

## ***In vitro* Antioxidant Activity of Methanol Extract of *Lantana camara* Leaves**

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**Abstract:** The plant kingdom offers a wide range of natural antioxidant and medicinal values. These are made possible as a result of plethora of different chemical constituents of these plants. The study was carried out to ascertain the antioxidant status of *Lantana camara* leaves and its potential for scavenging free radical species in the body. The quantitative phytochemical screening of *Lantana camara* showed that the leaves contain flavonoids (11.08±0.05 mg/g), tannins (9.0±0.03 mg/g), alkaloids (9.76±0.02 mg/g), saponin (6.07±0.06 mg/g), reducing sugar (4.86 ±0.05 mg/g) and carbohydrate (5.08± 0.03 mg/g). Micronutrients analysis showed vitamin A 0.50 mg/100g, vitamins C 6.5 mg/100g and vitamin E 1.6 mg/100g and total phenolic compounds 2.36 GAE (Gallic Acid Equivalent). The antioxidant activity of the extract was determined on 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), superoxide (O<sub>2</sub><sup>-</sup>), hydroxyl (OH) and nitric oxide (NO) radicals. The percentage inhibition of methanol extract of *Lantana camara* leaves extracts on DPPH radical was concentration dependent with an effective concentration at fifty percent (EC<sub>50</sub>) of 27.56±0.02µg/ml compared to standard (ascorbic acid) with EC<sub>50</sub> of 11.07±0.03µg/ml. The extract inhibited hydroxyl radical-induced 2-deoxy-ribose degradation, EC<sub>50</sub> (22.18 ± 0.02 µg/ml) compared to the standard (α-tocopherol) EC<sub>50</sub> (18.60 ± 0.02 µg/ml). The superoxide anion radical was inhibited in a concentration dependent manner. The extract had a significant O<sub>2</sub><sup>-</sup> anion radical scavenging ability, EC<sub>50</sub> was 27.94 ± 0.03 µg/ml compared to ascorbic acid standard EC<sub>50</sub> of 62.47 ± 0.02 µg/ml. The overall scavenging activity of the extract on nitric oxide radical showed that the extract at 500 µg/ml was most potent in scavenging nitric oxide radical compared to ascorbic acid standard at 500 µg/ml. Hydroxyl radical showed the highest anti-radical power (ARP) compared to DPPH and superoxide. Superoxide free radical recorded the lowest anti-radical power. The extract was found to be effective scavengers of the above-mentioned free radicals. These results show that the extract possesses antioxidant potential, which is very beneficial in tackling the damages caused by free radicals.

**Key words:** DPPH Radical • Hydroxyl Radical • Nitric Oxide Radical • Superoxide Radical • Ascorbic Acid Antioxidant Activity • Scavenging

### **INTRODUCTION**

Nature has provided an excellent source of remedies to cure many of the ailments of mankind. In ancient days, almost all the medicine used were from natural sources, particularly from plants. Plants continue to be important sources of new drugs even now. The importance of biological, chemical and pharmacological evaluation of plant derived agents used in the treatment of human ailments has been increasingly recognized in the last decades [1]. World health organisation (WHO) currently encourages, recommends and promotes traditional herbal medicines due to their ease of availability, low cost, safety and people's faith in such remedies [2]. In the last few decades, many of traditionally known plants have

been extensively studied by advanced scientific techniques and reported for various medicinal properties which include: anticancer activity, antioxidant activity, anti-inflammatory, antidiabetic, antibacterial, antifungal and hepato-protective activities [3].

