Victoria Devi. *et al.* / *International Journal of Phytopharmacology*. 5(6), 2014, 441-444.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

ANTIOXIDANT ACTIVITIES OF THE LEAVES OF *THUNBERGIA COCCINEA* WALL

S.H.Victoria[1]*, S. Das [2], H. Lalhlenmawia[1], L. Shantabi[3] and S.H. Sarda[4]

[1]*Department of Pharmacy, Regional Institute of Paramedical and Nursing Sciences, Zemabawk-796017, Aizawl, Mizoram, India.
[2] Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh-786004, Assam, India.
[3]Department of Zoology, Mizoram University, Tanhril-796004, Aizawl, India.
[4] Department of Medical Laboratory Technology, Regional Institute of Paramedical and Nursing Sciences, Zemabawk-796017, Aizawl, Mizoram, India.

ABSTRACT

To evaluate the in-vitro antioxidant potential of methanolic extract of *Thunbergia coccinea*. The leaves of *Thunbergia coccinea* Wall were dried and powdered. The methanol extract of the sample were prepared by using Soxhlet apparatus. The extract was filtered using Whatman filter paper No 42 (125 mm) and the extract was evaporated under reduced pressure using Rotary Vaccum evaporator . Anti-oxidant analysis such as DPPH, Hydroxyl, Superoxide and Nitric oxide of the above extract was done by standard methods. The IC_{50} values of scavenging activities of METC on DPPH, Hydroxyl, Superoxide and Nitric oxide radicals were found to be, 179.21 mcg/ml, 73.52 mcg/ml, 40.88 mcg/ml and 76.21mcg/ml as compared to Ascorbic acid and Gallic acid which were found to be 35.84mcg/ml and 45.45mcg/ml respectively. The data obtained in the present study suggests that the extracts of *Thunbergia coccinea* leaves have potent antioxidant activity against free radicals, prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage.

Key words: *In vitro,* Anti-oxidant, *Thunbergia coccinea,* METC.

INTRODUCTION

Antioxidants are important substances that possess the ability to protect the body from damage caused by free radical induced oxidative stress. A variety of free radical scavenging antioxidants are exists within the body, many of which are derived from dietary sources such as fruits, vegetables and tea (Romero A, Saavedra RG, 2005).The effects of free radicals on human beings are closely related to toxicity, diseases like chronic renal failure, diabetes mellitus, cancer, immune dysfunction and aging are closely related to the peroxidation reactions in living organisms (Maxwell SJ, 1995; Halliwell B *et al.,* 1996). Most living species have an efficient defense system to protect themselves against the oxidative stress induced by Reactive Oxygen Species (ROS) (Sato M *et al*., 1996). Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases including cancer, atherosclerosis and the aging process (Stajner D *et al.,* 1998). A free radical is defined as any atom or molecular possessing unpaired electrons. Antioxidants are agents which scavenge the free radicals and prevent the damage caused by reactive oxygen species (ROS), reactive nitrogen species (RNS). ROS are a group of highly reactive molecules due to the presence of unpaired electron. The example includes superoxide anions, hydroxyl and hydrogen peroxide radicals. They are often generated as byproducts of oxidative damage to the DNA molecules, lipids and proteins (Farber J L, 1994).

Corresponding Author

Sh. Victoria Devi
Email: victoriadevi09@gmail.com

Antioxidants can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells prevent damage to lipids, proteins, enzymes, carbohydrates DNA. Antioxidants can be classified into two major classes i.e., enzymatic and non-enzymatic. The enzymatic anti-oxidants are produced endogenously include dismutase, catalase, glutathione obtained from eroxidase. The non-enzymatic antioxidants include tocopherols, carotenoids, ascorbic acid, flavonoids and tannins which are obtained from natural plant sources. A wide range of antioxidants from both natural and synthetic origin has been proposed for use in the treatment of various human diseases. There are some synthetic antioxidant compounds such as butylated hydroxytoluene, butylated hydroxyanisole tertiary butylhydroquinone which are commonly used in processed foods. However, it has been suggested that these compounds have shown toxic effects like liver damage mutagenesis. Flavonoids and other phenolic compounds of plant origin have been reported as scavengers of free radicals. Hence, nowadays search for natural antioxidant source is gaining much importance.

