ISSN (Online) 2249-6084 (Print) 2250-1029

International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)
[Impact Factor – 0.852]

Journal Homepage: www.eijppr.com

Research Article

In Vitro Antioxidant Properties of *Eclipta Alba* (L.) Hassk. and *Lippia Nodiflora* Linn

T. Regupathi* and K. Chitra[1]
* Ultra College of Pharmacy, Madurai – 625 104, Tamil Nadu, India
[1] Department of Pharmaceutical Chemistry, Sri Ramachandra University, Chennai – 600 116, Tamil Nadu, India

Article info

Article History:
Received 28 June 2014
Accepted 9 August 2014

Keywords:
Antioxidant activity, *Ecliptaalba, Lippia nodiflora,* Nitric oxide radical scavenging assay, DPPH radical scavenging assay, Lipid peroxidation inhibitory activity, Hydrogen peroxide scavenging assay

Abstract

The aim of the study was to examine the possible antioxidant activities of the methanolic extracts of medicinal plants, *Eclipta alba* and *Lippia nodiflora*. We examined for such properties such as nitric oxide radical scavenging assay, DPPH radical scavenging activity, lipid peroxidationinhibitory activity and hydrogen peroxide scavenging assay. Our results showed thatthe nitric oxide radical scavenging activity of methanolic extract of *Eclipta alba* and *Lippia nodiflora* was 2.09, 4.20, 7.37, 7.56 and 13.88 % inhibition and 8.84, 11.64, 27.72, 52.98 and 62.52 % inhibition in 62.5, 125, 250, 500 and 1000 μg/ml of extract respectively. The IC_{50} value of the methanolic extract of *Eclipta alba, Lippia nodiflora* and standard ascorbic acid for nitric oxide radical scavenging was found to be > 1000, 475.00 ± 5.00 μg/ml and 64.67 ± 1.15 μg/ml respectively.In the DPPH assay conducted on the methanolic extracts, *Eclipta alba* had the lowest IC_{50} value (162.33 ± 2.52 μg/ml) among the two medicinal plants tested and *Lippia nodiflora*showed the highest IC_{50} value of > 1000 μg/ml. The positive control ascorbic acid had an IC_{50} value of 11.67 ± 0.58 μg/ml.The IC_{50} value of the methanolic extract of *Eclipta alba, Lippia nodiflora* and standard BHA for lipid peroxide scavenging was found to be > 1000, > 1000 μg/ml and 27 ± 1.00 μg/ml respectively. The IC_{50} value of the methanolic extract of *Ecliptaalba, Lippia nodiflora* and standard ascorbic acid for hydrogen peroxide scavenging was found to be > 1000 μg/ml, > 1000 μg/ml and 64.67 ± 1.15 μg/ml

1. INTRODUCTION

The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability[1]. Reactive oxygen species (ROS) or free radicals are mediators of process of ageing. They accelerate the ageing as well as cause tissue injury and they are continuously formed as a by – product of metabolisms in aerobic organisms and are also produced on exposure to tobacco smoke, ozone, radiations, organic solvents and other environmental pollutants[2]. ROS have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart diseases, stroke, atherosclerosis, diabetes, cancer and alopecia[3,4,5] (Hertog*et al.,* 1993; Alho and Leinonen, 1999 and Duh, 1998). As oxidative stress is a major cause in scalp and hair ageing, the antioxidants are believed to check the loss of hair. Antioxidant compounds may function as free radical scavengers, complexers of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation[6].

Medicinal plant parts are commonly rich in phenolic compounds, such as Flavanoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity[7]. *In vitro* experiments on antioxidant compounds in higher plants show how they protect against oxidation damage by inhibiting or quenching free radicals and reactive oxygen species[8]. The role of these compounds as potential antioxidants can be inferred by their similarity to synthetic antioxidants of related structures. *Eclipta alba* and *Lippia nodiflora* possess therapeutic potential and therefore we investigated these two plants and analyzed their antioxidant activities. In the present study, we collected *Eclipta alba* and *Lippia nodiflora* plants prepared their methanolic extracts, and analyzed their antioxidant and free radical scavenging activities using *in vitro* methods like Nitric Oxide (NO) radical scavenging activity, DPPH radical scavenging activity, Lipid peroxidation scavenging activity and H_2O_2 scavenging activity[9].

