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A comparative study on proximate analysis conducted on three *Viburnum* Linn. species

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ABSTRACT

The leaves, stem and the roots of Viburnum punctatum, Viburnum coriaceum and Viburnum erubescens were collected from Nilgiri Hills and Coimbatore, Tamilnadu and taxonomically authenticated. Herbarium of the species was submitted to the museum of the place of research studies. The samples were shade dried for a week. The materials were dried in the sun and then the shade for about 15 days. About 500g of the leaves, stems and roots of each V.punctatum, V.coriaceum and V.erubescens were powdered separately using a mechanical grinder in to a moderately coarse powder. By using the samples some of the proximate qualities were determined such as: Extractive values, Total ash and sulphated ash values, Acid insoluble ash value, Water soluble Ash value, Crude fibre content, Loss on Drying and including Fluorescence analysis (UV – 366 nm) and the results were tabulated to note down how far these species differ in their qualities each other.

Keywords: *Viburnum*; Extractive values; sulphated ash values; Crude fibre content; Loss on Drying.

INTRODUCTION

Viburnum Linn. Species contain sterols, sesqui and triterpenoids, phenolic compounds and their glycosides as their common chemical constituents [1-5]. A few species among 17 in India, namely: *Viburnum punctatum* Buch.-Ham.ex D.Don, *Viburnum coriaceum* Blume and *Viburnum erubescens* Wall.ex DC; have been reported in literature to posses uterine sedative, anti-asthmatic, astringent, anti-inflammatory and anti-microbial activities [6,7]. A verbal enquiry to the local community and plant vendors of Ooty and Coimbatore, Tamilnadu, also supported that

the above listed pharmacological activities were traditional and were promising with roots, stem barks and leaves of these species [8].

Among the above listed chemical constituents, phenolic compounds, terpenoids and their glycosides may be the cause for biological responses. In addition to this, a qualitative chemical screening and spectrophotometric analysis of extracts were performed to reveal that the stem part of these three species contains an appreciable amount and a wide range of phenolic compounds [9,10].

Radical scavenging activities of phenolic compounds play a key role in ameliorating healing and even preventing several ailments in living being. It is a well known fact that the plants synthesis phenolic compounds for diverse purposes, which may be of protective, functional or as metabolic end products in nature [11]. But, human exploit them as valuable medicines/ phyto-pharmaceuticals by focusing on their anti-oxidant potential with or without modification.

A quest for a search of herbal phenolic compounds is still a renewed interest in the science of natural products as a source of valuable medicines. The herbal phenolic molecules such as flavonoids, anthocyanins, bioflavones and other phenolic glycosides have, already, been explored and known for their applications against several human ailments-cardiovascular disorders, chronic inflammation and GIT related troubles [12-14].

MATERIALS AND METHODS

Collection of Specimens

The studies were undertaken on some three species of the genus *Viburnum* namely: *Viburnum punctaum* Buch.-Ham.ex D.Don. *Viburnum coriaceum* Blume. and *Viburnum erubescens* Wall.ex DC. The choices of plant parts were the leaves, stems and the roots of these species. The plant specimens for the study were collected from Nilgiri Hills, Tamil Nadu, India, and authenticated by Dr V Chelladurai, former Professor of Botany, Medicinal Plant Survey for Siddha, Government of India, as *Viburnum punctatum* Buch.-Ham.ex D.Don., *Viburnum coriaceum* Blume. and *Viburnum erubescens* Wall.ex DC. The voucher specimens were labelled (V181), (VC131) and (VE131), and deposited in the department of pharmacognosy at Nandini Nagar Mahavidyalaya College of Pharmacy, Uttar Pradesh. A care was taken to select healthy plants and the plant parts for the study were collected fresh and dried for a couple of weeks to be involved for further studies.

Proximate Analysis [15-19]

Extractive values

About 5g of air dried drug was coarsely powdered and mixed with 100 ml of 90% ethanol in a closed flask. The flask was frequently shaken during the first 6 hours and allowed to stand for 18 h. Then, the mixture was rapidly filtered to minimize the loss of ethanol and 25 ml of the filterate was evaporated to dryness in a tarred flat-bottomed shallow dish. The residue was dried at 105º C for minutes and then weighed. The procedure was performed twice more (with each 25 ml) from the filterate.

Ash values

Total ash and sulphated ash values

A silica crucible was heated for about 30 min to red hot and cooled in a desiccator to note down its weight. About 3g of the powdered specimen were weighed and then dried at 100 – 105º C for 1 h and ignited to constant weight in a muffle furnace at 600 - 625º C, until a carbon free ash

formed. The crucible was allowed to cool in a desiccator after each ignition and care was taken to avoid catching fire. The weight of the carbon free ash was determined. The procedure was repeated to obtain a standard deviation to ensure consistency and then tabulated. The same procedure was carried out adding dilute sulphuric acid to determine the yield of sulphated ash.

