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Histo-chemical analysis of the leaves, stem and roots of three *Viburnum* Linn. species

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ABSTRACT

Histochemical analysis is one of the valuable standardization processes of quality control of crude drugs to locate the presence of ergastic cell contents in the histological zones of the plant. In the current study, the leaves, stems and the roots of three Viburnum Linn. species were collected, authentificated and preserved fresh in Formalin-Aceto-Alcohol solution. A primary organic analysis was carried out using their extracts to identify the presence of diverse categories of phyto-constituents followed by subjecting hand sections of plant organs to histo-chemical analysis. The presence of some ergastic cell contents such as lignin, starch grains, saponins, druses, phenolic compounds, mucilage and suberin were micro-photographed and the results were tabulated. This article will be surely a referential tool for further scientific studies of their phyto-chemical and phyto-constituents based biological aspects.

Key words: *Viburnum*, Xylem, Lignin, Druses, Starch grains.

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, triterpenes and phytosterols; phenolic compounds and their derivatives 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 [3, 4]. In the late 1960s and early 1980s, the 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 on 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* [9]; iridoid glycosides from *Viburnum tinos;* antinociceptive and anti-inflammatory activities of *Viburnum lanata* [10], and *Viburnum opulus* [11], and an iridoid glucoside from *Viburnum rhytidophyllum* [12].

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 revealed that the leaves, stem bark and root barks of mature plants had been reliably in usage to the non-pregnant uterus, the GIT, microbial and inflammation related ailments, and are also in application as an ideal healing aid as well as one of the best home remedies. In view of the above, the current study centres at the finding of chemical nature of phyto-constituents and their location on the leaves, stems and roots of three *Viburnum* Linn. species, which may supplement some useful information regarding the species.

MATERIALS AND METHODS

The Leaves, stem and roots of *V.punctatum*, *V.coriaceum* and *V.erubescens* were collected from Nilgiri hills, Tamilnadu, India and authentificated by Dr.V.Chelladurai, Ex. Professor, (Botany), Medicinal plant survey for Siddha, Government of India, as *Viburnum punctatum* Buch.-Ham.ex D.Don (VP), *Viburnum coriaceum* Blume (VC) and *Viburnum erubescens* Wall.ex DC (VE). Herbarium of the specimens (labelled V181, VC131 and VE131 for VP, VC and VE respectively) was submitted to the museum of the department of Pharmacognosy, Nandini Nagar Mahavidyalaya College of Pharmacy.

Histo-chemical Studies

A proper care was taken to select healthy plant organs for the current study. The fresh form of the organs was separately placed in FAA (formalin-acetic acid-70% ethyl alcohol) in a ratio of 1:1:18. Twenty four hour later, they were subjected to histo-chemical analysis. A preliminary phyto-chemical analysis was conducted on these species using suitable chemical reagents just prior to histo-chemical analysis.

Hand sections of the leaf, stem and roots were mounted in suitable chemical reagents to determine the presence of various chemical substances and their zone of distribution. They were treated with the following reagents to study the histochemical reactions: 50% glycerin as temporary mountant; 2% phloroglucinol in 90% ethanol and Conc. HCl (1:1) for lignin; 5% alcoholic ferric chloride for phenolic compounds; 2% Iodine solution for starch grains; 0.08% Ruthenium red in 10% lead acetate for mucilage.; Sudan red-III for cuticle; Dragendorff's reagent for alkaloids and Sulphuric acid for saponins/ other steroidal compounds, if any.

Photomicrography

Photomicrographs were taken to better describe the histological features during microscopic examination, wherever necessary, at different magnifications using compound microscope and Nikon Labphot 2 unit. For normal observations, a bright field was used while for the study of calcium oxalate crystals, starch grains and lignified cells, polarized light was employed. Since these structures have bi-refringent properties under polarized light, they appear bright against dark background. Magnifications of the figures were indicated by scale-bars. The anatomical features were described taking botanical terminology in to account[13-16].