Eclipta alba is a plant used in folk and traditional medicine for cirrohosis and infectious diseases. It is believed to prevent aging and rejuvenate hair, teeth, bone, memory, sight, hearing. *Eclipta alba*has been reviewed for its pharmacological and insecticidal activities and phytochemical constituents, antioxidant, antihepatotoxic, antihyperlipedemic, anticancer immunomodulatory, analgesic, anti-inflammatory and antidiabetic activities[10]. *Lippia nodiflora* has been reviewed for its biological activities and phytochemical constituents, antioxidant, antinociceptive antimicrobial, antipyretic, antitumor, lipid peroxide scavenging and free radical scavenging activities[11].

2. MATERIALS AND METHODS

2.1 Collection and authentication of Plant Materials

The plant specimen for the proposed study, *Eclipta alba* (L) Hassk. and *Lippia nodiflora* Linn. , were collected from the paddy fields and other irrigated fields in and around Madurai District, Tamil Nadu, India.The herbarium of these plants was identified and authenticated by Dr. D. Stephen, Professor, Department of Botany, American college of Arts and Science, Madurai, Tamil Nadu and the specimen was deposited in Department of Pharmaceutical Chemistry, Ultra College of Pharmacy, Madurai, Tamil Nadu, India.

2.2 Preparation of plant extracts

The fresh whole plant of *Eclipta alba* and *Lippia nodiflora* was washed with distilled water separately to removed unwanted foreign materials like soil and dusts. After, washed plant material was dried under shade at room temperature without direct exposure of sunrays. It was then coarsely grounded by using mechanical device. The powdered plant material was passed through sieve no 40 and stored in an airtight container for further use.

Corresponding Author:
Mr. T. Regupathi,
Ultra College of Pharmacy,
4/235, College Road, Thasildar Nagar,
Madurai – 625 020, Tamil Nadu, India
Email : poornareg@yahoo.co.in ; Mobile Number: 09443432476

The coarsely powdered plant materials of *E.alba* (2000 g) and *L. nodiflora* (2000 g) were extracted separately to exhaustion in a soxhlet apparatus for 72 hours by using Petroleum ether (60 - 80˚C) and methanol (95%) solvent (Merk and Spectrum Chemicals, India) systems. All the extracts were filtered through a cotton plug followed by whattman filter paper (No.1) and then concentrated by using a rotary evaporator at low temperature (40 - 50˚C) and reduced pressure to get 24.4 g and 108.6 g respectively. The extracts were preserved in airtight containers and kept at 4˚C until further use.

2.3 Nitric Oxide radical scavenging assay

Sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of modified GriessIlosvay reaction. In the present investigation, GriessIlosvay reagent is modified by using Naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). Nitrite ions react with Griess reagent, which forms a purple azo dye. In presence of test components, likely to be scavengers, the amount of nitrites will decrease. The degree of decrease in the formation of purple azo dye will reflect the extent of scavenging. The absorbance of the chromophore formed was measured at 540 nm.

The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (PBS, pH 7.4, 1 ml) and extract in DMSO at various concentrations or standard was incubated at 25° C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 min for completion of diazotisation. Then, 1 ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A pink colouredchromophore was formed. The absorbance of these solutions was measured at 540 nm.

2.4 DPPH radical scavenging assay

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in color and upon reaction with hydrogen donor changes to yellow color. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured at 490nm.

The assay was carried out in a 96 well microtitre plate. To 200 µl of DPPH solution, 10 µl of each of the test sample or the standard solution was added separately in wells of the microtitre plate[12]. The final concentration of the test and standard solutions used were 1000, 500, 250, 125, 62.5, 31.25 and 15.625, 7.812 µg/ml. The plates were incubated at 37° C for 30 min and the absorbance of each solution was measured at 490 nm, using a microplate reader. The ability to scavange the DPPH radical was calculated using the formula given[13].

