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Total Phenolic Content and *in vitro* Antioxidant Potentials of Ethanolic Stem Extracts of Three *Viburnum* Linn. Species

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The stems of *Viburnum punctatum*, *Viburnum coriaceum* and *Viburnum erubescens* were collected from Nilgiri Hills and Coimbatore, Tamilnadu and taxonomically authenticated. Herbaria of the species were submitted to the museum of the place of research studies. The samples were shade dried for a week. About 500 g of powdered samples were extracted with petroleum ether (60-80 ºC), benzene and 75 % (v/v) ethanol successively in a soxhlator one by one and followed by determination of percentage extractives. The extracts were qualitatively tested for different chemical constituents present. The ethanolic extracts were selected for further investigations, such as total phenolic content and *in vitro* antioxidant potential. It was revealed that all the three species exhibited a significant antioxidant potential against the reference compounds subjected and antioxidant potential among three species was ascending in series: *V. coriaceum* > *V. punctatum* > *V. erubescens*.

Key Words: *Viburnum*, Phenolic compounds, Antioxidant, *In vitro*, Nitric oxide, DPPH.

INTRODUCTION

Radical scavenging activities of phenolic compounds play a key role in ameliorating and healing and even preventing several ailments in living being. It is a well known fact that plants synthesize phenolic compounds for diverse purposes, which may be of protective, functional or metabolic end products in nature. But, human exploit them as valuable medicines both in pure and crude forms, with a focus on their antioxidant potential, so as to eradicate several discomforts elated or caused by unbalanced oxidation in their physiological systems. The total phenolic content is conventionally assessed by spectrophotometry and *in vitro* antioxidant potential is usually screened accounting radical scavenging activity, nitric oxide scavenging activity and reducing power as parameters against a suitable antioxidant as reference.

A quest for a search of herbal phenolic compounds is still a renewed interest in the science of natural products as source of medicines and an easy tool for modeling, inventing and making of bioactive synthetic molecules.

The herbal phenolic molecules such as flavonoids, anthocyanins, biflavones and other phenolic glycosides have, already, been recognized for their wide vital roles against many human ailments.

The genus *Viburnum* Linn.[1,2], belonging to the family Caprifoliaceae, contains about 200 species throughout the world and some 17 of them have been reported to grow in India. The stem of those species elaborates many bioactive molecules such as glycosides, sesquiterpenes[3,4], triterpenoids[5], phytosterols and diverse classes of phenolics such as phenolic acids and flavonoids; proanthocyanidins and anthocyanidins and their glycosides. These components have been, in several species, proven to possess antiulcer, antiinflammatory, astringent and uterine relaxant activities. The current study is aimed at a comparative study on some three *Viburnum* Linn. species namely, *Viburnum punctatum* Buch.-Ham.ex D. Don, *Viburnum coriaceum* Blume. and *Viburnum erubescens* Wall.ex DC, for their phenolic content and antioxidant potential. The pharmacognostical investigations on these species have been recorded in literatures[6-9]. However, a study on their phenolics, which are their major constituents, has not yet been dealt with.

EXPERIMENTAL

The research specimens for the present study was collected from Nilgiri Hills at an altitude of 1500-1800 ft and taxonomically authenticated by Dr. Chelladurai, (Ex. Professor) Medicinal plants supply for siddha, Govt. of India, Tamilnadu

as *Viburnum punctatum* Buch.-Ham.ex D.Don, *Viburnum coriaceum* Blume. and *Viburnum erubescens* Wall.ex DC. The specimens were dried in the sun and shade for a week, and separately grounded in a mechanical grinder to obtain moderately coarse powder. About 500 g of stem powder of each species were soxhlated for 15-18 h successively with petroleum ether (60-80 ºC), benzene and 75 % v/v aqueous ethanol followed by determination of percentage extractives[10]. The ethanolic extracts of *V. punctatum*, *V. coriaceum* and *V. erubescens* were labelled as VPEE, VCEE and VEEE, respectively and the extracts were screened for their chemical fractions with aid of suitable reagents[11].