RESULT

A primary organic analysis conducted on the non-polar (petroleum ether and chloroform) and moderately polar to polar solvent (alcohol and water) extracts revealed the presence of phyto-sterols and triterpenes in the former and saponins, phenolic compounds, carbohydrates and glycosides (phenolic glycosides which was confirmed after a hydrolytic test) in the later with the leaves, stems and roots of VP, VC and VE. However, in case of VC triterpenes and phytosterol test in leaf and root were not observed to be well pronounced (Table 1).

Presence of lignin, tannins and starch grains were prevalent in case of all the organs of all the three species subjected for study. However, presence of saponins, druses and suberin were found rarely in the histological zones of Transverse Section (T.S.) of all the species. Alkaloids were totally absent in all the three species.

Table 1. Primary organic analysis on the leaves, stem and roots of three *Viburnum* Linn species

Plant species	Plant organ	Solvent extracts*	Phyto-constituents positive
V.punctatum	Leaf	Non-polar	Sterols, Triterpenes
		Moderately polar & polar	Saponins, phenolic compounds, reducing sugar, flavonoids, Glycosides
	Stem	Non-polar	Sterols, Triterpenes
		Moderately polar & polar	Saponins, phenolic compounds, reducing sugar, flavonoids, Glycosides
	Root	Non-polar	Sterols, Triterpenes
		Polar	Saponins, phenolic compounds, reducing sugar, flavonoids, Glycosides
V.coriaceum	Leaf	Non-polar	Sterols
		Polar	Saponins, phenolic compounds, reducing sugar, flavonoids, Glycosides
	Stem	Non-polar	Sterols, Triterpenes
		Polar	Saponins, phenolic compounds, reducing sugar, flavonoids, Glycosides
	Root	Non-polar	Triterpenes
		Polar	Saponins, phenolic compounds, reducing sugar, flavonoids, Glycosides
V.erubescens	Leaf	Non-polar	Sterols, Triterpenes
		Polar	Saponins, phenolic compounds, reducing sugar, flavonoids, Glycosides
	Stem	Non-polar	Sterols, Triterpenes
		Moderately polar & polar	Saponins, phenolic compounds, reducing sugar, flavonoids, Glycosides
	Root	Non-polar	Sterols
		Moderately polar & polar	Saponins, phenolic compounds, reducing sugar, flavonoids, Glycosides

** - Polar: Pet.ether, chloroform; Non-polar: Ethanol (75% v/v), water*

DISCUSSION

T.S of leaves, stems and roots represented the presence of ergastic cell contents such as: tannins, mucilage, lignin, starch grains, calcium oxalate crystals (druses), saponins and suberin in their concerned histological zones or regions (Table 2).

Table 2. Histo-chemical analysis on the leaves, stem and roots of three *Viburnum* Linn. species

Plant species	Plant organ	Histological region	Phyto-constituents present
V.punctatum	Leaf	Phloem region Mesophyll Vascular region	Tannins Mucilage Lingin
	Stem	Xylem ray cells Phloem parenchyma Xylem region Pith cells	Tannins Starch grains Lignin Saponins
	Root	Xylem ray cells Xylem parenchyma Xylem region	Tannins Starch grains Lignin & saponins
V.coriaceum	Leaf	Mesophyll Ground tissue Xylem parenchyma	Tannins Druses Saponins & lignin
	Stem	Xylem parenchyma Phloem parenchyma Xylem ray cells Cortex Pith cells	Lignin Starch grains Tannins Druses Saponins
	Root	Xylem vessel Xylem parenchyma Xylem region Xylem ray cells	Lignin Starch grains Saponins Tannins
V.erubescens	Leaf	Phloem region Trichomes Vascular bundle Phloem parenchyma Ground tissue	Tannins Lignin Lignin Saponins Starch grains (rarely)
	Stem	Phloem ray cells Phloem region Xylem ray cells Periderm Phloem region Xylem	Tannins Starch grains Starch grains Suberin Saponin Lignin
	Root	Phloem ray cells Xylem Cortical parenchyma Phloem region Periderm	Tanins Lignin Starch grains Sapoins Suberin