The present study was therefore carried out to investigate the antioxidant potential of methanol extract of the leaves of *Thunbergia coccinea* on different in vitro models.

MATERIALS AND METHODS

Leaves of *Thunbergia coccinea* were collected from Maulpheng area of Mizoram during July 2009. The plant material was identified by Botanical Survey of India, Shillong and the voucher specimen (BSI/ERC/2010)/RP/080 was deposited in the herbarium of the Department of Pharmacy, Regional Institute of Paramedical and Nursing Sciences (RIPANS)

Test material

Various concentrations of METC (10-100 mcg/ml in methanol) were prepared and used for the antioxidant studies on different in vitro models. For reductive ability study, 10-100 mcg/ml concentrations of the extracts were used. Ascorbic acid and Gallic acid were used as standard.

Chemicals

1.1-diphenyl-2-picryl- hydrazyl (DPPH) from Sigma Chemicals, Nitrobluetetrazolium (NBT), sodium nitroprsside, ascorbic acid, trichloroacetic acid (TCA), potassium ferricyanide [$K_3Fe(CN)_6$], Folin-Ciocalteu's phenol reagent (FCR) from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. All other chemicals and solvents used were of analytical grade.

(i) DPPH radical scavenging activity

To different concentrations (10-100 mcg/ml) METC methanol (0.5 ml each) was added to 1ml of methanol solution of 0.2 mM DPPH (Yen GC, Duh PD, 1994). After mixing thoroughly, the mixture was allowed to stand in the dark for 30 min and the absorbance at 523 nm was measured using methanol for the baseline correction. The results were compared with that of the control prepared as above without sample. Radical scavenging activity was expressed as a percentage and was calculated using the following formula.

% Scavenging = (A control-A sample)/A control X 100

Where A sample is the absorbance of the test sample and A control is the absorbance of the control.

Hydroxyl radical scavenging activity

Methanolic extract at different concentrations (10-100 mcg/ml) was placed in a test tube and evaporated to dryness. One ml of iron –EDTA solution (0.13 %ferrous ammonium sulfate and 0.26 % EDTA), 0.5 ml 0f 0.018 % EDTA, 1 ml of DMSO (0.85 %, v/v in 0.1 mol L^{-1} phosphate buffer, pH 7.4) and 0.5 ml of 0.22 % ascorbic acid were added to each tube (Klein SM *et al.,* 1981). The tubes were capped tightly and heated in a water bath at 80-90°C for 15 min. The reaction was terminated by adding 1 ml of ice –cold TCA (17.5%m/V). Three ml of Nash reagent (75.0 g ammonium acetate, 3 ml glacial acetic acid and 2 ml, acetyl acetone were mixed and water was added to a total volume of 1 L) was added to each tube; the tubes were left at room temperature for 15 min for colour development .The intensity of the yellow colour formed was measured at 412 nm against a blank of the reagent. Percentage inhibition was determined by comparing the results of the test and standard compounds. Radical scavenging activity was expressed as a percentage and was calculated using the following formula.

% Scavenging = (A control-A sample)/A control X 100

Where A sample is the absorbance of the test sample and A control is the absorbance of the control.

(iii) Superoxide anion scavenging activity

Superoxide scavenging activity was estimated by using a standard method. To the reaction mixture containing 0.2 ml of NBT (1mg/ml of solution in DMSO) 0.6 ml of the extract and standard of the DMSO, 2ml of alkaline DMSO (1ml DMSO containing 5 mM NAOH in 0.1 ml H_2O) was added to give final volume of 2.8 ml and the absorbance was measured at 560 nm (Hyland K *et al.,* 1983). Superoxide free radical was formed by alkaline DMSO which reacts with NBT to produce coloured diformazan. The methanolic extract of *Thunbergia coccinea* scavenges superoxide radical and thus inhibits formazan formation which increases in dose dependent manner.

The blank consisted of pure DMSO instead of alkaline DMSO. The absorbance was read at 560 nm using a UV_VIS double beam spectrophotometer. Radical

scavenging activity was expressed as a percentage and was calculated using the following formula.