Determination of total phenolics: Aliquot of 100 µg of each sample were pipetted out in series of test tubes and volume was made up to 3 mL with distilled water. Folin-Ciocalteau reagent (0.5 mL) was added to each tube and incubated for 3 min at room temperature. Sodium carbonate (20 %; 2 mL) solution was added, mixed thoroughly and the tubes were incubated for 1 min in boiling water bath. Absorbance was measured at 650 nm using a UV-visible double beam spectrophotometer (Systronic) against a reagent blank. Standard curve using different concentrations of standard phenolic compound (catechol) was prepared. From the standard curve (Fig. 1), concentration of phenols in the test samples was determined and expressed as µg of catechol equivalent[12].

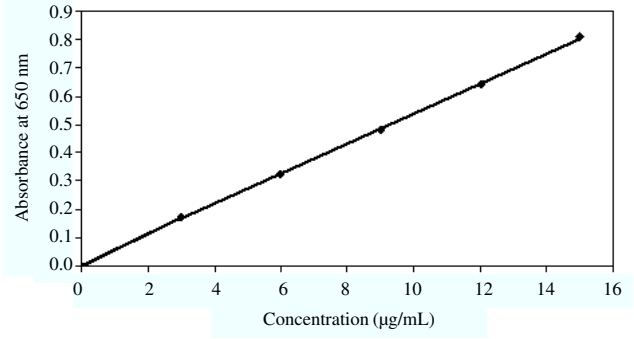


Fig. 1. Standard curve of different concentrations of standard phenolic (catechol) against ethanolic extracts (catechol calibration curve)

In vitro antioxidant evaluation

Diphenyl 1,2-picryl hydrazyl (DPPH) radical scavenging activity: DPPH scavenging activity was measured by spectrophotometric method. 0.1 mM solution of DPPH was prepared in ethanol. To this solution, 3 mL of the test solution was added at different concentration (25-800 µg/mL). Equal amount of distilled water was added to the control. The mixture was shaken well and incubated at room temperature for 0.5 h. The absorbance was read at 517 nm using a spectrophotometer[13].

Nitric oxide (NO) scavenging: Nitric oxide scavenging activity was measured by using a spectrophotometer. Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with different concentrations of the ethanolic extracts (25-800 µg/mL) dissolved in distilled water and incubated at 25 ºC for 0.5 h. A control without test compound but with equivalent amount of distilled water was taken. After 0.5 h, 1.5 mL of the incubation solution was removed and diluted with 1.5 mL of Griess reagent (1 % w/v sulphanilamide, 2 % v/v phosphoric acid and 0.1 % w/v naphthyl ethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine was measured at 546 nm[14].

Hydroxyl radical scavenging activity: The hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system (Fenton reaction). The hydroxyl radicals attack deoxyribose that eventually results in TBARS formation. The reaction mixture contained deoxyribose (2.8 mM), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (1 mM), ascorbic acid (0.1 mM), KH_2PO_4-KOH (20 mM, pH 7.4) and various concentrations (25-800 µg/mL) of the extract in a final volume of 1 mL. The reaction mixture was incubated for 1 h at 37 ºC. Deoxyribose degradation was measured as TBARS and percentage inhibition was calculated[15].

The percentage reduction was calculated by comparison with the control using the formula:

$$\text{Inhibition (\%)} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

Determination of reducing power: 10 mg of ethanolic extracts in 1 mL of distilled water was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1 % w/v). The mixture was incubated at 50 ºC for 20 min. A portion (2.5 mL) of trichloro acetic acid (10 % w/v) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride, (0.5 mL, 0.1 % w/v). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture signifies increased reducing power[16].

Statistical analysis: The statistical analysis of studies were carried out using analysis of variance (ANOVA) followed by Dunnett's 't' test and linear regression analysis to calculate IC_{50} values, $p < 0.001$ were considered significant.