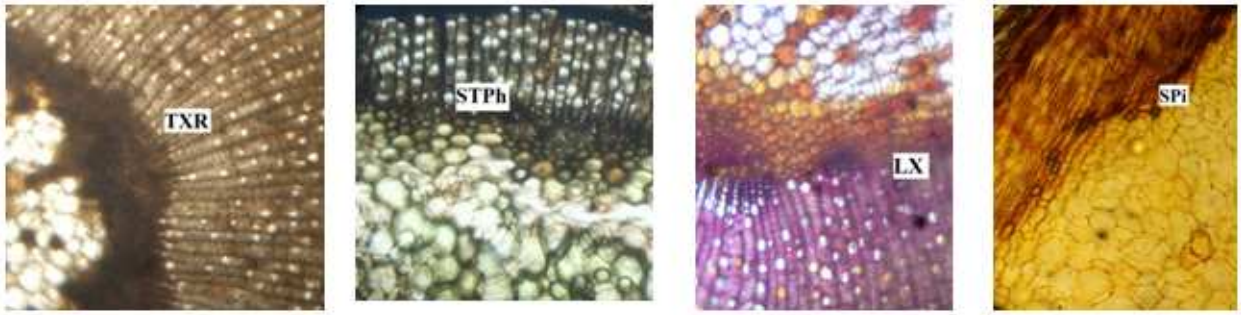
Histo-chemical analysis on *V.punctatum*

Presence of tannins in phloem region; mucilage in mesophyllic region; lignin in vascular bundle was found in leaves (Figure 1). In case of the stem xylem, ray cells, phloem parenchyma, xylem region and pith cells represented the distribution of tannins, starch grains, lignin and saponins respectively (Figure 2). Tannins, starch grains, lignin and saponins were observed with xylem ray cells, xylem parenchyma, xylem fibres and vessels and entire region of xylem as far as the root of the species was concerned (Figure 3).

Figure 1. Histochemical analysis of T.S. of leaves of *V.punctatum*

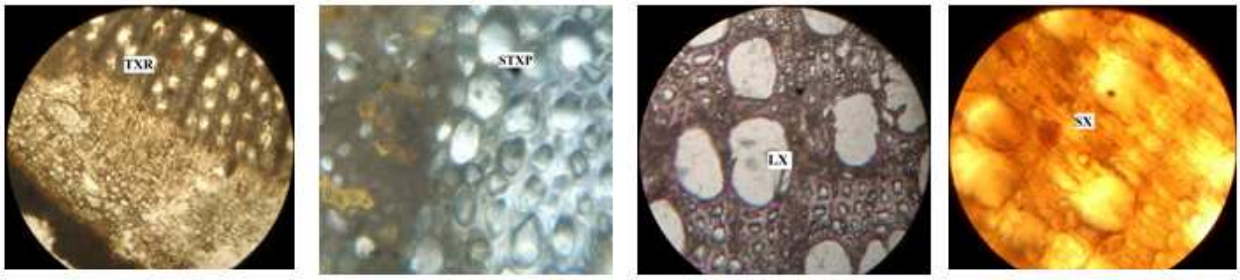
TPh – Tannins in Phloem; M– Mucilage Mesophyll; LV – Lignin in Vessels; Magnification: 10x×45x

Figure 2. Histochemical analysis of T.S. of Stems of *V.punctatum*



TXR – Tannins in Xylem Ray; STPh – Starch grains in Phloem parenchyma; LX – Lignin in Xylem; SPi – Saponins in Pith cells; Magnification: 10x×45x