Acid insoluble ash

About 1g of the total ash (from total ash) was boiled with 25 ml of 2M hydrochloric acid for 5min. The acid insoluble ash was separated by filteration on an ash less filter paper in a Gooch crucible. The content on the ash less filter paper was washed with hot water and ignited and then weighed to obtain the percentage of ash with reference to the air dried drugs.

Water soluble Ash

About 1g of the total ash was boiled with 25 ml of water for 5 min then filterated to retain the insoluble matter on an ash less filter paper. The content was ignited for 15 min at a temperature not exceeding 450º C then weighed. The difference between the amount of ash subjected and weight of insoluble ash was accounted as the water soluble ash value.

Fluorescence analysis (UV – 366 nm)

Various extracts of specimens and powdered drugs were exposed to ultraviolet radiation (366 nm, ultraviolet cabinet-MAC, MSW-508, Long UV, India) to visualize fluorescence or a change in colour, if any.

Crude fibre content (Dutch's Method)

About 2 g of the powdered drug specimen was used and 0.128 M and 0.313 M solutions of sulphuric acid, and sodium hydroxide respectively for boiling (the ungainly strengths derive from the 1.25% solutions originally used in the assay). The dried residue finally obtained was weighed (A) and then washed (B), the weight difference (A-B) represents the crude fibre for calculation on a percent dry weight basis.

Loss on Drying

About 10 g of the each specimens under study were accurately weighed and transferred to a tarred china dish which was known for its weight and kept in a hot air oven at 100 – 105 º C for an. Then, the sample was weighed along with china dish to deduct the actual weight of tarred china dish. The weight of the powder was noted to calculate the percentage loss on drying with reference to air dried specimen.

RESULTS AND DISCUSSION

The results obtained were recorded and tabulated to discuss in detail under the next section and the values are presented as mean±S.D.

Proximate analysis on *V.punctatum* (leaves, stem and root)

Alcoholic and aqueous extractives

In case of *V.punctatum*, alcoholic extractive value of stem was higher than that of aqueous extractive (5.44±0.106%, aqueous being 2.12±0.108%). The leaves assumed the highest percentage extractives when compared to that of the stem and root **(Table 1).**

Table 1. Proximate analysis on *V.punctatum* (leaves, stem and root)

Extractive Values	Specimen	Colour of the Residue	Extractives % w/w
Alcoholic extractives	Leaves	Brownish green	5.53±0.065
	Stems	Light brown	5.44±0.106
	Roots	Brown	1.69±0.112
Aqueous extractives	Leaves	Green	6.45±0.093
	Stems	Light brown	1.67±0.108
	Roots	Light brown	2.07±0.141

Values are presented as mean±S.D

Ash figures

The leaves of *V.punctatum* showed about 7.55±0.046%w/w of total ash when compared to the percentage ash value of stem and root, which lied between 3 – 4% w/w. However, sulphated ash values of leaf and stem showed proximity i.e., 8.1±0.171 and 8.38±0.089 respectively (**Table 2)** . However, it is highly controversial and unusual that the water soluble ash value assumed more than 80% of the total ash value (for an unknown reason).

Crude fibre content and Loss on drying

Crude fibre content of *V.punctatum* stem and root posed a closeness, between 10 – 11% w/w, while loss on drying for leaves, stem ad root, 3 – 4% w/w.

Table 2. Proximate analysis on *V.punctatum* (leaves, stem and roots)

S.No.	Expérimental studies	Percentage w/w		
		Leaves	Stem	Root
1.	Total ash value	3.8±0.072	7.55±0.046	3.05±0.184
2.	Water soluble ash	1.1±0.124	6.64±0.068	2.60±0.126
3.	Acid insoluble ash	3.3±0.143	1.55±0.087	1.85±0.105
4.	Sulphated ash	8.1±0.171	8.38±0.089	4.80±0.214
5.	Crude fibre content	-	11.34±0.060	10.95±0.311
6.	Loss on drying	4.19±0.207	3.82±0.168	3.27±0.241

Values are presented as mean±S.D

Fluorescence analysis

Under UV-365 nm, 75% v/v ethanolic extracts of the leaves, stem and roots were turned yellowish orange, reddish orange and pale yellow respectively. The methanolic (75%) extract of the stem turned reddish orange. Ethylacetate extract of stem turned yellowish brown. Excepting the above, most of the other solvent extracts of this species showed a whitish opalescence or a pale pink colouration.

Proximate analysis *V.coriaceum* (leaves, stem and root)

Aloholic and aqueous extractives

The aqueous extract of leaf showed a maximum yield ever, about 8.21±0.164%. But, the alcoholic stem and root extracts were higher than that of the aqueous stem and root extracts 3.30±0.127 and 1.90±0.104 against the aqueous being, 1.82±0.089 and 1.64±0.151% w/w with reference to air dried samples (**Table 3**).