% Scavenging = (A sample- A control)/A sample X 100

Where A sample is the absorbance of the test sample and A control is the absorbance of the control.

Nitric oxide scavenging activity

Nitric Oxide generated by sodium nitroprusside gets converted to nitrous acid on contact with air which is estimated using Greiss reagent . Sodium nitroprusside (5mM) in PBS was mixed with different concentrations of extract (10-100 mcg/ml) and incubated at 25ºC for 150 min. The samples from the above were reacted with Greiss reagent (1% Sulfanilamide, 2 % H_3PO_4, and 0.1 % napthylenediaminedihydrochloride) (Marcocci L, Packer L, 1994; Sreejayan Rao MNA, 1997). The absorbance of the chromophore formed during diazotization of nitrite with Sulfanilamide and subsequent coupling with napthylenediamine was read at 546 nm and referred to the absorbance of standard solutions of potassium nitrite treated in the same way with Greiss reagent. Radical scavenging activity was expressed as a percentage and was calculated using the following formula.

% Scavenging = (A control-A sample)/A control X 100

Where A sample is the absorbance of the test sample and A control is the absorbance of the control.

RESULTS AND DISCUSSION

The free radical scavenging activity was evaluated by using various in vitro assays. DPPH radical was used as a substrate to evaluate the free radical scavenging activity of the methanol extract of *Thunbergia coccinea* (METC) The scavenging effect of METC extract at different concentrations (10-100 mcg/ml), on the DPPH radical were 8.45±0.04, 14.56±0.07, 23.22±0.04, 30.23±0.38, 38.24±0.04 and 48.44 ±0.03compared to the scavenging effects of Ascorbic acid at concentrations (10-100 mcg/ml) were 6.04±0.03, 10.8 ±0.03, 18.67±0.03, 24.45±0.03, 32.34±0.08, 42.88±0.02 respectively. The IC_{50} values of scavenging activities on DPPH radical were found to be 100.40 mcg/ml and 118.20 mcg/ml for METC and Ascorbic acid respectively (Fig 1). The METC shows potent activity and indicated the presence of compounds with strongest radical-scavenging activity and of high polarity. Values were represented as mean ±SD of three parallel measurements.

Hydroxyl radicals are the major active oxygen species that cause lipid oxidation and enormous biological damage (Aurand LW *et al*., 1997). The percentage of hydroxyl radical scavenging increased with the increasing concentration of extract. The percentage of H_2O_2 scavenging activity of METC(10-100 mcg/ml) were found to be 6.65±0.08, 16.65±0.32, 29.89±0.08, 40.49±0.07, 48.87±0.09, 66.34±0.12 compared to the scavenging effects of Gallic acid at concentrations (10-100 mcg/ml) were 18.44±0.08, 23.34±0.08, 36.47±0.08, 46.56±0.12, 68.37±0.08, 75.89±0.07 respectively. The IC_{50} values of scavenging activities on Hydroxyl radical were found to be 75.8 mcg/ml and 52.57 mcg/ml for METC and Gallic acid respectively (Fig 2).

The super oxide anion radical scavenging activity of METC was assayed using alkaline DMSO method. The percentage inhibition of super oxide generation by METC(10-100μg/ml) were found to be 6.04±0.52, 10.8±0.18, 18.67±0.02, 24.45±0.08, 32.34 ±0.05, 42.88 ±0.52 compared to the scavenging effects of Ascorbic acid at concentrations (10-100mcg/ml) were 6.04±0.03, 10.8 ±0.03, 18.67±0.03, 24.45±0.03, 32.34±0.08, 42.88±0.02 respectively. The IC_{50} values of scavenging activities on Superoxide radical were found to be 107.5 mcg/ml and 118.20 mcg/ml for METC and Ascorbic acid respectively (Fig 3)