Figure 3. Histochemical analysis of T.S. of Roots of *V.punctatum*



TXR – Tannins in Xylem Ray cells; STXP – Starch grains in Xylem Parenchyma; LX – Lignin in Xylem; SX – Saponins in Xylem region; Magnification: 10x×45x

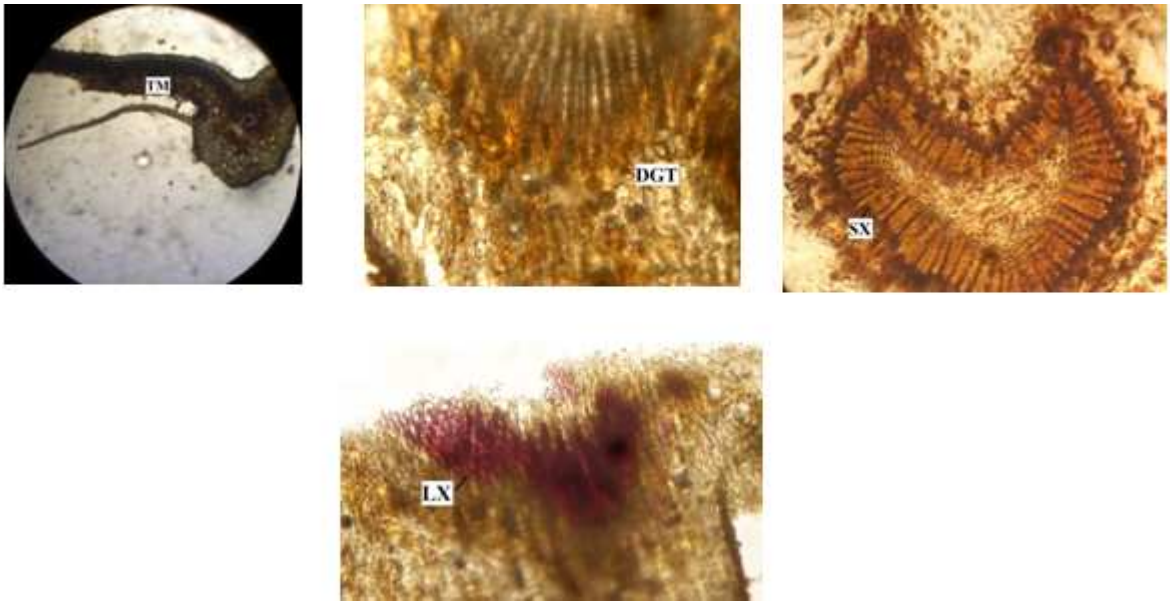
Histo-chemical analysis on *V.coriaceum*

The leaves represented the presence of tannins, druses, saponins and lignin at mesophyllic region, groun tissue and xylem parenchyma respectively (Figure 4).

The T.S. of stem showed the presence of lignin, starch grains, tannins, druses and saponin in xylem parenchyma, phloem parenchyma, xylem ray cells, cortex and in pith cells respectively (Figure 5).