The capability to scavenge the DPPH radical was calculated using following equation:

$$\text{DPPH Scavenged (\%)} = \frac{(A_{cont} - A_{test})}{A_{cont}} \times 100$$

Where A_{cont} is the absorbance of the control reaction,
A_{test} is the absorbance in the presence of the sample of the extracts

2.5 Lipid peroxidation inhibitory activity

Lipid peroxidation can be initiated by ROS such as hydroxyl radicals by extracting a hydrogen atom from lipids and forming a conjugated lipid radical. This reacts rapidly with oxygen to form a lipid radical until the chain reaction is terminated. The lipid peroxidation adducts may induce the oxidation of biomolecules such as DNA, proteins and other lipids resulting in cellular damage.

The reaction mixture containing egg lecithin (1ml), ferric chloride (0.02ml), ascorbic acid (0.02ml) and extract or standard (0.1ml) in DMSO at various concentrations was kept for incubation for 1 hour at 37ºC. After incubation 2 ml of 15% TCA and 2ml of 0.37% TBA were added. Then the reaction mixture was boiled for 15 min, cooled, centrifuged and absorbance of the supernatant was measured at 532 nm.

2.6 Hydrogen peroxide scavenging assay

Hydrogen peroxide is generated *in vivo* by several oxidase enzymes. There is increasing evidence that hydrogen peroxide, either directly or indirectly via its reduction product hydroxyl radical (OH^-) causes severe damage to biological system. In this method, when a scavenger is incubated with hydrogen peroxide, the decay or loss of hydrogen peroxide can be measured spectrophotometrically at 230 nm[14].

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Various concentrations of 1 ml of the extracts or standards in methanol were added to 2 ml of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm, after 10 min.

3. STATISTICAL ANALYSIS

Tests were carried out in triplicates. The mean values were calculated from the triplicate values. Values are expressed as the Mean ± SD and differences between groups were considered to be significant if $p < 0.05$.

4. RESULTS AND DISCUSSION

The Nitric Oxide radical scavenging activity of the methanolic extract of *Eclipta alba* and *Lippia nodiflora* were tabulated in Table 1 and illustrated in Fig 1 and 2. The NO radical scavenging activity of methanolic extract of *Eclipta alba* and *Lippia nodiflora* was 2.09, 4.20, 7.37, 7.56 and 13.88 % inhibition and 8.84, 11.64, 27.72, 52.98 and 62.52 % inhibition in 62.5, 125, 250, 500 and 1000 µg/ml of extract respectively. The IC_{50} value of the methanolic extract of *Eclipta alba, Lippia nodiflora* and standard ascorbic acid for nitric oxide radical scavenging was found to be > 1000, 475.00 ± 5.00 µg/ml and 64.67 ± 1.15µg/ml respectively.

The DPPH radical scavenging activity of the methanolic extract of *Eclipta alba* and *Lippia nodiflora* were tabulated in Table 2 and Fig 3 and 4. The relative stable organic radical DPPH is widely used in modeling systems to investigate the scavenging activities of several natural compounds, such as phenolics and anthocyanins, as well as crude mixtures, such as methanol or water extracts from plants. The DPPH radical is scavenged by antioxidants through the donation of electrons forming the reduced DPPH[15]. The color changes from purple to yellow after reduction, and the accompanying decrease in absorbance can be quantified at wavelength 490 nm. Table 1 shows the IC_{50} values for radical scavenging activities of methanolic extract fractions of *Eclipta alba and Lippia nodiflora* and ascorbic acid using the DPPH colorimetric method.In the DPPH assay conducted on the methanolic extracts, *Eclipta alba had* the lowest IC_{50} value (162.33 ± 2.52µg/ml) among the two medicinal plants tested and *Lippia nodiflora* showed the highest IC_{50} value of > 1000µg/ml. The positive control ascorbic acid had an IC_{50} value of 11.67 ± 0.58 µg/ml. The above IC_{50} values showed that *Eclipta alba* demonstrated higher radical scavenging activities compared to *Lippia nodiflora* which showed higher IC_{50} value.

