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In-vitro antioxidant activity and free radical scavenging potential of roots of *Vitex trifoliate*

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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, DPPH radical activities. The IC_{50} values for superoxide radical with chloroform and methanolic extracts of *Vitex trifoliata* were found to be 191.41 µg, 249.32 µg. The IC_{50} values for hydroxyl radical with chloroform and methanolic extracts of *Vitex trifoliata* were found to be 290.12 µg, 348.50 µg. The IC_{50} values for inhibition of lipid peroxidation activity with chloroform and methanolic extracts of *Vitex trifoliata* were found to be 262.30 µg, 310.72 µg. The IC_{50} values for DPPH radical with chloroform and methanolic extracts of *Vitex trifoliata* were found to be 122.20 µg, 169.32 µg.

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

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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 pro-duced in the mitochondrial respiratory chain, liver mixed function oxidases, through xanthine oxi-dase 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 inincreased radical activity and damage. Free radi-calsor 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 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 trifoliata* (Verbenaceae) is an aromatic shubby tree which can grow to 4m. in the Seychelles, bearing trifoliate leaves, or more rarely 1-2 leaves. It is found from Chintapalli, Anantagiri forest, Vizag to Nallamadala forests in Andhra Pradesh. Exhaustive and up to date review of literature for anti-oxidant their methods of screening and pharmacological review of the selected plant was conducted. Dried powdered roots of *Vitex trifoliata* 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].

MATERIALS AND METHODS

All the chemicals were used analytical grade 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 trifoliata* 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 trifoliata*, was separately extracted in a Soxhlet apparatus for 6 hrs successively with chlorform and methanol and concentrated to dryness under reduced pressure .Later, the quantity of root powder taken for extraction. These extract used to test the free radical scavenging activity, superoxide radicals, hydroxyl radicals, lipid peroxidation, and DPPH radical activities.

***In-vitro* anti oxidant study**

The methanolic & chloroform Extracts of *Vitex trifoliata* roots tested for its 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 560nm. 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 calorimetrically 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 3ml of 0.004% DPPH solution in ethanol and 0.1ml of plant extract at various concentrations were mixed and incubated at 37˚c for 30 min. and absorbance of the test mixture was read at 517nm.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].

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Calculation of percentage inhibition

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

$$\text{Inhibitory ratio} = \frac{(A_o - A_1)\,x100}{A_o}$$

Where, A_0 is the absorbance of control

A_1 is the absorbance with addition of test sample.

Statistical analysis

Linear regression analysis was used to calculate IC50 values [21].

RESULTS AND DISCUSSION

Superoxides are produced from molecular oxygen due to oxidative enzymes [22] of body as well as via non enzymatic reactions such as auto oxidation by catecholamines [23].In the present study, the total methanolic and chloroform extracts of *Vitex trifoliata* root was found to scavenge the superoxides generated by photoreduction of riboflavin. The chloroform extract and methanol extract of *Vitex trifoliata* root produced dose dependent inhibition of superoxide radicals. The IC50 values for superoxide radical with chloroform extract and methanol extract of *Vitex trifoliata* root were found to be 191.41 µg, 249.32 µg; with ascorbic acid were found to be 128.54µg respectively. The chloroform extract of *Vitex trifoliata* root was found to have better superoxide radical scavenging activity when compared to methanol extract of *Vitex trifoliata* root*, as* shown in Table-1, Fig-1.

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 hydro peroxides 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 trifoliata* 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 trifoliata* were found to be 290.12 µg, 348.50 µg. The chloroform extract of *Vitex trifoliata* root was found to have better hydroxyl radical scavenging activity when compared to methanol extract of *Vitex trifoliate, as* shown in Table-2, Fig-2.

Free radicals induce lipid peroxidation in polyunsaturated lipid rich areas like brain and liver [24].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 trifoliata* 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 trifoliata* were found to be 262.30 µg, 310.72 µg. The chloroform extract of *Vitex trifoliata* root was found to have higher lipid peroxidation inhibition than the methanol extract of *Vitex trifoliata as* shown in Table-3, Fig-3.

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 [25].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 trifoliata* were investigated in comparison with the known antioxidant ascorbic acid. The IC_{50} values for DPPH radical with chloroform and methanolic extracts of *Vitex trifoliata* were found to be 122.20 µg, 169.32 µg. The chloroform extract of *Vitex trifoliata* root was found to have better DPPH radical scavenging activity when compared to methanol extract of *Vitex trifoliata, as* shown in Table-4, Fig-4.

CONCLUSION

The data obtained reveal that the activity of Chloroform extract of *Vitex trifoliata* in superoxide radicals, hydroxyl radicals, lipid peroxidation, DPPH radical activities were found to possess higher anti-oxidant activity when compared to methanolic extracts.

