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IN VITRO ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF STEM OF *MUSSAENDA ERYTHROPHYLLA*

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ABSTRACT

Mussaenda erythrophylla is native to western tropical Africa, occasionally seen in gardens and parks as ornamental plant in India and belongs to Rubiaceae family. The present study was concentrated on the in vitro antioxidant methods like superoxide radical, hydroxyl radical, lipid peroxidation and DPPH radical methods. The ethylacetate and methanolic extracts of *Mussaenda erythrophylla* were subjected for the above methods. The results of anti oxidant activity revealed that, the ethylacetate extract has lower IC_{50} values than the methanolic extract of *Mussaenda erythrophylla.* The lower IC_{50} value indicates the higher free radical scavenging ability. So, the ethylacetate extract has better antioxidant activity than methanolic extract. The results were compared with the standard ascorbic acid. The plant contains phytosterols, triterpenoids, flavonoids, glycosides, saponins and tannins. These active constituents alone or in combination may be responsible for the observed antioxidant activity.

INTRODUCTION: *Mussaenda erythrophylla* (Rubiaceae) is native to western tropical Africa, occasionally seen in gardens and parks as ornamental plant in India and is commonly known as mussenda (telugu), nagavalli (Sanskrit) and red flag bush (English) [1]. It is a perennial, evergreen shrub with branched tap root system. The roots are useful for cough, jaundice and when chewed acts as an appetizer [2]. A number of triterpenoids and glycosides were reported. Mussaenda genus viz., contains mussaendosides U(1) and V(2) [3], mussaendosides G(1) and K(2) are two new triterpenoid saponins [4], mussaendosides A-C, M and N with cyclolanostene type aglycone [5-6] and aureusidin[7], iridoid glycosides [8].

The pharmacological activities reported from Mussaenda species were diuretic, antiphlogistic, antipyretic and effective in laryngopharyngitis, acute gastroenteritis and dysentery [9] and also anti-fertility activity [10]. It is established that plants which have anti oxidant property exert hepatoprotective [11-14] and anti diabetic actions [15-17]. It is in the light of this fact that antioxidant and free radical scavenging potential of stem of *Mussaenda erythrophylla* (M.E) was investigated.

MATERIAL AND METHODS: All the chemicals used were of analytical grade obtained from S.D. Fine Chemicals Pvt. Ltd., Mumbai, Sigma chemical company, USA and Loba chemicals, Mumbai.

Plant Material: The stem of *Mussaenda erythrophylla* were procured from M.V.P colony, Visakhapatnam.The authentification of the plant was done by Prof. M. Venkaiah, Dept. of Botany, Andhra University, Visakhapatnam. The Voucher specimen was deposited in the herbarium of our department.

Preparation of Extract: Freshly collected plant material was shade dried at room temperature and coarsely powdered in Wiely mill. The powdered stem (1kg) was extracted successively with hexane, ethylacetate and methanol using soxhlet apparatus. The crude extract was evaporated to dryness in a rotary film evaporator (Roteava,Equitron, Medica instrument, India) and found to be 2.5, 30 and 25gms respectively. Preliminary phytochemical screening of ethylacetate extract of *Mussaenda erythrophylla* stem revealed the presence of steroids, triterpenoids and flavonoids; methanol extract tested positive for glycosides, tannins and saponins.The constituents present in the ethylacetate extract and methanolic extracts of *M. erythrophylla* stem initiated to carry out the anti oxidant activity for the above said extract.

***In vitro* Anti Oxidant Study:** The ethylacetate extract and methanolic extract of *M. erythrophylla* stem 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 [18], 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 [19].

Hydroxyl Radical Scavenging Activity: Hydroxyl radical scavenging activity was measured according to the method of Elizabeth and Rao[20], by studying the competition between deoxyribose and test extracts 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.

Lipid Peroxidation Inhibition Activity: The inhibition of lipid peroxidation was performed as per the method described by Ohkawa et al**.,** [21]. Rat liver homogenate was used as the source of polyunsaturated fatty acids for determining the extent of lipid peroxidation. The absorbance was measured at 532nm. Percentage of inhibition was calculated using the formula.