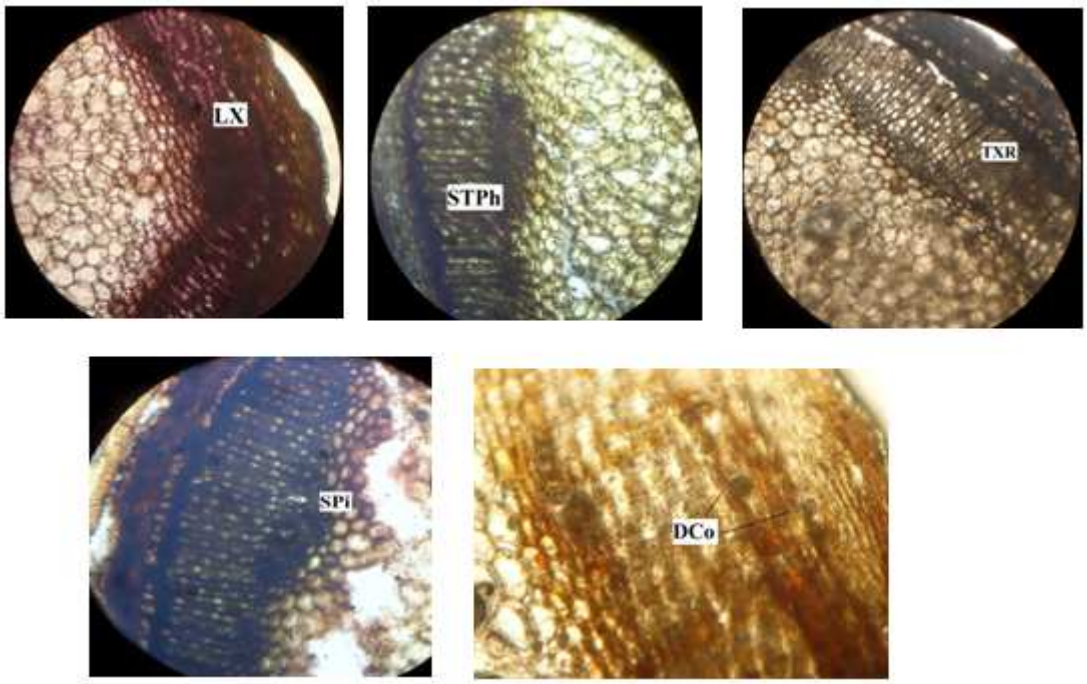
In case of root, all ergastic cell content such as lignin, starch grains, saponins and tannins were confined within xylem region of the organ (Figure 6).

Figure 4. Histochemical analysis of T.S. of leaves of *V.coriaceum*



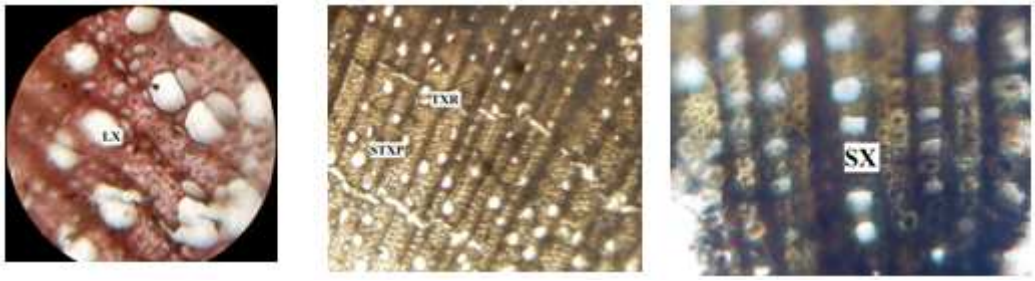
TM – Tannins in Mesophyll (10x×10x); DGT – Druses in Ground Tissue; SX – Saponins in Xylem parenchyma; LX – Lignins in Xylem parenchyma; Magnification: 10x×45x

Figure 5. Histochemical analysis of T.S. of stems of *V.coriaceum*



LX – Lignins in Xylem parenchyma; STPh – Starch in Phloem; TXR – Tannins in Xylem Ray cels; SPi – Saponins in Pith cells; DCo – Druses in Cortex; Magnification: 10x×45x

Figure 6. Histochemical analysis of T.S. of roots of *V.coriaceum*



LX – Lignin in Xylem vessels; STXP – Starch grains in Xylem parenchyma; SX – Saponins in Xylem; TXR – Tannins in Xylem Ray cells; Magnification: 10x×45x