The effect of methanolic extract of *Eclipta alba and Lippia nodiflora* and standard antioxidant BHA on the *in vitro* inhibition of lipid peroxidation is illustrated in Fig 5 and 6 and values are tabulated in Table 3. Lipid peroxide scavenging was monitored by the change in optical density of the prepared concentrations (62.5 – 1000µg/ml) and the percentage inhibition was calculated. Ascorbate/FeSO4 induced peroxidation was inhibited by methanolic extract of *Eclipta alba and Lippia nodiflora and* standard antioxidant such as BHA. It emphasized that the percentage inhibition of the methanol extract increased with concentration dependent manner. The IC_{50} value of the methanolic extract of*Eclipta alba, Lippia nodiflora and* standard BHA for lipid peroxide scavenging was found to be > 1000, > 1000 µg/ml and 27 ± 1.00 µg/ml respectively. Lipid peroxidation is the oxidative deterioration of polyunsaturated lipids. Peroxidation reactions for nonbiologicalolefinic substances are known. The peroxidation process leads to the formation of free radical intermediates, which can lead to autocatalysis[16].

The hydrogen peroxide scavenging activity of the methanolic extract of *Eclipta alba and Lippia nodiflora* were tabulated in Table 4 and illustrated in Fig 7 and 8. The hydrogen peroxide scavenging activity of methanolic extract of *Eclipta alba and Lippia nodiflora* was 21.18, 27.12, 28.38, 30.08 and 31.35 % inhibition and 16.89, 23.38, 27.36, 37.82 and 46.29 % inhibition in 62.5, 125, 250, 500 and 1000 µg/ml of extract respectively. The IC_{50} value of the methanolic extract of *Eclipta alba, Lippia nodiflora* and standard ascorbic acid for

hydrogen peroxide scavenging was found to be > 1000µg/ml, > 1000 µg/ml and 64.67 ± 1.15 µg/ml respectively.

Antioxidants are very essential for averting degenerative reactions produced by free radicals and reactive oxygen species, which have been concerned with many diseases and in food deterioration and spoilage[17]. However the safety of some of the synthetic antioxidants used in the food industry and cosmetics industry has been questioned, because recent studies recognized they might be carcinogenic[18]. Hence, there is an emerging interest in natural antioxidants, which might help to prevent oxidative damage[19]. Previous findings suggested that the *Lippia nodiflora* extract and compound isolated from the extract were considered as an effective free radical inhibitor as well as the primary antioxidants, which may limit free radical damage that takes place in the body[20].

Table 1: Nitric oxide radical scavenging activity of methanolic extract of *Eclipta alba* and *Lippia nodiflora*

Methanolic concentration of extract (µg/ml)	*Eclipta alba*		*Lippia nodiflora*		Ascorbic acid IC_{50} value (µg/ml)
	% Inhibition	IC_{50} value (µg/ml)	% Inhibition	IC_{50} value (µg/ml)	
1000	13.88	>1000	62.52	475.00 ± 5.00	64.67 ± 1.15
500	7.56		52.98		
250	7.37		27.72		
125	4.20		11.64		
62.5	2.09		8.84		

Table 2: DPPH radical scavenging activity of methanolic extract of *Eclipta alba and Lippia nodiflora*

Methanolic concentration of extract (µg/ml)	*Eclipta alba*		*Lippia nodiflora*		Ascorbic acid IC_{50} value (µg/ml)
	% Inhibition	IC_{50} value (µg/ml)	% Inhibition	IC_{50} value	
1000	80.71	162.33 ± 2.52	87.57	>1000	11.67 ± 0.58
500	68.15		84.01		
250	56.97		71.05		
125	46.55		62.38		
62.5	39.01		53.29		

Table 3: Lipid peroxidation inhibitory activity of methanolic extract of *Eclipta alba and Lippia nodiflora*