DPPH Radical Scavenging Activity: DPPH radical scavenging activity was measured according to the method of Braca et al., [22]. 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;

$$\text{Percentage inhibition} = \frac{A_o - A_1 \times 100}{A_o}$$

Where A_o = Absorbance of the control; A_1 = Absorbance of the plant extract/ standard.

Statistical Analysis: Linear regression analysis was used to calculate IC_{50} values [23].

RESULTS AND DISCUSSION: Superoxides are produced from molecular oxygen due to oxidative enzymes of body as well as via non enzymatic reactions such as auto oxidation by catecholamines [24]. In the present study ethylacetate extract and methanol extract of *M. erythrophylla* stem was found to scavenge the superoxides generated by photo reduction of riboflavin. The ethylacetate extract and methanol extract of *M. erythrophylla* stem produced dose dependent inhibition of superoxide radicals. The IC_{50} values for superoxide radical with ethylacetate extract and methanol extract of *M. erythrophylla* stem were found to be 216.63µg, 271.38µg with ascorbic acid were found to be 140.76 µg respectively. The ethylacetate extract of *M. erythrophylla* stem was found to have better superoxide radical scavenging activity than methanol extract of *M. erythrophylla, as* shown in **Table 1, Fig. 1.**

TABLE 1: PERCENTAGE INHIBITION OF SUPEROXIDE RADICAL SCAVENGING ACTIVITY *IN VITRO* BY ETHYLACETATE AND METHANOL EXTRACTS OF *MUSSAENDA ERYTHROPHYLLA* STEM

Extract/ Compound	Quantity in micrograms (µg)					IC_{50} values
	10	50	100	200	300	
AA	15.56±1.76	31.93±2.91	47.63±4.79	69.7±1.93	76.1±1.38	140.76
EAEME	6.46±0.86	17.1±1.43	30.3±1.60	47.16±1.48	65.13±0.26	216.63
MEME	5.06±1.51	13.8±1.46	23.0±1.44	39.76±1.07	53.26±1.50	271.38

AA – Ascorbic acid, EAEME-Ethyl acetate extract of *M erythrophylla*,MEME-Methanol extract of *M. erythrophylla*

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. The ethylacetate extract and methanol extract of *M. erythrophylla* stem showed concentration dependent activity and the ascorbic acid at various concentrations produced dose dependent inhibition of hydroxyl radicals. The IC_{50} values for hydroxyl radical with ethylacetate extract and methanol extract of *M. erythrophylla* stem were found to be 250.82µg, 321.42µg, with ascorbic acid was found to be 231.96 µg respectively. The ethylacetate extract of *M. erythrophylla* stem was found to have better hydroxyl radical scavenging activity when compared to methanol extract of *M. erythrophylla, as* shown in **Table 2, Fig. 2.**

TABLE 2: PERCENTAGE INHIBITION AND IC_{50} VALUES OF HYDROXYL RADICAL SCAVENGING ACTIVITY *IN VITRO* BY ETHYL ACETATE AND METHANOL EXTRACTS OF *MUSSAENDA ERYTHROPHYLLA* STEM

Extract/ Compound	Quantity in micrograms (µg)						IC_{50} values
	10	50	100	200	300	400	
AA	2.53±1.51	17.9±3.52	28.83±2.22	52.9±1.63	63.13±0.37	74.83±2.25	231.96
MEME	2.76±2.9	11.76±1.94	23.13±5.36	38.0±4.06	48.93±1.43	56.43±0.63	321.42
EAEME	3.16±1.16	15.47±0.63	26.1±2.25	47.4±0.71	60.28±0.21	70.88±0.91	250.82

AA – Ascorbic acid, EAEME-Ethyl acetate extract of *M erythrophylla*, MEME-Methanol extract of *M. erythrophylla*

Free radicals induce lipid peroxidation in polyunsaturated lipid rich areas like brain and liver [25]. In this study, *in vitro* lipid peroxidation was induced in rat liver by using ammonium ferrous sulphate and ascorbic acid. The extract showed concentration dependent prevention towards generation of lipid peroxides. The IC_{50} values for the lipid peroxidation inhibiting activity with ethyl acetate extract and methanol extract of *M. erythrophylla* stem were found to be 321.32µg, 355.13µg; with ascorbic acid was found to be 183.51 µg respectively. The lower the IC_{50}, the higher the free radical scavenging ability. The ethylacetate extract of *M. erythrophylla* stem was found to have higher lipid peroxidation inhibition than the methanol extract of *M. erythrophylla as* shown in **Table 3, Fig. 3.**

