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IN VITRO ANTIOXIDANT ACTIVITY AND FREE RADICAL SCAVENGING POTENTIAL OF ROOTS OF *VITEX QUINATA*

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ABSTRACT

The selected plant extracts and known antioxidant ascorbic acid at various concentrations produced dose dependent inhibition of superoxide radicals, hydroxyl radicals, lipid peroxidation and DPPH radical activities. The IC_{50} values for superoxide radical with chloroform and methanolic extracts of *Vitex quinata* were found to be 208.17 µg, 269.32 µg. The IC_{50} values for hydroxyl radical with chloroform and methanolic extracts of *Vitex quinata* were found to be 265.32 µg, 312.56 µg. The IC_{50} values for inhibition of lipid peroxidation activity with chloroform and methanolic extracts of *Vitex quinata* were found to be 281.23 µg, 336.81 µg. The IC_{50} values for DPPH radical with chloroform and methanolic extracts of *Vitex quinata* were found to be 110.12 µg, 154.50 µg.

Key words: *Vitex quinata***,** Antioxidant activity, Ascorbic acid.

INTRODUCTION

In living systems, free radicals are generated as part of the body's normal metabolic process, and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, through xanthine oxidase activity, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics. In addition, chemical mobilization of fat stores under various conditions such as lactation, exercise, fever, infection and even fasting, can result in increased radical activity and damage. Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders. Oxygen free radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other

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membranes, and play a role in the long term complication of diabetes[1-4]. Antioxidants may be defined as radical scavengers, which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties[5-12]. *Vitex quinata* (Verbenaceae) trees 4-12 m tall, evergreen; bark brown. Branchlets pubescent and glandular, when young, glabrescent leaves 3-5-foliolate; petiole 2.5-6 cm; petiolules 0.5-2 cm; leaflets obovate-elliptic to obovate or oblong to elliptic, thickly papery, both surfaces shiny. It is found from Chintapalli, Anantagiri forest, Vizag to Nallamadala Forests in Andhra Pradesh. Exhaustive and up to date review of literature for antioxidant, their methods of screening and pharmacological review of the selected plant were conducted. Dried powdered roots of *Vitex quinata* were separately extracted in a Soxhlet apparatus for 6 h successively with chloroform and methanol. The concentrate is dried under vacuum in a rotary evaporator[13,14].

EXPERIMENTAL

Materials and methods

All the chemicals used (Analytical grade) were obtained from S.D. Fine Chemicals Pvt. Ltd., Mumbai, Sigma Chemical Company, U.S.A. and Loba Chemicals, Mumbai.

Plant material

The roots of *Vitex quinata* were collected from Ananthagiri Forest region, Visakhapatnam District, Andhra Pradesh, India in the months of March and May, 2008. These plant species were authenticated by Dr. M. Venkaiah, Taxonomist, Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India. The voucher specimens (VTR-14-03-2008) were deposited in the institutional museum, College of Pharmaceutical Sciences, Andhra University, Visakhapatnam. The collected plants were washed and air-dried under the shade, cut into small pieces, powdered by a mechanical grinder and passed through 40-mesh sieve and stored in a closed vessel for future use.

Preparation of extract

Shade dried root powder of *Vitex quinata*, was separately extracted in a Soxhlet apparatus for 6 hrs succesively with chloroform and methanol and concentrated to dryness under reduced pressure. Later, the quantity of root powder was taken for extraction. These extracts were used to test the free radical scavenging activity, superoxide radicals, hydroxyl radicals, lipid peroxidation, and DPPH radical activities.

In vitro antioxidant study

The methanolic and chloroform extracts of *Vitex quinata* roots were tested for their free radical scavenging property using different *in vitro* models. All experiments were performed thrice and the results were averaged.

Superoxide radical scavenging activity

Superoxide radical scavenging activity of the plant extract was measured according to the method of Mc Cord and Fridovich[15,20], which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium. All the solutions were prepared in phosphate buffer (pH 7.8). The optical density was measured at 560 nm. The percentage inhibition was calculated from formula[16].

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured according to the method of Elizabeth and Rao[17,20] by studying the competition between deoxyribose and test extract for hydroxyl radicals generated by Fenton's reaction. The damage imposed on deoxyribose due to the free radicals was determined colorimetrically by measuring the thiobarbituric acid reactive substances (TBARS) at 532 nm. Percentage of inhibition was calculated using the formula[16].

Lipid peroxidation inhibition activity

The inhibition of lipid peroxidation was performed as per the method described by Ohkawa et al.[18,20] Rat liver homogenate was used as the source of polyunsaturated fatty acids for determining the extent of lipid peroxidation. The absorbance was measured at 532 nm. Percentage of inhibition was calculated using the formula[16].