Free radical reactions have been implicated in the pathology of many human diseases such as atherosclerosis, ischemic heart disease, diabetes, neurodegenerative diseases and disease conditions such as ageing process, inflammation and immune-suppression. A number of plants and plant products have been reported to protect against free radical induced damages in various experimental models [4]. Exposure to ionizing radiation, smoking, herbicides, pesticides and fried foods generate free radicals. Reactive oxygen species (ROS) are

chemically reactive molecules containing oxygen which includes superoxide ( $O_2^-$ ), hydroxyl ( $OH^\bullet$ ), peroxy ( $ROO^\bullet$ ). Nitric oxide ( $NO^\bullet$ ) and nitrogen dioxide ( $NO_2^\bullet$ ) are two nitrogen free radicals. Oxygen and nitrogen free radicals can be converted to other non-radical reactive species, such as hydrogen peroxide ( $H_2O_2$ ), hypochlorous acid ( $HOCl$ ), hypobromous acid ( $HOBr$ ) and peroxyxynitrite ( $ONOO^\bullet$ ) [5]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in animals and humans under physiologic and pathologic conditions. During energy transduction, a small number of electrons “leak” to oxygen prematurely, forming the oxygen free radical superoxide, which has been implicated in the patho-physiology of a variety of diseases [6]. Superoxide anion,  $O_2^-$  can capture further electron to form hydrogen peroxide. Hydrogen peroxide ( $H_2O_2$ ) is toxic, injurious and can further react with “superoxide” anion, in the presence of ferrous ion or copper to form “hydroxyl” radical and “singlet oxygen”. Whenever superoxide anion,  $O_2^-$  is formed in the tissues, it will lead to the formation of another free radical and hydrogen peroxide [7]. Nitric oxide ( $NO^\bullet$ ) has also been involved in a variety of biological functions, including neurotransmission, vascular homeostasis, antimicrobial and antitumor activities [8]. When antioxidants react with DPPH $^\bullet$ , which is a stable free radical, the radical becomes paired off in the presence of a hydrogen donor and is reduced to the DPPHH and as consequence the absorbance decreases from the DPPH (hydrazyl radical) to the DPPH-H (hydrazine) form [9]. The hydroxyl radical has a high reactivity, making it a very dangerous radical [10]. ROS induced cell death can result from oxidative processes such as membrane lipid peroxidation, protein oxidation, enzyme inhibition, DNA and RNA damage [11].

Oxidative stress represents an imbalance between the production of reactive oxygen species and a biological system’s ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of tissues can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including protein lipids and DNA. Chemically, oxidative stress is associated with increased production of oxidizing species or significant decrease in the capability of oxidants defences, such as Gluthathione [12].

An antioxidant is a molecule capable of preventing or inhibiting the oxidation of other molecules [13]. Synthetic antioxidants such as butylated hydroxyl-toluene and butylated hydroxyl-anisole have recently been reported to

be dangerous for human health [14]. Both enzymatic and non-enzymatic antioxidants exist in the intracellular and extracellular environment to detoxify free radicals. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Examples of antioxidants are vitamin C, vitamin E, selenium and carotenoids [15]. Natural antioxidants such as phenols, flavonoids and tannins are increasingly attracting attention because they are natural disease preventing, health promoting and anti-ageing substances [16]. Antioxidants may serve the task of reducing oxidative damage in human induced by free radicals and reactive oxygen species under oxidative stress conditions.

*Lantana camara* Linn, (*Verbenaceae*) is an ornamental weed with aromatic leaves, orange, blue, yellow and bright red flowers with dark blue and black fruits (drupes). It is a low, erect vigorous shrub which can grow up to 2 - 4 meters in height [17]. *Lantana camara* has been used in many parts of the world to treat a wide variety of disorders [18]. Fevers, colds, rheumatism, asthma and high blood pressure were treated with preparations from the plant [19]. The leaves are also used to treat cuts, rheumatism, ulcers and intestinal worms. Leaf extracts of *Lantana* exhibit antioxidant, antihypertensive, antimicrobial, fungicidal, insecticidal and nematicidal properties [20- 24]. *Lantana camara* oil is sometimes used for the treatment of skin itches, as an antiseptic for wounds and externally for leprosy and scabies [25]. Pharmacological investigations indicated that extracts of leaves of *Lantana camara* exhibited strong antioxidant activities [26]. Thus, the search for effective, non-toxic natural compounds with antioxidant activity has been intensified in recent years- hence the rationale of the study.