Similar to the previous species (*V.PUNCTATUM*), the stem of *V.CORIACEUM* yielded 7.81±0.077% of total ash, of which 5.83±0.074% were soluble in water minimizing percentage fraction of acid insoluble ash. In case of the leaves, 4.88±0.221% of total ash, about 3.40±0.213% was acid insoluble (**Table 4**)

The fibre content of stem was higher than that of the root (11.24±.061 against 9.95±0.042). Loss on drying of the leaves was comparable to rest of the parts (leaf, 4.06±0.210; stem, 3.02±0.053; and root, 3.08±0.261).

Table 3.Proximate analysis on *V.coriaceum* (leaves, stem and root)

Extractive Values	Specimen	Colour of the Residue	Extractives % w/w
Alcoholic extractives	Leaves	Green	7.47±0.210
	Stems	Brown	3.30±0.127
	Roots	Light brown	1.90±0.104
Aqueous extractives	Leaves	Green	8.21±0.164
	Stems	Dark brown	1.82±0.089
	Roots	Light brown	1.64±0.151

Values are presented as mean±S.D

Table 4. Proximate analysis on *V.coriaceum* (leaves, stem and root)

S.No.	Ash values	Percentage w/w		
		Leaves	Stem	Root
1.	Total ash value	4.88±0.221	7.81±0.077	3.33±0.255
2.	Water soluble	2.10±0.108	5.83±0.074	2.80±0.190
3.	Acid insoluble	3.40±0.213	1.53±0.054	1.45±0.086
4.	Sulphated ash	9.20±0.151	11.98±0.053	5.93±0.311
5.	Crude fibre content	–	11.24±0.061	9.95±0.042
6.	Loss on drying	4.06±0.210	3.02±0.053	3.80±0.261

Values are presented as mean±S.D

Fluorescence analysis

The leaf, stem and root extracts of petroleum ether, benzene, chloroform, ethanol (75%) and water, when exposed to UV-365 nm, resulted an yellowish orange colouration to the alcoholic fraction; and orange to reddish orange to the ethereal and chloroform fraction. Except the above most of the extract under UV-365 nm turned whitish (opalescent by nature).

Proximate Analysis *V.erubescens* (leaves, stem and root)

Alcoholic and aqueous extractives

The leaves of VE was found to show the highest percentage of extractives, when alcohol (ethanol 92%) was the choice of solvent. But, the percentage aqueous extractives were approximately one half of the value of the percentage extractive of the former. However, the stem and root both the solvents yielded a comparable result, i.e., 7 – 8% w/w for alcohol, while for aqueous being 8 – 8.1% w/w with reference to air dried samples (**Table 5)**.

Table 5.Proximate analysis on *V.erubescens* (leaves, stem and root)

Extractive Values	Specimen	Colour of the Residue	Extractives % w/w
Alcoholic extractives	Leaves	Brownish green	15.23±0.143
	Stems	Brown	7.76±0.089
	Roots	Light brown	7.50±0.074
Aqueous extractives	Leaves	Green	7.62±0.159
	Stems	Brown	8.00±0.671
	Roots	Brown	8.10±0.452

Values are presented as mean±S.D

Table 6.Proximate analysis on *V.erubescens* (leaves, stem and root)

S. No.	Experimental studies	Percentage w/w		
		Leaves	**Stem**	**Root**
1.	Total ash value	15.38±0.324	4.03±0.074	3.00±0.052
2.	Water soluble	7.46±0.143	5.02±0.089	7.61±0.068
3.	Acid insoluble	4.06±0.114	3.90±0.106	4.28±0.072
4.	Sulphated ash	21.10±0.025	10.08±0.134	11.20±0.142
5.	Crude fibre content	–	4.50±0.063	6.00±0.091
6	Loss on drying	12.25±0.175	15.00±0.156	15.12±0.122

Values are presented as mean±S.D

Ash values

The percentage total ash of stem and root was to the extent of one half of the leaves. The leaves and roots were found to containing higher water soluble ash and so the acid insoluble ash value also showed a hike. In these species the percentage ash of *V.erubescens* was observed to be lower than that of the rest (**Table 6).**

The fibre content of root being 6.00±0.091% w/w, the stem showed 4.50±0.063% w/w, slightly lower than the former. The percentage loss on drying of this species was 3 fold quantities of the previous two species.

Fluorescence analysis

The chloroform fraction of leaf, stem and root appeared violet and dark reddish; the aqueous fraction expressed a pale orange to fluorescent orange colouration except the orange colouration except the above, other fractions gave a transition to yellow to golden yellow colouration.

CONCLUSION

The leaves, stems and roots of each *V.punctatum*, *V.coriaceum* and *V.erubescens* were powdered separately using a mechanical grinder in to a moderately coarse powder. By using the samples some of the proximate qualities were determined such as: Extractive values, Total ash and sulphated ash values, Acid insoluble ash value, Water soluble Ash value, Crude fibre content, Loss on Drying and including Fluorescence analysis (UV – 366 nm) and the results were tabulated to note down how far these species differ in their qualities each other. Determination of proximate qualities of a species will give a finger print of whether the species is adulterated or not.

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