Methanolic concentration of extract (µg/ml)	*Eclipta alba*		*Lippia nodiflora*		Ascorbic acid IC_{50} value (µg/ml)
	% Inhibition	IC_{50} value (µg/ml)	% Inhibition	IC_{50} value	
1000	27.57	>1000	35.63	>1000	27 ± 1.00
500	19.03		24.38		
250	10.49		20.92		
125	7.77		20.40		
62.5	5.63		18.84		

Table 4: Hydrogen peroxide scavenging activity of methanolic extract of *Eclipta alba and Lippia nodiflora*

Methanolic concentration of extract (µg/ml)	*Eclipta alba*		*Lippia nodiflora*		Ascorbic acid IC_{50} value (µg/ml)
	% Inhibition	IC_{50} value (µg/ml)	% Inhibition	IC_{50} value (µg/ml)	
1000	31.35	>1000	46.29	>1000	64.67 ± 1.15
500	30.08		37.82		
250	28.38		27.36		
125	27.12		23.38		
62.5	21.18		16.89		

Fig. 1. Nitric oxide radical scavenging assay of methanolic extract of *Eclipta alba*

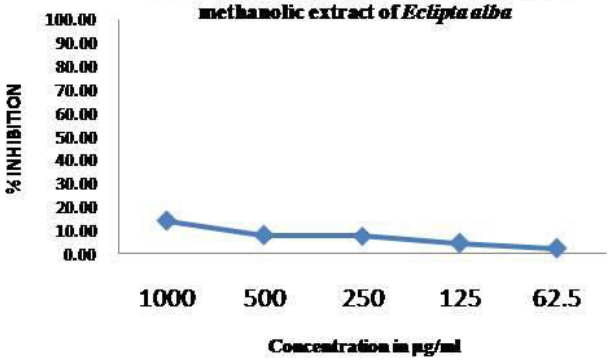


Fig. 2. Nitric oxide radical scavenging assay of methanolic extract of *Lippia nodiflora*

