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ANTIOXIDANT POTENTIALS OF METHANOLIC ROOT FRACTIONS OF THREE *VIBURNUM* LINN. SPECIES

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ABSTRACT

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. About 500 g of powdered samples were extracted with petroleum ether (40 - 60º C), chloroform and 80 % v/v methanol successively in a soxhlator one by one and followed by determination of percentage extractives. The extracts were qualitatively tested for different chemical constituents. The methanolic extracts were selected for further investigations such as total phenolic content and *in vitro* anti-oxidant potential. It was revealed that all three species exhibited an appreciable anti-oxidant potential against reference compound subjected, and anti-oxidant potential among three species was ascending in series *V. Erubescens*>*V. Coriaceum*>*V. punctatum.*

KEYWORDS: *Viburnum*; Phenolic compounds; Anti-oxidant; *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 synthesis 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 anti-oxidant potential, so as to eradicate several discomforts elated or caused by oxidation in their physiological systems. The total phenolic content is conventionally assessed by spectrophotometry, and *in vitro* anti-oxidant potential is usually screened accounting radical scavenging activity, nitric oxide scavenging activity and reducing power as parameters against suitable anti-oxidant as reference standard.

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 stems 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, anti-inflammatory, astringent and uterine relaxant. The current study is aimed at a comparative study on roots of 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 anti-oxidant potential. The pharmacognostical investigations on these species have been recorded in literatures.[6,7,8and9] But, study on their phenolics, being their major constituents, has not yet been dealt with.

MATERIALS AND METHODS

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 then separately ground in a mechanical grinder to obtain moderately coarse powder. About 500 g of root powder of each species were soxhlated for 15 – 18 h successively with petroleum ether (60 - 80º C), benzene and 80 % v/v aqueous methanol followed by determination of percentage extractives.[10] The methanolic extracts of *V. punctatum, V. coriaceum* and *V. erubescens* were named as VPMRE, VCMRE and VEMRE 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 with aid of a UV-visible double beam spectrophotometer against a reagent blank.[12]

In vitro antioxidant evaluation

Hydroxyl Radical scavenging activity[13]

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_2PO4-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 hr at 37^0 C. Deoxyribose degradation was measured as TBARS and percentage inhibition was calculated.

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

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

Determination of Reducing Power[14]

10 mg of methanolic extract of *Helicteres isora* in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K_3 Fe (CN) $_6$] (2.5 ml, 1% w/v). The mixture was incubated at 50 ºC for 20 min. A portion (2.5 ml) of trichloroacetic 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.

Linoleic Acid auto-oxidation[15]

The total antioxidant activity was determined by linoleic acid auto-oxidation. A mixture of 4 mg of test drug in 4 ml absolute ethanol, 8 ml of 0.05 M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in a vial with a screw cap and then placed in an oven at 40^0 C in the dark. To 1 ml of this solution, 2 ml of 20% w/v trichloroacetic acid and 2 ml of thiobarbituric acid aqueous solution were added. The mixture was placed in a boiling water bath for 10 min. After cooling, it was centrifuged at 3000 rpm for 20 min and the absorbance of the supernatant was measured at 532 nm for every 24 hrs for a period of 6 days. Antioxidant activity was based on the absorbance on the final day.

Evaluation of Hydrogen peroxide (H_2O_2) scavenging activity[16]

H_2O_2 radical scavenging assay The ability of the extract to scavenge hydrogen peroxide was determined according to the method given by Ruch et al., (1989). A solution of hydrogen peroxide (2mmol/l) was prepared in phosphate buffer (pH 7.4). Extracts (1–10µg/ml) were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide and compared with ascorbic acid, the reference compound.

$$\text{H2O2 activity (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where, Abs (control): Absorbance of the control and

Abs (test): Absorbance of the extracts/standard.

RESULTS AND DISCUSSION

The total phenolic content of VCMRE was observed to be as high 13.5 ± 0.44 mg/g of methanolic residue. Nevertheless the total phenolics with VPMRE was proximal to VCMRE being 11.5±0.50 mg/g; however, VEMRE showed about 11.8±0.50 mg/g (Table1).

In vitro antioxidant screening of extracts also showed significant results. The VCMRE, VEMRE and VPMRE significantly scavenged the Hydroxyl radicals generated by the EDTA/H_2O_2 system, when compared with control. The percentage scavenging of OH radicals by VCMRE, VEMRE and VPMRE increased in a dose dependent manner. Results were comparable with standard having IC_{50} value 192.22 ± 1.08 µg/ml (Table 2).

The reducing power increased with increasing concentration of VCMRE, VEMRE and VPMRE. All the tested concentrations of VCMRE, VEMRE and VPMRE showed significant ($p<0.001$) activity than control. Results were comparable with the standard (BHT) ($p<0.001$) (Table 3).