DPPH radical scavenging activity

DPPH radical scavenging activity was measured according to the method of Braca et al.[19,20]. An aliquot of 3 mL of 0.004% DPPH solution in ethanol and 0.1 mL of plant extract at various concentrations were mixed and incubated at 37^{o}C for 30 min. and absorbance of the test mixture was read at 517 nm.The percentage of inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the formula[16].

Calculation of percentage inhibition

The percentage inhibition of radical production by the test sample was calculated

using the formula:

$$\text{Inhibitory ratio} = \frac{(A_o - A_1) \times 100}{A_o}$$

Where, A_o is the absorbance of control and A_1 is the absorbance with addition of test sample.

Statistical analysis

Linear regression analysis was used to calculate IC_{50} values[21].

RESULTS AND DISCUSSION

Superoxides are produced from molecular oxygen due to oxidative enzymes[21] of body as well as via non-enzymatic reactions such as auto-oxidation by catecholamines[22]. In the present study, the total methanolic and chloroform extracts of *Vitex quinata* root was found to scavenge the superoxides generated by photoreduction of riboflavin. The chloroform extract and methanol extract of *Vitex quinata* root produced dose dependent inhibition of superoxide radicals. The IC_{50} values for superoxide radical with chloroform extract and methanol extract of *Vitex quinata* root were found to be 208.17 µg, 269.32 µg; with ascorbic acid were found to be 212.48 µg, respectively. The chloroform extract of *Vitex quinata* root was found to have better superoxide radical scavenging activity, when compared to methanol extract of of *Vitex quinata* root, (Table 1, Fig. 1).

Table 1: Percentage inhibition and IC_{50} values of superoxide radical scavenging activity *in vitro* by methanolic and chloroform extracts of *vitex quinata* root and ascorbic acid.

Extract	Quantity in micrograms (µg), Mean ± SEM					IC_{50} values
	25	50	100	200	400	
AA	20.38 ± 2.11	31.69 ± 1.14	45.44 ± 1.28	64.77 ± 2.31	76.94 ± 2.26	212.48
VQME	5.60 ± 0.80	19.04 ± 1.55	24.15 ± 0.80	31.53 ± 1.37	38.01 ± 0.79	269.32
VQCE	11.83 ± 0.49	26.95 ± 0.88	41.86 ± 1.14	51.76 ± 1.37	60.40 ± 1.20	208.17

AA- Ascorbic acid, VQME-Methanol extract of *V. quinata*,
VQCE- Chloroform extract of *V. quinata*

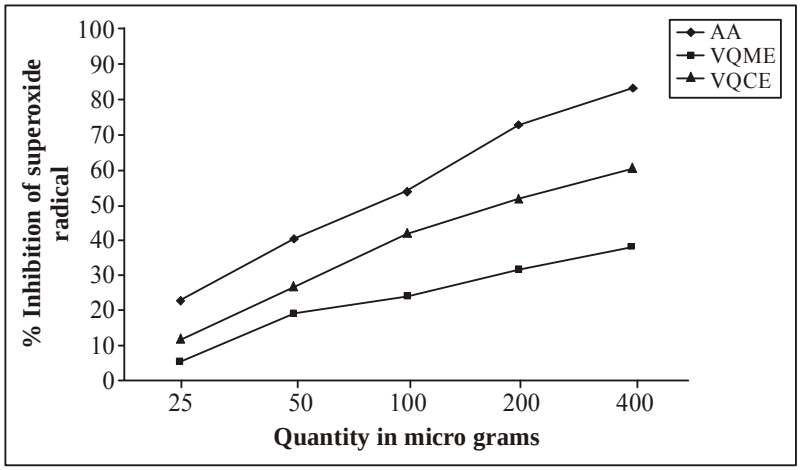

Fig. 1: *In vitro* concentration dependent percentage inhibition of superoxide radical *in vitro* by methanolic and chloroform extracts of *vitex quinata* root and ascorbic acid

Hydroxyl radical is an extremely reactive oxidising radical that will react with most biomolecules at diffusion controlled rates. It has extremely short half life but is capable of causing damage within a small radius of its site of production. A single hydroxyl radical can result in formation of many molecules of lipid hydroperoxides in the cell membrane, which may severely disrupt its function, and lead to cell death. In the present study, the methanolic and chloroform extracts of *Vitex quinata* roots were investigated in comparison with the known antioxidant ascorbic acid. The IC_{50} values for hydroxyl radical with chloroform and methanolic extracts of *Vitex quinata* were found to be 265.32 µg, 312.56 µg. The chloroform extract of *Vitex quinata* root was found to have better hydroxyl radical scavenging activity, when compared to methanol extract of *Vitex quinata* (Table 2, Fig. 2).