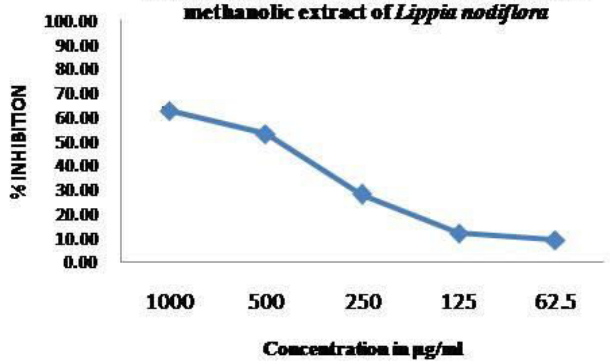


Fig. 3. DPPH scavenging assay of methanolic extract of *Eclipta alba*

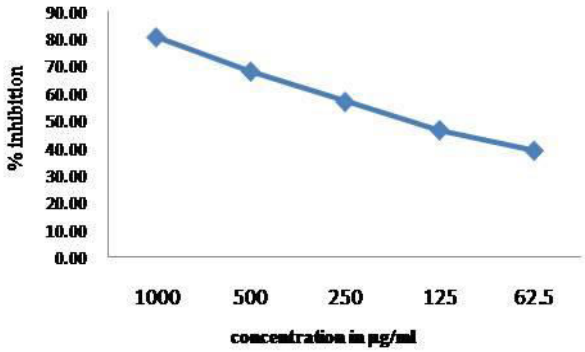
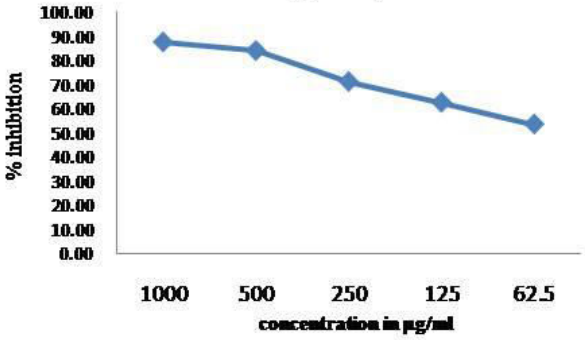
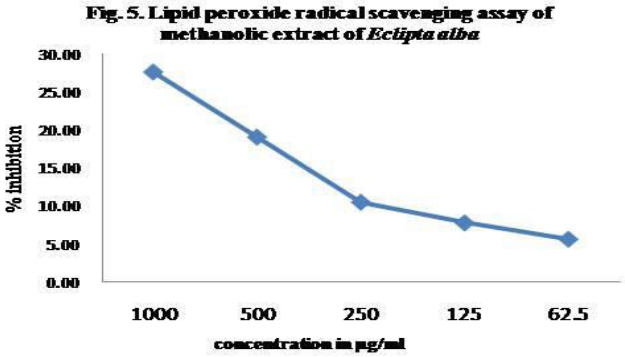
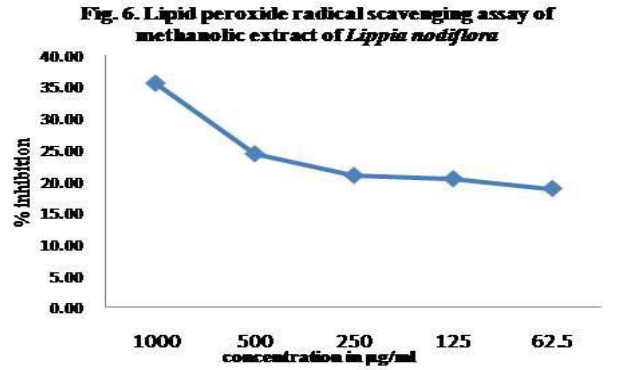
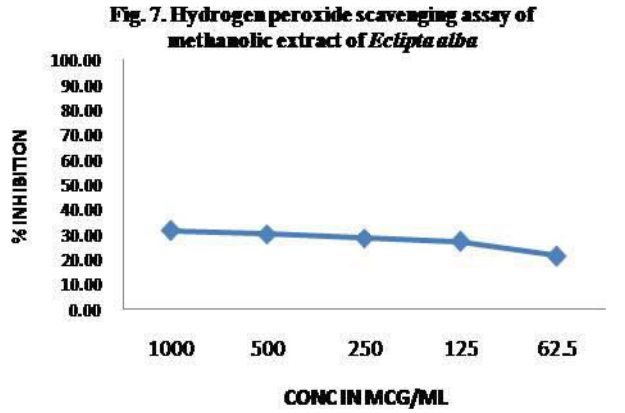


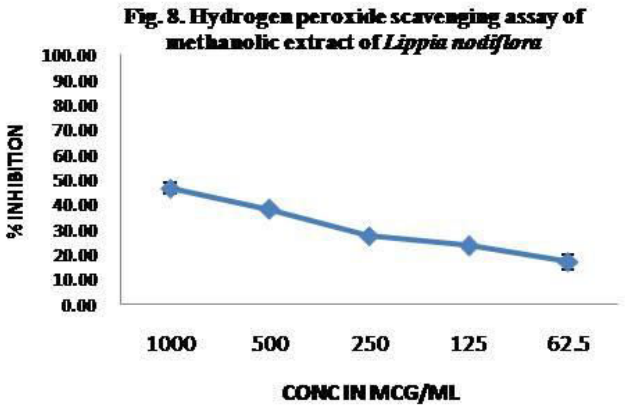
Fig. 4. DPPH scavenging assay of methanolic extract of *Lippia nodiflora*











5. CONCLUSION

The present study was projected to assess the antioxidant and free radical scavenging activities of extracts and fractions from aerial parts of *Eclipta alba and Lippia nodiflora* by using *in vitro* antioxidant methods. From the present results, it concludes that methanolic extract of *Eclipta alba*and *Lippia nodiflora* had significant inhibition of *in vitro* nitric oxide scavenging activity, DPPH scavenging activity, lipid peroxidation and hydrogen peroxide scavenging activity. Therefore these extracts can be considered as a potential source of natural antioxidant agent.