TABLE 3: PERCENTAGE INHIBITION AND IC_{50} VALUES OF LIPID PEROXIDATION *IN VITRO* BY ETHYL ACETATE AND METHANOL EXTRACTS OF *MUSSAENDA ERYTHROPHYLLA* STEM

Extract/ Compound	Quantity in micrograms (µg)						IC_{50} values
	10	50	100	200	300	400	
AA	7.63±1.90	25.5±3.17	42.73±5.24	66.83±2.10	71.06±1.45	79.0±0.90	183.51
MEME	1.33±1.64	8.3±1.02	17.6±0.95	34.26±0.89	40.8±0.55	54.6±0.83	355.13
EAEME	5.33±0.90	11.10±0.80	21.50±0.86	38.46±0.80	46.33±4.89	58.66±0.75	321.32

AA – Ascorbic acid, EAEME-Ethyl acetate extract of *M erythrophylla*,MEME-Methanol extract of *M. erythrophylla*

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 [26]. 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. The mean IC_{50} values for DPPH radical with ethylacetate extract of *M. erythrophylla* stem and methanol extract of *M. erythrophylla* stem were found to be 159.86 µg, 174.35 µg; with ascorbic acid were found to be 75.22 µg respectively. The lower the IC_{50}, the higher the freeradical scavenging ability. The ethylacetate extract of *M. erythrophylla* stem was found to have better DPPH radical scavenging activity when compared to methanol extract of *M. erythrophylla, as* shown in **Table 4, Fig. 4.**

TABLE 4: PERCENTAGE INHIBITION AND IC_{50} VALUES OF DPPH RADICAL SCAVENGING ACTIVITY *IN VITRO* BY ETHYL ACETATE AND METHANOL EXTRACTS OF *MUSSAENDA ERYTHROPHYLLA* STEM

Extract/ Compound	Quantity in micrograms (µg)				IC_{50} values
	10	50	100	200	
AA	27.23±1.41	46.3±0.81	63.16±3.35	78.7±1.37	75.22
MEME	19.26±0.73	30.36±0.72	40.66±0.85	52.5±0.39	174.35
EAEME	21.03±1.34	32.03±0.72	43.46±0.66	54.9±0.63	159.86

AA – Ascorbic acid, EAEME-Ethyl acetate extract of *M erythrophylla*,MEME-Methanol extract of *M. erythrophylla*

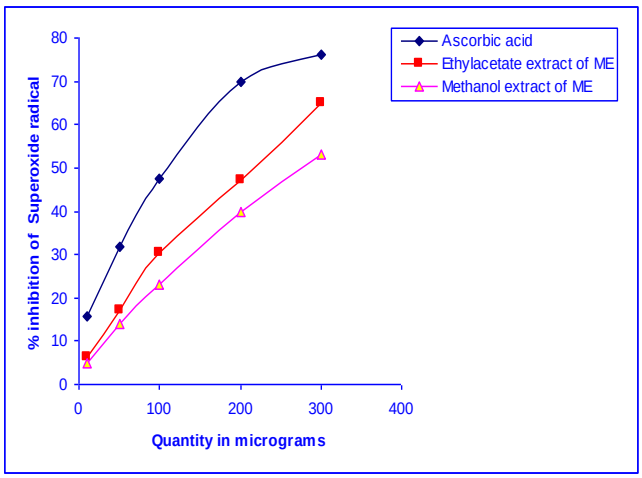

FIG. 1: *IN VITRO* CONCENTRATION DEPENDENT PERCENTAGE INHIBITION OF SUPEROXIDE RADICAL SCAVENGING ACTIVITY BY ETHYL ACETATE AND METHANOL EXTRACTS OF *MUSSAENDA ERYTHROPHYLLA* STEM AND ASCORBIC ACID