Table 2: Percentage inhibition and IC_{50} values of hydroxyl radical *in vitro* by methanolic and chloroform extracts of *Vitex quinata* and ascorbic acid

Extract	Quantity in micrograms (µg), Mean ± SEM					IC_{50} values
	25	50	100	200	400	
AA	20.38 ± 2.11	31.69 ± 1.14	45.44 ± 1.28	64.77 ± 2.31	76.94 ± 2.26	212.48
VQME	5.05 ± 0.49	14.18 ± 1.85	23.71 ± 1.21	32.04 ± 1.01	40.73 ± 0.98	312.56
VQCE	14.81 ± 0.97	20.28 ± 1.18	28.16 ± 1.21	39.39 ± 1.32	47.11 ± 1.46	265.32

AA – Ascorbic acid, VQME-Methanol extract of *V. quinata*,
VQCE – Chloroform extract of *V. quinata*

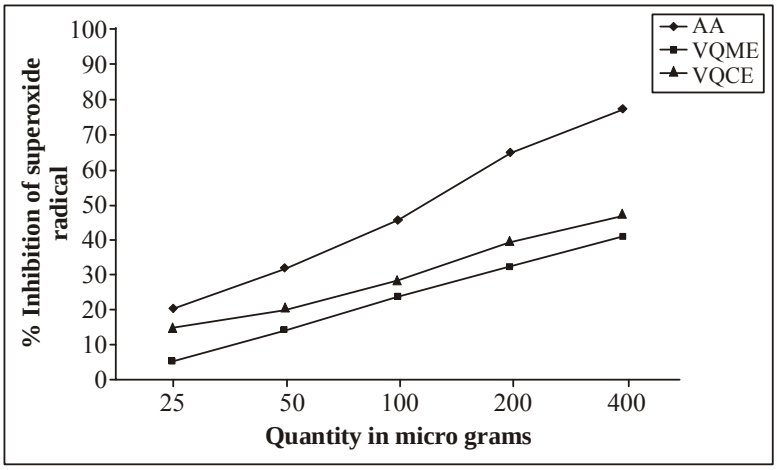

Fig. 2: *In vitro* concentration dependent percentage inhibition of hydroxyl radical *in* vitro by methanolic and chloroform extracts of *Vitex quinata* and ascorbic acid

Free radicals induce lipid peroxidation in polyunsaturated lipid rich areas like brain and liver[23]. Lipid peroxidation, which involves a series of free radical mediated chain reaction processes, is also associated with several types of biological damage. Therefore, much attention has been focused on the use of natural antioxidants to inhibit lipid peroxidation and to protect from damage due to free radicals. In the present study, the methanolic and chloroform extracts of *Vitex quinata* were investigated in comparison with the known antioxidant ascorbic acid. The IC_{50} values for inhibition of lipid peroxidation activity with chloroform and methanolic extracts of *Vitex quinata* were found to be 281.23 µg, 336.81 µg. The chloroform extract of Vitex quinata root was found to have higher lipid peroxidation inhibition than the methanol extract of *V. quinata* (Table 3, Fig. 3).

Table 3: Percentage inhibition and IC50 values of lipid peroxidation radical *in vitro* by methanolic and chloroform extracts of *Vitex quinata* and Ascorbic acid

Extract	Quantity in micrograms (µg), Mean ± SEM					IC_{50} values
	25	50	100	200	400	
AA	20.38 ± 2.11	31.69 ± 1.14	45.44 ± 1.28	64.77 ± 2.31	76.94 ± 2.26	212.48
VQME	2.27 ± 0.62	13.66 ± 0.92	23.15 ± 0.57	29.81 ± 1.71	51.29 ± 2.57	336.81
VQCE	8.53 ± 0.63	22.65 ± 0.81	35.14 ± 1.03	41.19 ± 0.88	63.28 ± 2.10	281.23

AA – Ascorbic acid, VQME-Methanol extract of *V. quinata*,
VQCE – Chloroform extract of *V. quinata*