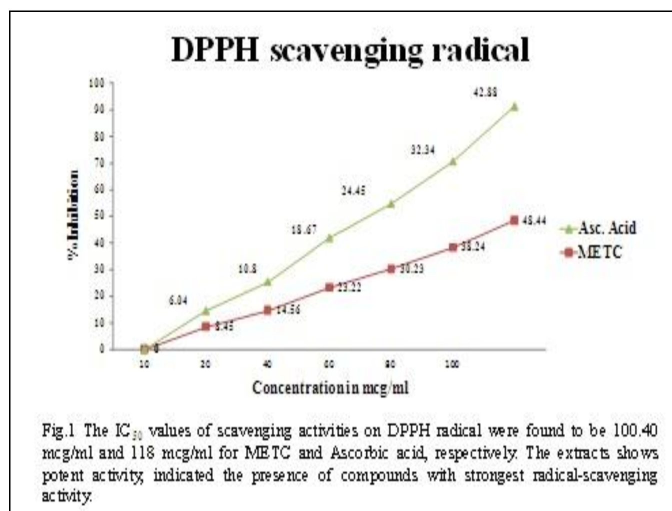

Fig.1 The IC_{50} values of scavenging activities on DPPH radical were found to be 100.40 mcg/ml and 118 mcg/ml for METC and Ascorbic acid, respectively. The extracts shows potent activity, indicated the presence of compounds with strongest radical-scavenging activity.

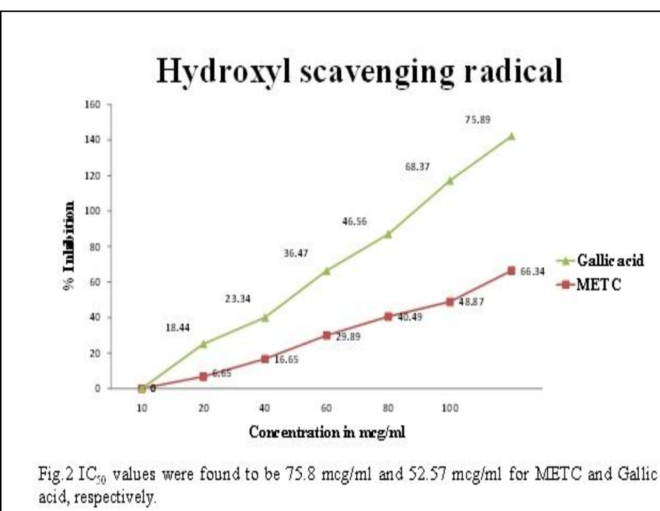

Fig.2 IC_{50} values were found to be 75.8 mcg/ml and 52.57 mcg/ml for METC and Gallic acid, respectively.

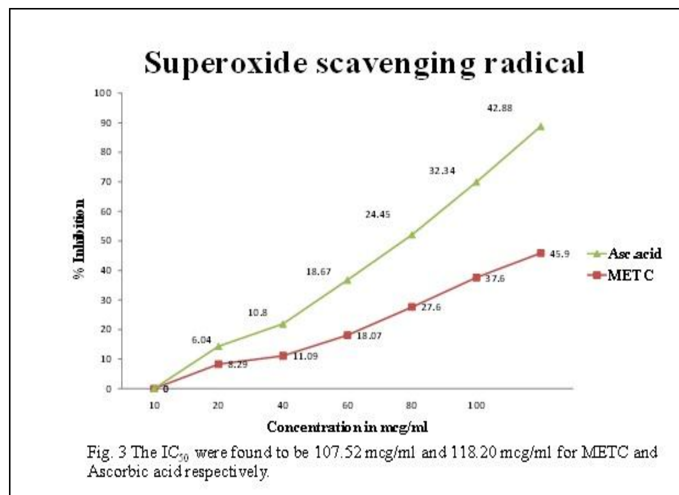

Fig. 3 The IC_{50} were found to be 107.52 mcg/ml and 118.20 mcg/ml for METC and Ascorbic acid respectively.