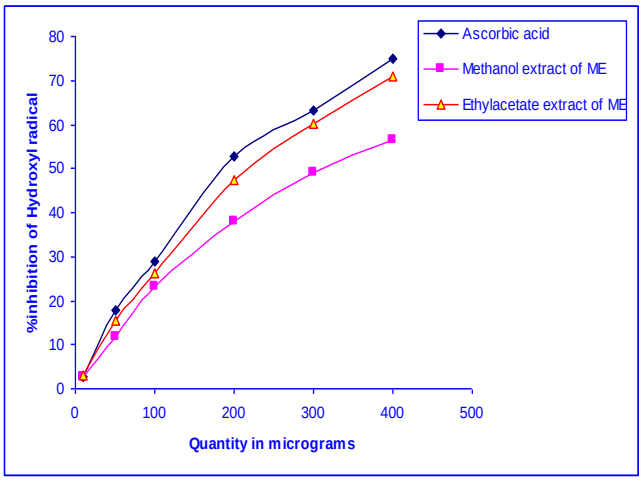

FIG. 2: *IN VITRO* CONCENTRATION DEPENDENT PERCENTAGE INHIBITION OF HYDROXYL RADICAL SCAVENGING ACTIVITY BY ETHYLACETATE AND METHANOL EXTRACTS OF *MUSSAENDA ERYTHROPHYLLA* STEM AND ASCORBIC ACID

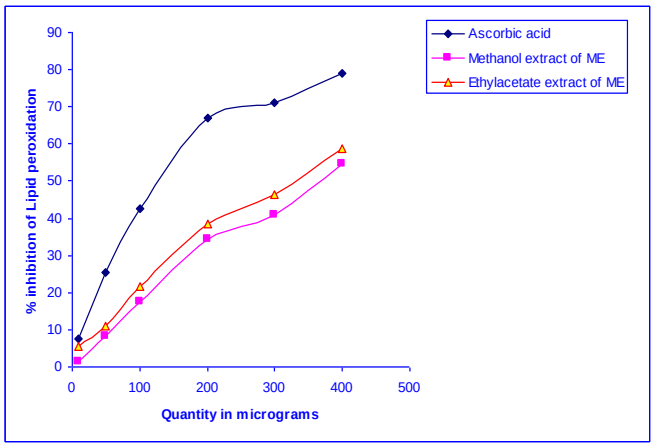

FIG. 3: *IN VITRO* CONCENTRATION DEPENDENT PERCENTAGE INHIBITION OF LIPID PEROXIDATION BY ETHYL ACETATE AND METHANOL EXTRACTS OF *MUSSAENDA ERYTHROPHYLLA* STEM AND ASCORBIC ACID

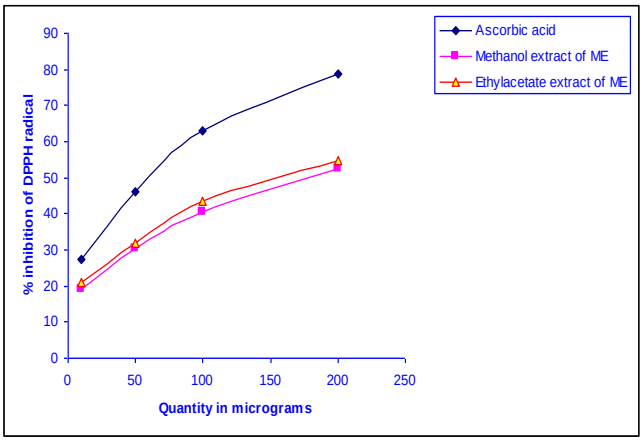

FIG. 4: *IN VITRO* CONCENTRATION DEPENDENT PERCENTAGE INHIBITION OF DPPH RADICAL SCAVENGING ACTIVITY BY ETHYLACETATE AND METHANOL EXTRACTS OF *MUSSAENDA ERYTHROPHYLLA* STEM AND ASCORBIC ACID

CONCLUSION: Natural antioxidants such as phenolic acids, flavonoids and tannins possess potent antioxidant activity [27].Sterols like β-sitosterol have been reported for antioxidant activity [28].Terpenoids are also reported to possess antioxidant activity [29].Phytochemical analysis reveal that *Mussaenda erythrophylla* stem contains phytosterols, triterpenoids, flavonoids, saponins, glycosides and tannins; hence, the observed activity may be due to the presence of any of these constituents. The extracts merits further experiments *in vivo.*

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