Methanolic extracts showed dose dependent activity in prevention of linoleic acid also comparable with standard (Table 4). Hydrogen peroxide radical scavenging is a very important function of extracts to prevent cytotoxicity and activity may be attributed to the phenolics groups present in the extracts (Table 5).

Table: 1. Estimation of total phenolic content from methanolic extracts of *V.punctatum*, *V.coriaceum* and *V. erubescens*

S.No.	Test Sample	Absorbance OD	mg of catechol equivalent phenolics mg/g of sample
1.	VCMRE	0.63±0.55	13.5 ± 0.44 mg/g
2.	VEMRE	0.55±0.40	11.8±0.50 mg/g
3.	VPMRE	059±0.55	11.5±0.50 mg/g

Values are mean ± SD, n=3

Table: 2. Hydroxyl radical scavenging activity of MERF

Concentration (µg)	25	50	100	200	400	800	IC_{50} Value (µg)
VCMRF	9.45± 0.38	14.52± 0.41	33.00± 0.94	43.22± 0.32	51.77± 0.64	71.85± 0.11	341.88± 3.41
VEMRF	10.44± 0.83	17.51± 0.22	36.85± 0.31	49.61± 0.70	57.84± 0.48	74.17± 0.36	311.45± 1.68
VPMRF	8.97± 0.32	12.34± 0.18	31.82± 0.44	40.89± 0.62	49.56± 0.37	68.21± 0.19	367.94±4.01
Vitamin-E	14.11± 0.20	29.33 ± 0.39	41.02 ± 0.24	58.07 ± 0.67	73.24± 0.18	89.28± 0.61	192.22 ± 1.08

Values are mean ± SEM of 6 replicates

Table: 3. Reducing power of MERF

Concentration (µg)	125	250	375	500
VCMRF	0.229± 0.007**	0.578± 0.004**	0.684± 0.024**	0.834± 0.001*
VEMRF	0.366± 0.005**	0.622± 0.014**	0.755± 0.003*	0.898± 0.007*
VPMRF	0.270± 0.001**	0.492± 0.004**	0.701± 0.005*	0.841± 0.006*
BHT	0.541± 0.005*	0.710± 0.002*	0.851± 0.0012*	0.977± 0.0015*

BHT: Butylated hydroxytoluene ; Values are Mean ± SEM of 6 parallel measurements.

** P<0.01, * P< 0.001 when compared against control. Spectrophotometric deduction of the $Fe^{3+} – Fe^{2+}$ transformation.

Table: 4. Anti-oxidant activity of MERF and reference drug in linoleic acid emulsion.

Concentration	Mean absorbance ± SEM					
	1st day	2nd day	3rd day	4th day	5th day	6th day
Control	0.039 ± 0.001	0.097 ± 0.002	0.13 ± 0.003	0.241 ± 0.005	0.524 ± 0.0025	0.741 ± 0.0028
VCMRF	0.054 ± 0.006	0.081 ± 0.0013@	0.089 ± 0.0007*	0.148 ± 0.0008*	0.216 ± 0.0016*	0.242 ± 0.0009*
VEMRF	0.049± 0.0051	0.074± 0.006	0.083± 0.001	0.104± 0.007	0.184± 0.002	0.204± 0.006
VPMRF	0.044± 0.0065	0.069± 0.008	0.079± 0.006	0.091± 0.003	0.126± 0.007	0.216± 0.005
α-tocopherol (0.02%)	0.061 ± 0.0062	0.079 ± 0.001*	0.088 ± 0.005*	0.147 ± 0.001*	0.181 ± 0.002*	0.3.1± 0.0012*

Values are mean ± SEM of 6 replicates

Comparison with control

@- P< 0.01, *- P< 0.001

Table: 5. H_2O_2 scavenging activity of MERF

Concentration (µg/ml)	25	50	100	200	400	800	IC_{50} Value (µg /ml)
VCMRF	17.90 ± 0.12	28.88 ± 0.051	40.59 ± 0.211	57.93 ± 0.389	70.57 ± 0.160	85.12 ± 0.117	292.15 ± 1.094
VEMRF	10.15 ± 0.210	20.29 ± 0.154	33.82 ± 0.323	48.59 ± 0.327	62.64 ± 0.323	74.00 ± 0.346	304.14 ± 1.330
VPMRF	10.32 ± 0307	20.29± 0.257	38.26 ± 0.360	48.79 ± 0.361	62.39 ± 0.348	80.25 ± 0.265	373.89 ± 1.068
Vitamin-E	9.59 ± 0.289	26.26 ± 0.324	34.34 ± 0.462	47.08 ± 1.287	65.22 ± 0.309	79.45 ± 0.224	254.80 ± 1.377

Values are mean ± SEM of 6 replicates

CONCLUSIONS

From the results of experimentation, it can be concluded that the phenolic content in VCMRE was higher than that of VEMRE and VPMRE. The scavenging of Hydrogen peroxide and hydroxyl radical, and reducing power, and prevention of linoleic acid of all three species were mainly, concentration dependent against reference compounds involved.

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