RESULTS AND DISCUSSION

Petroleum ether, benzene and ethanol yielded 0.84 ± 0.038, 2.49 ± 0.071 and 1.16 ± 0.142; 0.64 ± 0.051, 0.73 ± 0.083 and 5.71 ± 0.078 and 2.32 ± 0.106, 3.12 ± 0.211 and 2.99 ± 0.078 % w/w extractives with reference to dried specimens, respectively.

Ethereal and benzene fractions gave positive tests for sterol and triterpenoids; while alcoholic fraction showed phenolics and their glycosides and also free reducing sugar. To assess the total phenolic content, the ethanolic fraction of *V. punctatum*, *V. coriaceum* and *V. erubescens* was selected (Table-1).

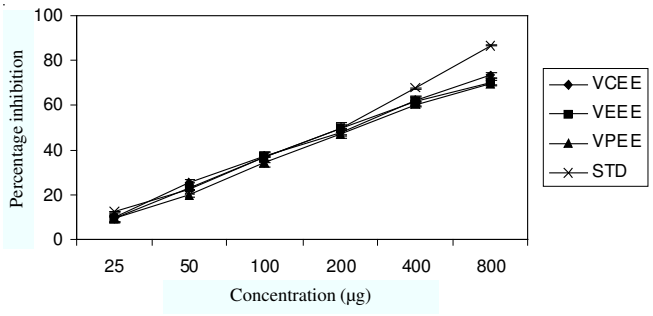
TABLE-1
ESTIMATION OF PHENOLICS FROM AQUEOUS ETHANOLIC EXTRACTS OF *V. punctatum*, *V. coriaceum* AND *V. erubescens*

Test sample	Absorbance OD	mg of Catechol equivalent phenolics mg/g of sample
V. coriaceum	0.72 ± 0.41	13.00 ± 0.52 mg/g
V. erubescens	0.52 ± 0.22	10.50 ± 0.42 mg/g
V. punctatum	0.66 ± 0.13	12.50 ± 0.50 mg/g

Values are mean ± standard deviation (n = 3).

The total phenolic content of VCEE was observed to be as high 13.00 ± 0.52 mg/g of ethanolic residue. Nevertheless the total phenolics with VPEE was proximal to VCEE being 12.50 ± 0.50; however, VEEE showed about 10.50 ± 0.42 mg/g.

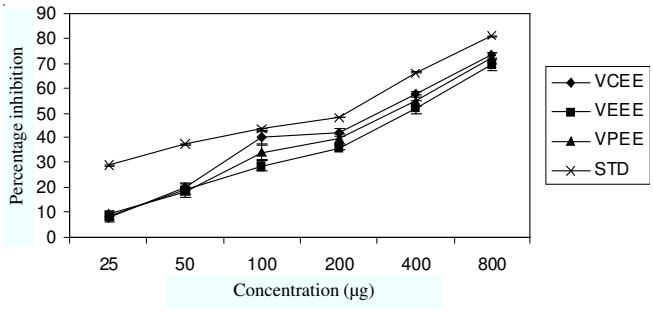
In vitro antioxidant screening of extracts also showed significant results. The scavenging of nitric oxide by the VCEE, VEEE and VPEE was concentration dependent and the IC_{50} values were found to be 318.68 ± 2.02, 343.41 ± 2.34 and 331.43 ± 2.15 µg/mL, respectively. The percentage scavenging of nitric oxide was as high as 86.62 % at the concentration of 800 µg/mL. Results were comparable to the standard, vitamin-E having IC_{50} value of 238.4 ± 1.33 µg/mL (Fig. 2).