Table–1: Percentage inhibition and IC50 values of superoxide radical scavenging activity in-vitro by methanolic and chloroform extracts of V.trifoliata root and Ascorbic acid.

Extract	Quantity in micrograms (µg),Mean±s.e.m					IC_{50}values
	25	50	100	200	400	
AA	22.69±1.29	40.50±1.07	53.59±1.52	72.99±1.97	83.15±1.57	128.54
VTME	10.20±1.44	19.21±0.80	32.69±1.25	36.66±0.54	41.21±0.92	249.32
VTCE	20.11±1.41	31.39±0.77	53.27±1.72	60.03±0.94	69.52±1.06	191.41

AA – Ascorbic acid, VTME-Methanol extract of V.trifoliata, VTCE- Chloroform extract of V.trifoliata.

Table–2: Percentage inhibition and IC50 values of hydroxyl radical in-vitro by methanolic and chloroform extracts of V.trifoliata and Ascorbic acid.

Extract	Quantity in micrograms (µg),Mean±s.e.m	

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	25	50	100	200	400	IC_{50}values
AA	20.38±2.11	31.69±1.14	45.44±1.28	64.77±2.31	76.94±2.26	212.48
VTME	11.09±0.90	19.80±1.67	24.88±1.12	31.08±1.69	43.80±1.17	348.50
VTCE	14.63±1.73	24.46±0.77	32.50±0.82	43.63±2.07	52.88±1.06	290.12

AA – Ascorbic acid, VTME-Methanol extract of V.trifoliata, VTCE- Chloroform extract of V.trifoliata.

Table–3: Percentage inhibition and IC50 values of lipid-peroxidation radical in-vitro by methanolic and chloroform extracts of V.trifoliata and Ascorbic acid

Extract	Quantity in micrograms (µg),Mean±s.e.m					IC_{50}values
	25	50	100	200	400	
AA	19.17±1.47	32.38±1.49	44.49±1.69	64.23±2.43	19.17±1.47	173.56
VTME	2.97±0.42	12.36±1.58	22.52±0.94	27.74±1.11	2.97±0.42	310.72
VTCE	10.17±0.68	16.89±1.70	32.06±1.12	37.36±1.52	10.17±0.68	262.30

AA – Ascorbic acid, VTME-Methanol extract of V.trifoliata, VTCE- Chloroform extract of V.trifoliata.

Table-4: Percentage inhibition and IC50 values of DPPH radical in-vitro by methanolic and chloroform extracts of V.trifoliata and Ascorbic acid.

Extract	Quantity in micrograms (µg),Mean±s.e.m					IC_{50}values
	25	50	100	200	400	
AA	26.48±1.11	40.85±0.80	54.98±1.79	72.42±2.37	87.85±2.05	71.56
VTME	10.59±0.70	18.58±0.88	28.71±0.53	34.47±0.83	40.36±0.64	169.32
VTCE	19.44±2.33	31.13±1.26	37.92±1.13	46.53±1.01	49.23±0.83	122.20

AA – Ascorbic acid, VTME-Methanol extract of V.trifoliata, VTCE- Chloroform extract of V.trifoliata

Figure: - 1: **In vitro concentration dependent Percentage inhibition of superoxide radical in-vitro by methanolic and chloroform extracts of v.trifoliata root and ascorbic acid.**

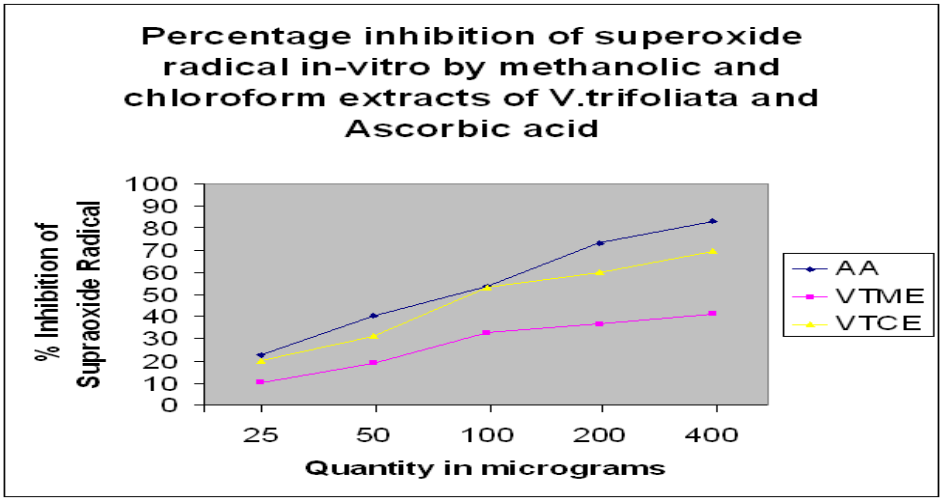

Figure: - 2: **In vitro concentration dependent Percentage inhibition of hydroxyl radical in-vitro by methanolic and chloroform extracts of V.trifoliata root and ascorbic acid**