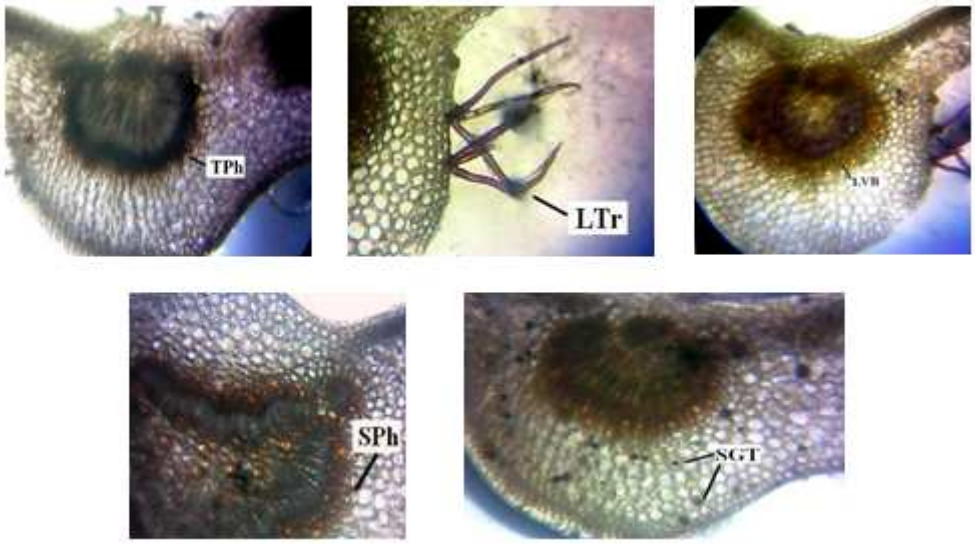
Histo-chemical analysis on *V.erubescens*

In case of leaves, tannins and saponins were represented in phloem region; the both trichomes and vascular region showed the presence of lignin but rarely seen were the grains in the ground tissue (Figure 7).

The stems showed the presence of tannins and starch grains and lignin in phloem region; saponins in xylem ray cells and suberin in periderm cells (Figure 8).

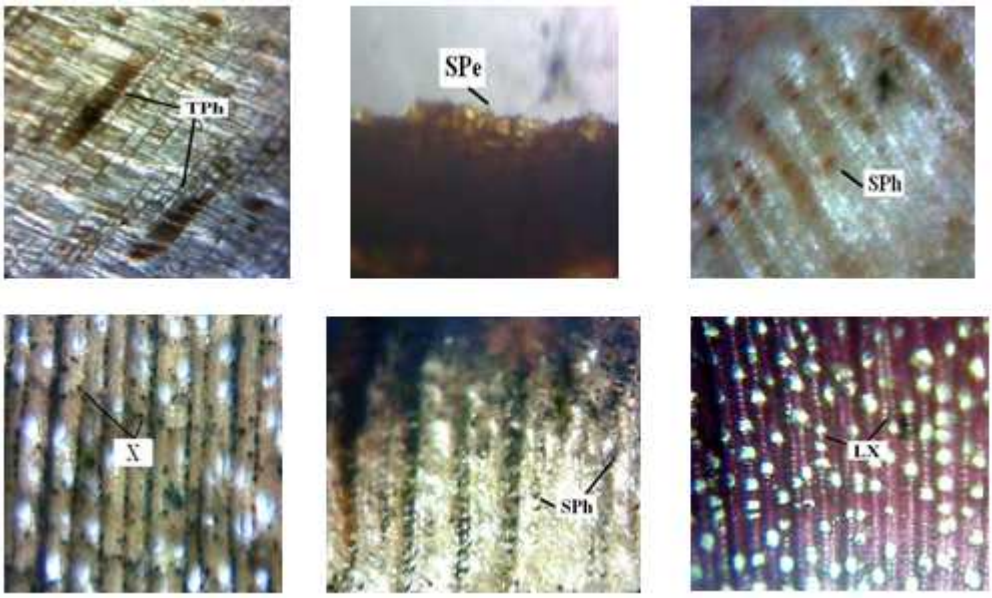
The T.S. of root, upon treatment with suitable reagents, showed the presence of tannins, lignin, grains, saponins and suberin with phloem ray cells, xylem region, cortical parenchyma, phloem region and with periderm (Figure 9).