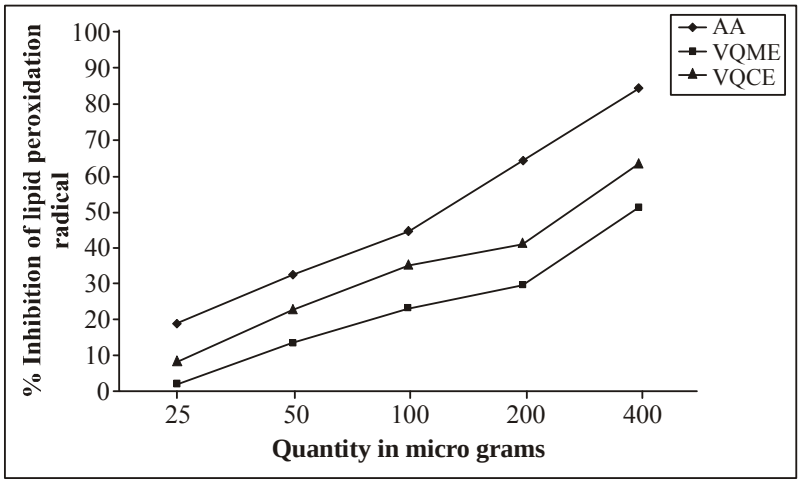

Fig. 3: *In vitro* concentration dependent percentage inhibition of lipid peroxidation radical *in vitro* by methanolic and chloroform extracts of *Vitex quinata* root and ascorbic acid

DPPH assay has been extensively used for screening antioxidant activity because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentration[24]. When DPPH radicals encounter a proton donating substance such as an antioxidant, it would be scavenged and the absorbance is reduced. Thus, the DPPH radicals were widely used to investigate the scavenging activity of some natural compounds. In the present study, the total methanolic and chloroform extracts of *Vitex quinata* were investigated in comparison with the known antioxidant ascorbic acid. The IC_{50} values for DPPH radical with chloroform and methanolic extracts of *Vitex quinata* were found to be 110.12 µg, 154.50 µg. The chloroform extract of *Vitex quinata* root was found to have better DPPH radical scavenging activity, when compared to methanol extract of *Vitex quinata* (Table 4, Fig. 4).

Table 4: Percentage inhibition and IC_{50} values of DPPH radical *in vitro* by methanolic and chloroform extracts of *Vitex quinata* and ascorbic acid

Extract	Quantity in micrograms (µg), Mean ± SEM					IC_{50} values
	25	50	100	200	400	
AA	20.38 ± 2.11	31.69 ± 1.14	45.44 ± 1.28	64.77 ± 2.31	76.94 ± 2.26	212.48
VQME	13.43 ± 3.21	23.23 ± 0.91	31.90 ± 1.13	37.60 ± 1.40	43.93 ± 1.18	154.50

Cont…

Extract	Quantity in micrograms (µg), Mean ± SEM					IC_{50} values
	25	50	100	200	400	
VQCE	20.96 ± 1.20	33.84 ± 0.84	40.43 ± 1.27	46.64 ± 1.70	54.30 ± 0.99	110.12

AA – Ascorbic acid, VQME-Methanol extract of *V. quinata*,
VQCE- Chloroform extract of *V. quinata*

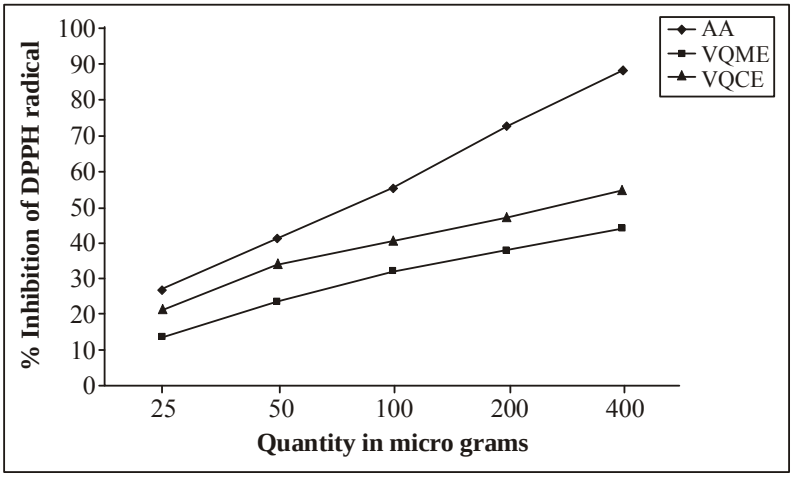

Fig. 4: *In vitro* concentration dependent percentage inhibition of DPPH radical *in vitro* by methanolic and chloroform extracts of *Vitex quinata* root and ascorbic acid

CONCLUSION

The data obtained reveal that the activity of chloroform extract of *Vitex quinata* in superoxide radicals, hydroxyl radicals, lipid peroxidation and DPPH radical activities were found to possess higher antioxidant activity, when compared to methanolic extracts.

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