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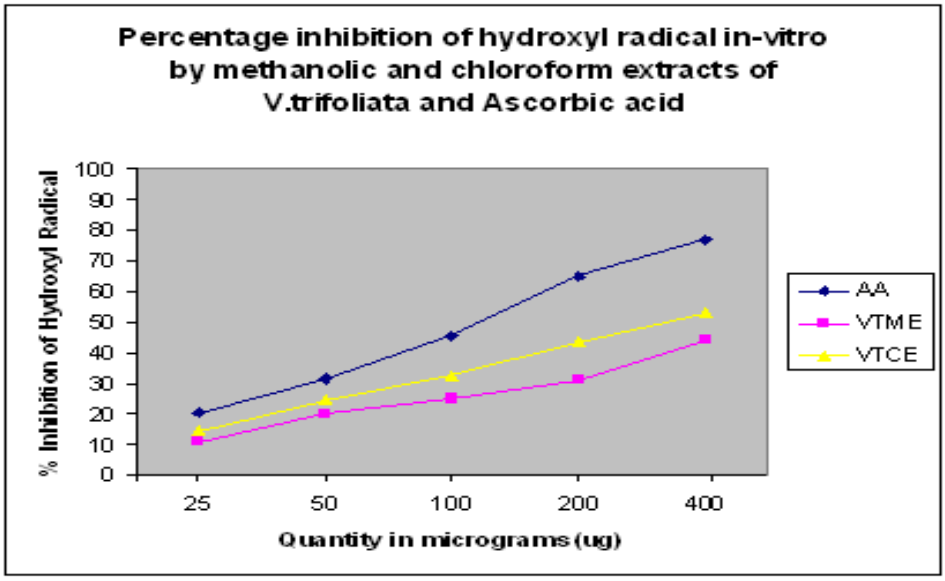

Figure: - 3: ***In vitro*** **concentration dependent Percentage inhibition of lipid-peroxidation radical in-vitro by methanolic and chloroform extracts of V.trifoliata root and Ascorbic acid**