Figure 7. Histochemical analysis of T.S. of leaves of *V.erubescens*



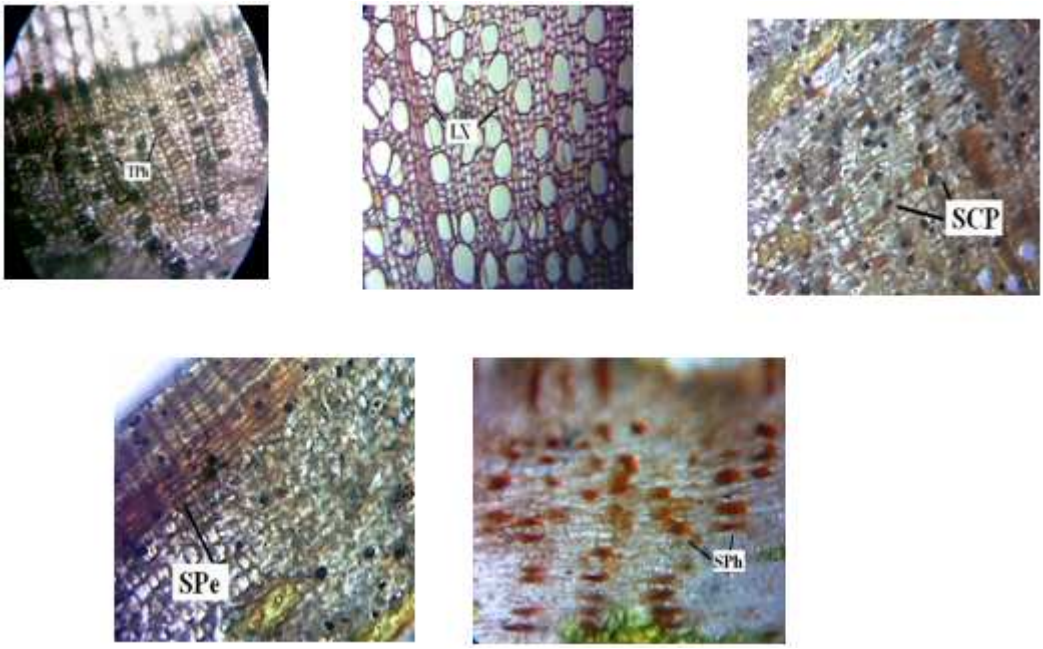
TPh – Tanniferous content in Phloem region; LTr – Lignified Trichome; LVB – Lignified Vascular Bundle; SPh – Saponins in Phloem parenchyma; SGT – Starch grains in Ground Tissue; Magnification: 10x×10x

Figure 8. Histochemical analysis of T.S. of Stems of *V.erubescens*



TPh – Tannins in Phloem ray cells; SPh – Starch grains in Phloem region; X – Xylem region with starch grains; SPe – Suberised Periderm; SPh – Saponins in Phloem region; LX – Lignified Xylem; Magnification: 10x×45x

Figure 9. Histochemical analysis of T.S. of Roots of *V.erubescens*



TPh – Tannins in Phloem ray cells; LX – Lignified Xylem; SCP – Starch grains in Cortical Parenchyma; SPh – Saponins in Phloem Region; SPe – Suberised Periderm; Magnification: 10x×45x

CONCLUSION

Upon primary organic analysis, presence of some phyto-constituents in all the three species was divulged. The presence of phyto-constituents was located using suitable chemical reagents employing histo-chemical investigation on the T.S. of the organs. The current study can be useful to carry out some advanced phyto-chemical studies in order to unfold the nature of different classes of phyto-constituents and suitable method of their isolation to scrutinize their biological potential then.

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