ACKNOWLEDGEMENT

The authors are grateful to the Ultra College of Pharmacy, Madurai for providing the necessary facilities to carry out the studies.

REFERENCES

1. Karthikumar S, Vigneswari K et al, "Screening of antibacterial and antioxidant activities of leaves of *Ecliptaprostrata* (L)". *Sci. Res. Essays*. 2007; 2(4): 101-04.
2. Singh N, Singh BR et al. "Oxidative DNA damage protective activity, antioxidant and anti – quorum sensing potentials of *Moringa oleifera". Food and Chemical Toxicology,* 2009, 47(6): 1109-1116.
3. Hertog MGL, Feskens EJM et al "Dietary antioxidant flavonoids and risk of coronary heart disease: The zupthen elderly study", *Lancet,* 1993, 342: 1007-1014.
4. Alho H, Leinonen J. "Total antioxidant activity measured by chemiluminescence method". *Methods Enzymol*. 1999, 299: 3-15.
5. Duh PD. "Antioxidant activity of Burdock: Its scavenging effect on free-radical and active oxygen". *JAOCS*. 1998. 75: 455-463.
6. Andlauer W, Furst P. "Antioxidative power of phytochemicals with special reference to cereals". *Cereal Foods World,* 1998, 43:356-359.
7. Packer L, Rimbach G et al. "Antioxidant activity and biologic properties of a procyanidin rich extract from pine (*Pinus maritima*) bark, pycnogenol". *Free Radic. Biol. Med*. 1999, 27: 704 - 724.
8. Ali BH, Blunden G, et al. "Some Phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale*): a review of recent research". *Food Chem. Toxicol*. 2008, 46: 409 - 420.
9. SangitaShukla, Saluja AK, et al. "*In vitro* antioxidant activity of aerial parts of *Lippia nodiflora* Rich Pharmacologyonline". *J. Pharm*. 2009, 2: 450-459.
10. Jadhav VM, Thorat RM, et al "*Eclipta alba* (L.) Hassk. – Kesharaja : A Review". *J. Pharmacy Res.,* 2009, 2(8): 1236 – 1241.
11. Faheem Amir, Wan Sinn Yam et al. "Chemical constituents and biological applications of *Lippia nodiflora*". *J. Pharm*, 2011, 2(3): 101-105.
12. Yen GH, Chen HY. "Antioxidant activity of various extract in relation to their antimutagenicity". *J. Agric. Food Chem.,* 1995, 43: 27-32.
13. Duan XJ, Zhang WW et al. "Evaluation of antioxidant property of extract and fraction obtained from a red algae, *Polysiphoniaurceolata*". *Food Chem.,* 2006, 95: 37-43.
14. Jayaprakasha GK, Lingamallu JR et al. "Antioxidant activities of flavidin in different *in-vitro* model systems". *Organic Medicinal Chemistry*. 2004, 12: 5141-5146.
15. Singh RP, Murthy KNC, et al. "Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* models". *J. Agricul. Food Chemistry*. 2002, 50(1): 81-86.
16. Plaa GL, Witschi. "Chemicals, drugs and lipid peroxidation". *Annu. Rev. Pharmacol. Toxicol.,*1976, 16: 125-142.
17. Koleva II, Niederlander HAG et al. "An online HPLC method for detection of radical scavenging compounds in complex mixtures". *Analytical Chemistry,* 2000, 72(10): 2323-2328.
18. Whysner J, Wang CX, et al. "Dose response of promotion of butylatedhydroxyanisole in chemically initiated tumors of the rat forestomach". *Food and Chemical Toxicology,* 1994, 32(3): 215-222.
19. Silva F, Ferreres JO et al. "Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts". *Food Chemistry,* 2005, 90(1): 157-167.
20. Sudha A, Srinivasan P. "Bioassay guided isolation and antioxidant evaluation of flavonoid compound from aerial parts of *Lippia nodiflora* Linn." *International J. Pharmaceutical Sci. Res.,* 2013, 4(11): 4263-4271.