in ray cells	
Secondary xylem with vessels, lignified fibres that are thick-walled with a thin lumen	*V. cori.*	Periderm	200 µm wide, dark and tanniniferous, phellodermis suberised	Sclereids 25-55 µm; starch grains 7-20 µm; fibres 650 µm-1 mm; druses 40 µm; rosettes 50 µm; vessels up to 100 µm
		Cortex	Inner narrow zone of cortex with scattered sclerenchyma cells	
		Secondary phloem	Narrow rays with tannin-filled cells, polygonal, thin-walled	
		Secondary xylem	Radially oblong cells; vessels angular, thin-walled, solitary, wide, diffuse, and lignified	
Collapsed phloem, elongated sclereids, thick-walled fibres with wide lumen and lignified; xylem parenchyma scanty.	*V. erub.*	Periderm	Broken, obliterated, phellem 250 µm thick with 20 layers of tabular cells with suberised walls	Sclereids 45-60 µm; starch grains 6-12 µm; fibres up to 700 µm; druses 12-25 µm; vessels 45-60 µm
		Cortex	Brachysclereids, isodiametric and tangentially oblong, 60 µm thick	
		Secondary phloem	Di- or triseriate rays, tangentially oblong cells and dilated rays 150 µm wide, brick shaped (ray cells)	
		Secondary xylem	Dense, solid cylinder; parenchyma associated with fibres and vessels; vessels solitary, angular, elliptical, diffuse, thin-walled, and up to 60 µm in diameter	

the presence of phytosterols and triterpenoids in petroleum ether and benzene fractions. In addition, triterpenoids were noted in chloroform fractions; glycosides and other phenolic compounds were found in hydroalcoholic and aqueous fractions, as was reducing sugar (Table 2). After exhausting the free sugars, the extracts were investigated for the chemical nature of the nonsugar (aglycone) part of their glycosides; the results were positive for phenolic compounds.

Table 2. Qualitative chemical tests for solvent root extracts of *Viburnum punctatum* (*V. punc.*), *V. coriaceum* (*V. cori.*), and *V. erubescens* (*V. erub.*). All results indicate the findings of 3 repetitions.

Solvent extracts	Species	Alkaloids	Phytosterols	Triterpenes	Free reducing sugar	Glycosides	Flavonoids	Other phenolics
Petroleum ether (60-80 °C)	*V. punc.*	–	++	++	–	–	–	–
	V. cori.	–	++	++	–	–	–	–
	V. erub.	–	+	+	–	–	–	–
Benzene	*V. punc.*	–	++	+++	–	–	–	–
	V. cori.	–	++	+++	–	–	–	–
	V. erub.	–	–	++	–	–	–	–
Chloroform	*V. punc.*	–	+	+++	–	–	–	–
	V. cori.	–	+	+	–	–	–	–
	V. erub.	–	+	++	–	–	–	+
75% aqueous ethanol	*V. punc.*	–	–	–	++	+	+++	+++
	V. cori.	–	–	–	++	+	+++	+++
	V. erub.	–	–	–	+	+	++	+++
Double distilled water	*V. punc.*	–	–	–	+++	+	++	++
	V. cori.	–	–	–	+++	+	++	++
	V. erub.	–	–	–	+++	+	+	++

Symbols as follows: –, negative test; +, positive test (low intensity); ++, positive test (moderate intensity); +++, positive test (high intensity).

Alongside the folkloric claims about *Viburnum*, due to the presence of triterpenoids, phenolic compounds, saponins, tannins, flavonoids, and anthocyanins, the plant root may have antiinflammatory, antioxidant, antidiabetic, cytotoxic, antiulcer, antispasmodic, and antimicrobial properties.

Conclusion

The current study can be useful in offering valuable basic information about how to differentiate the roots of the 3 species on a micromorphological basis. In addition, we hope to draw attention to the species so that further scientific studies can be performed to isolate the plants' biologically valuable phytoconstituents and utilise them for the treatment of illnesses.

Acknowledgements

The authors of this paper are thankful to Prof. R.L. Khosa, Prof. A.K. Wahi, and the late Dr. Y. Mohan, Reader in Pharmacognosy, IT-BHU, Varanasi, Uttar Pradesh, India, without whom the present work on the *Viburnum* species would have remained a dream for us.

References

Altun ML, Saltan Çitoğlu G, Sever Yılmaz B & Özbek H (2009). Antinociceptive and anti-inflammatory activities of *Viburnum opulus*. *Pharm Biol* 47: 653-658.

Angiosperm Phylogeny Group (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Bot J Linn Soc* 161: 105-121.

Babu K, Gokul Shankar S & Rai S (2010). Comparative pharmacognostic studies on the barks of four *Ficus* species. *Turk J Bot* 34: 215-224.

CSIR (2003). *The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products – Raw Material Series*, Vol. 10, pp. 437-446. New Delhi: Council of Scientific and Industrial Research.

Easu K (1964). *Plant Anatomy*. New York: John Wiley and Sons.

Fukuyama Y, Kubo M, Minami H, Yuasa H, Matsuo A, Fujii T, Morisaki M & Harada K (2005). Rearranged vibsane-type diterpenes from *Viburnum awabuki* and photochemical reaction of vibsanin-B. *Chem Pharm Bull* 53: 72-80.

Gamble JS (1935). *Flora of the Presidency of Madras*, Vol. I. Calcutta, India: Botanical Survey of India.

Güvenç A & Duman H (2010). Morphological and anatomical studies of annual taxa of *Sideritis* L. (Lamiaceae), with notes on chorology in Turkey. *Turk J Bot* 34: 83-104.

Hoerhammer L, Wagner H & Reinhardt H (1965). Isolation of flavonoids from the barks of *Viburnum prunifolium* Dent. *Apothekerzer* 105: 1371.

Johansen DA (1940). *Plant Microtechnique*. New York: McGraw-Hill Book Co.

Khosa RL, Wahi AK, Mohan Y & Ray AB (1979). Isolation of Bergenin from roots of *Viburnum nervosum* Hook. *Indian J Pharm* 41: 120.

Lala PK (1981). *Practical Pharmacognosy*, Vol. 1, pp. 136-153. India: Lina Guha Publication.

Mutlu B (2010). New morphological characters for some *Erysimum* (Brassicaceae) species. *Turk J Bot* 34: 115-121.

Nadkarni KM (2002). *Indian Materia Medica*, Vol. 1, pp. 1271-1272. Bombay, India: Popular Prakashan.

Prabhu K, PK Karar, K Ponnudurai & S Hemalatha (2009). Pharmacognostic and preliminary phytochemical investigations on the leaves of *Viburnum punctatum* Buch.-Ham. ex D.Don. *J Pharm Sci Res* 1: 43-50.

Sass JE (1940). *Elements of Botanical Microtechnique*. New York: McGraw-Hill Book Co.

Scott FI (1950). Polyploidy among plant species extraneous in Indiana. *Butler University Botanical Studies* 9: 177-185.

Tomassini L, Brkic D, Foddai S & Nicoletti M (1997). Iridoid glucosides from *Viburnum rhytidophyllum*. *Phytochemistry* 44: 751-753.

Tomassini L, Gao J, Foddai S, Serafini M, Ventrone A & Nicoleti (2006). Iridoid glycosides from *Viburnum chinshanense*. *Nat Prod Res* 20: 697-700.

Wallis TE (2005). *Text Book of Pharmacognosy*, pp. 559-618. New Delhi, India: CBS Publishers and Distributors.