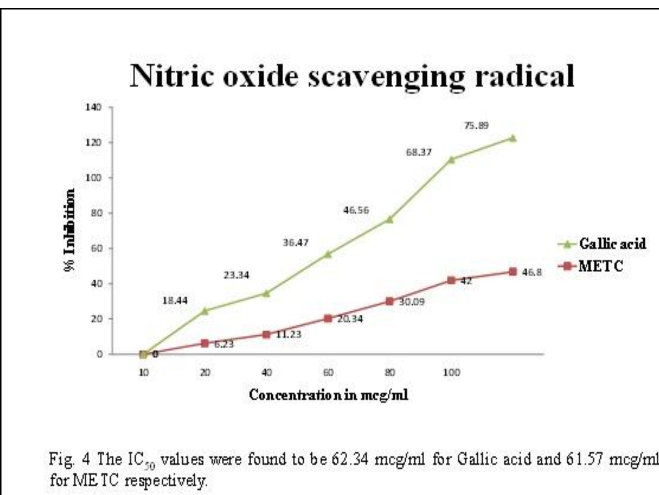

Fig. 4 The IC_{50} values were found to be 62.34 mcg/ml for Gallic acid and 61.57 mcg/ml for METC respectively.

Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity (Hagerman AE et al., 1998). The percentage inhibition of nitric oxide generation by METC(10-100 mcg/ml) were found to be 6.23 ±0.02, 11.23±0.48, 20.34±0.01, 30.09±0.02, 42.0±0.02, 46.8±0.01 compared to the scavenging effects of Gallic acid at concentrations (10-100 mcg/ml) were 18.44±0.08, 23.34±0.08, 36.47±0.08, 46.56±0.12, 68.37±0.08, 75.89±0.07 respectively. The IC_{50} values of scavenging activities on Nitric oxide scavenging radical were found to be 67.38 mcg/ml and 61.57mcg/ml for METC and Gallic acid respectively (Fig 4).

CONCLUSION

In vitro antioxidant study of the methanolic extracts of *Thunbergia coccinea* had shown a powerful scavenging activity in a dose dependent manner. It may be due to the presence of various phytochemical constituents and can be the potent source of natural antioxidants.

REFERENCES

Aurand LW, Boonme NH, Gidding GG. Superoxide and singlet oxygen in milk lipid peroxidation. *J Diary Sci,* 60, 1997, 363-369.

Farber, J L. Mechanisms of cell injury by activated oxygen species. *Environ Health Perspect,* 102, 1994, 17-24.

Hagerman AE, Riedl KM, Jones GA, Svik KN. Ritchard NT, Hartzfeld PW, Riechel TL. High molecular weight plant polyphenolics (tannins) as biological anti- Oxidants. *J Agric Food Chem*, 46, 1998, 1887-1892.

Halliwell B, Gutteridge JMC, Cross CE. Free radicals, antioxidants and human disease: where are we now?. *J Lab Clin Med,* 1996, 119, 598.

Hyland K, Voisin E, Banoun H, Auclair, C. Superoxide dismutase assay using dimethylsulfoxide as superoxide anion-generating system. *Anal Biochem*, 135, 1983, 280-287.

Klein SM, Cohen G, Cederbaum AI. Production of formaldehyde during metabolism of dimethylsulfoxide by hydroxyl radical generating system. *Biochem,* 20, 1981, 6006-6012

Marcocci L, Packer L. Antioxidant action of *Gingko biloba* extract EGB 761. *Methods Enzymol,* 234, 1994, 462-475.

Maxwell SJ. Prospects for the use of antioxidant therapies. *Drugs*, 49, 1995, 345.

Romero A, Saavedra RG. Screening Bolivian plants for antioxidant activity. *Pharmaceutical Biol*, 43, 2005, 79-86.

Sato M, Ramarathnam N, Suzuki Y, Ohkhubo T, Takeuchi M, Ochi H. Varietal differences in the phenolic content and superoxide radical scavenging potential of wines from different sources. *J Agri Food,* 44, 1996, 37-41.

Sreejayan Rao MNA. Nitric oxide scavenging by curcuminoids. *J Pharm Pharmacol,* 49, 1997, 105-107.

Stajner D, Milic N, Mimica-Dukic N, Lazic, lgic R. Antioxidant abilities of cultivated and wild species of garlic. *Phytother Res,* 12, 1998, 513-514.

Yen GC, Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. *J Agric Food Chem,* 42, 1994, 629-632.