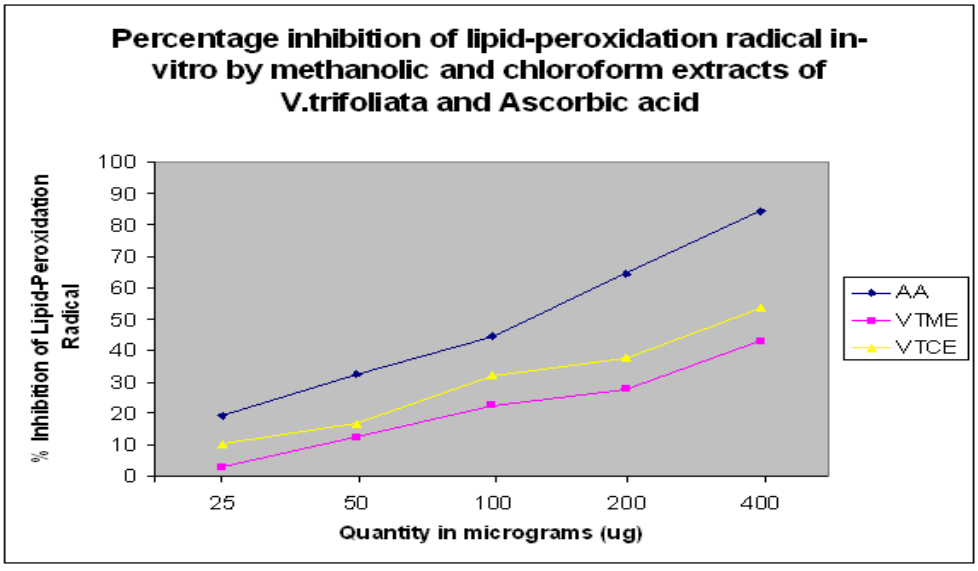

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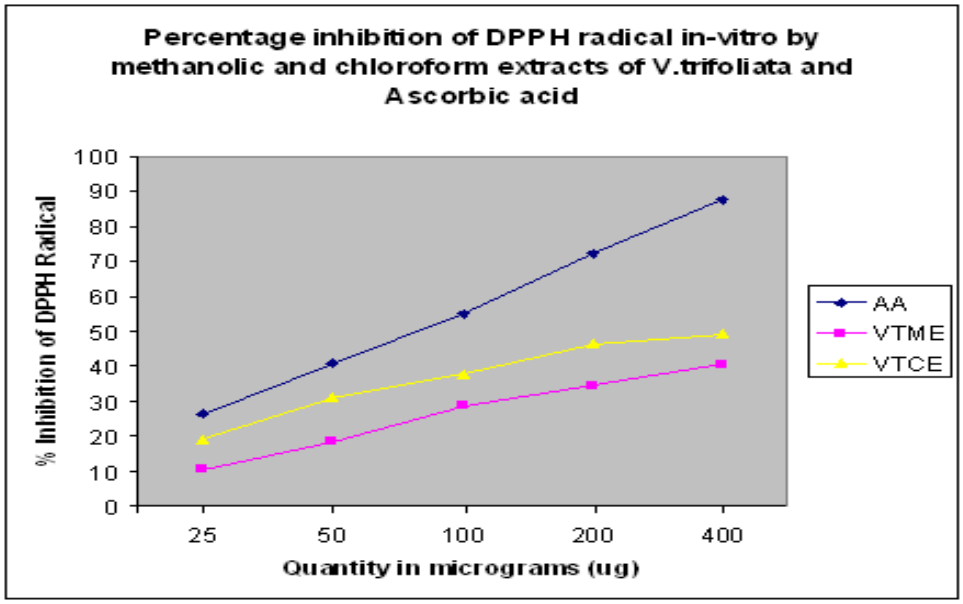
Figure: - 4: ***In vitro*** **concentration dependent Percentage inhibition of DPPH radical in-vitro by methanolic and chloroform extracts of V.trifoliata root and Ascorbic acid**



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REFERENCES

[1] Saha MN, Alam MA, Aktar R, Jahangir R. Bangladesh Journal Pharmacology 2008; 3: 90-96.
[2] Polterait O. Curr Org Chem 1997; 1: 415-440.
[3] Prior RL. American J Clin Nut 2003; 78: 570S-578S.
[4] Lee SE, Hwang HJ, Ha JS. Life Sci 2003; 73: 167-179.
[5] Makari HK, Haraprasad N, Patil HS, Ravikumar. The Internet J Aesthetic and Antiaging Medicine 2008; 1: 1-10.
[6] Augustin S, Claudine M, Christine M, Christian R. Crit Rev Food Sci 2005; 45: 287-306.
[7] Aviram M. Free Rad Res 2000; 33: S85-S87.
[8] Polterait O. Curr Org Chem 1997; 1: 415-440.
[9] Prior RL. American J Clin Nut 2003; 78: 570S-578S.
[10] Trease GE and Evans WC. Pharmacognosy: A Physicians's Guide to Herbal Medicine. Bailliere, Tindall. London. 1989; 13: 886-893.
[11] Lu Y and Foo Y. Food Chem 2000; 75: 197-202.
[12] Koleva II, Beek TV, HLinssen JP, Groot AD and Evstatieva LN. Phytochem Anal 2002; 13: 8-17.
[13] Krishna Rao RV, Ranjit J. Indian J Nat Prod 1996; 12(2): 26-28.

[14] Rakesh K, Sharma and Rajesh Arora. 2006, Herbal Drugs. Jaypee Brothers, Medical Publishers, 1st Edn.
[15] Mc Cord JM, Fridovich I. J Biol Chem 1969; 244(22):6049-6055.
[16] Shir waikar A, Rajendran K, Dinesh K, Indian J Exp Biol 42,2004,803.
[17] Elizabeth Kand, Rao MNA. Int J Pharm 1990; 58: 237-240.
[18] Ohkawa H, Ohishi N, Yagi K. Anal Biochem 1979; 95(2):351-358.
[19] Braca A,Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. J Nat Prod 2001; 64:892-895.
[20] T Satyanarayana, M Chinna Eswaraiah. Res J Pharm Biol Chem Sci 2010;1(2): 117
[21] Pratt DE, Natural anti-oxidants from plant material in phenolic compounds in food and their effects on health, ii: Antioxidants and cancer prevention (Acs symposium series 507), edited by Hang M, Ho C, lee C, (American chemical society, Washington DC,), 1992, 54.
[22] Pratt DE, Natural anti-oxidants from plant material in phenolic compounds in food and their effects on health, ii: Antioxidants and cancer prevention (Acs symposium series 507), edited by Hang M, Ho C, lee C, (American chemical society, Washington DC,), 1992, 54.
[23] Sai nani, GS, Manika JS, Sainani RG. Med Update 1997; 1:1.
[24] Hemmani T, Parihar MS. Indian J Physiol Pharmacol 1998; 42:440.
[25] Coyle JT, Put Farcken P. Science 1993; 219:1184.