DPPH: Diphenyl,2-picryl hydrazyl; VCEE: ethanolic stem extract of *V. coriaceum*; VEEE: ethanolic stem extract of *V. erubescens*; VPEE: ethanolic stem extract of *V. punctatum*; STD: standard

Fig. 2. DPPH radical scavenging of ethanolic extracts and vitamin C

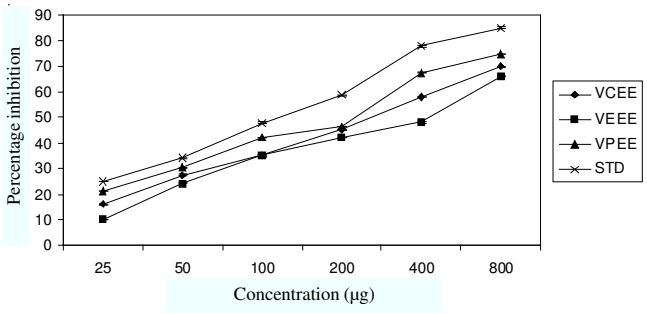
The reduction of DPPH radicals can be observed by the decrease in the absorbance at 517 nm. The scavenging capacity of the VCEE, VEEE and VPEE was found to be 73.43, 70.43 and 69.43 %, respectively while corresponding IC_{50} being 294.94 ± 1.749, 331.94 ± 1.560 and 303.94 ± 1.294 µg/mL. Result of the test drug was comparable with standard (vitamin-C) having IC_{50} value of 210.97 ± 0.822 µg/mL (Fig. 3).



VCEE: Ethanolic stem extract of *V. coriaceum*; VEEE: ethanolic stem extract of *V. erubescens*; VPEE: ethanolic stem extract of *V. punctatum*; STD: standard

Fig. 3. Determination of nitric oxide scavenging activity of ethanolic extracts and vitamin-E

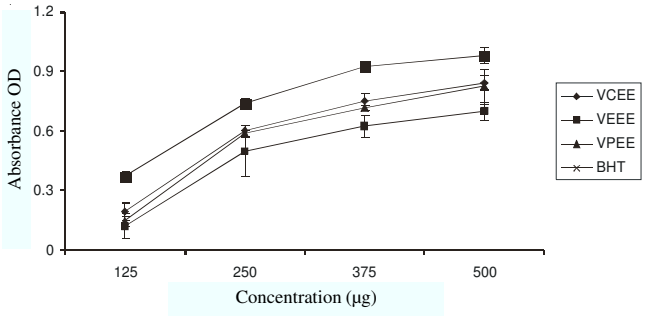
The VCEE, VEEE and VPEE (at all tested doses 100, 200 and 400 µg/mL) significantly scavenged the hydroxyl radicals generated by the EDTA/H_2O_2 system, when compared with control. The percentage scavenging of OH radicals by VCEE, VEEE and VPEE increased in a dose dependent manner. Results were comparable with standard whose IC_{50} value 165.55 ± 0.162 µg/mL (Fig. 4).



VCEE: Ethanolic stem extract of *V. coriaceum*; VEEE: ethanolic stem extract of *V. erubescens*; VPEE: ethanolic stem extract of *V. punctatum*; STD: standard

Fig. 4. Hydroxy radical scavenging of ethanolic extracts and vitamin E

The reducing power increased with an increase in concentration of VCEE, VEEE and VPEE. All the tested concentrations of VCEE, VEEE and VPEE showed a significant ($p < 0.001$) activity when compared with the control. Results were comparable with the standard (BHT) ($p < 0.001$) (Fig. 5).



VCEE: Ethanolic stem extract of *V. coriaceum*; VEEE: ethanolic stem extract of *V. erubescens*; VPEE: ethanolic stem extract of *V. punctatum*; BHT: butylated hydroxytoluene; OD: optical density

Fig. 5. Reducing power of ethanolic extracts of three *Viburnum* species and butylated hydroxytoluene

Conclusion

It can be concluded that the phenolic content in VCEE was higher than that of VEEE and VPEE. However, nitric oxide, DPPH scavenging activities of VEEE were noted to be parallel to VCEE and VPEE. The scavenging of hydroxyl radicals and reducing power of all three species were mainly, concentration dependent against the reference compounds involved.

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