## MATERIALS AND METHODS

**Plant Material:** The leaves of *Lantana camara* were used for the study. They were collected from Amokwe village Nsukka and were identified by Mr. Alfred Ozioko of the Bioresources Development and Conservation Programme (BDCP) Research Centre Nsukka, Enugu State.

**Equipment:** The equipment used were obtained from the Department of Biochemistry, University of Nigeria and other scientific shops in Nsukka.

**Chemicals and Reagents:** The chemicals and reagents used were of analytical grade.

**Preparation of Plant Material:** The fresh leaves of *Lantana camara* were collected, washed with clean water to remove dirt and drained. They were dried under shade for several days and then pulverised into powder.

**Extraction of Plant Material:** Powdered leaves (500 g) of *Lantana camara* was macerated in one litre of methanol for 24 h. The solution was filtered with Whatman no.1 filter paper and the filtrate was concentrated to a semi-solid residue using rotary evaporator.

**Determination of Total Phenolic Contents:** Total phenolics were determined using Folin-ciocalteu reagent (FCR) as described by Velioglu *et al.* [27]. Folin-ciocalteu reagent (FCR) consists of a yellow acidic solution containing complex polymeric ions formed from phosphomolybdic and phosphotungstic heteropoly acids. Dissociation of a phenolic proton in a basic medium leads to a phenolate anion, which reduces FCR forming blue coloured molybdenum oxide. The colour intensity is directly proportional to the phenolic contents. The extract (100µl) dissolved in methanol (1mg/ml) was mixed with 750µl of Folin-Ciocalteu reagent (diluted 10-fold in dH<sub>2</sub>O), shaken slightly and allowed to stand at 22°C for 5mins; 750µl of Na<sub>2</sub>CO<sub>3</sub> (60g/l) solution was then added to the mixture. After 90 min the absorbance was measured at 725nm. Results were expressed as gallic acid equivalents. For the standard; 100µl of diluted gallic acid (0.1g gallic acid salt in 10ml of 80% methanol) was mixed with 750µl of FCR as was with the extract. The blank was made up of 1ml of FCR and 1ml of Na<sub>2</sub>CO<sub>3</sub>.

**Quantitative Scavenging Assay of 1, 1-diphenyl-2-Picrylhydrazyl (DPPH) Radical:** Scavenging activity of DPPH free radicals by the extract was assessed according to the method of Gyamfi *et al.* [28]. A solution of the extract (1.0ml) at different concentrations (2.5-80µg/ml) in 80% methanol was mixed with 1.0ml of 0.3mM DPPH in methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 25 min. The negative control was 1.0ml of 0.3mM DPPH solution plus 2.0ml of methanol. L-Ascorbic acid was used as positive control. Thereafter, the absorbance of the assay mixture was measured at 518 nm against each blank with UV/Vis spectrophotometer. Lower absorbance of the reaction mixture indicated higher radical scavenging activity. DPPH radical scavenging activity was calculated using the equation:

$$\% \text{ scavenging activity} = 100 - \frac{(\text{ABS sample} - \text{ABS blank})}{\text{ABS control}} \times 100$$

The EC<sub>50</sub> value was calculated using a plot of % inhibition against different concentrations of the extract and it represented the concentration of the sample leading to 50% reduction of the initial DPPH concentration.

**Hydroxyl Radical (OH) Scavenging Assay:** The 2-deoxyribose assay was used to determine the scavenging effect of the extract on the hydroxyl (OH) radical as reported by Halliwell *et al.* [29]. Each reaction mixture contained the following concentration of reagents in a volume of 1.0ml: 2-deoxyribose (2.5µM) potassium phosphate buffer (pH 7.4, 20mM), FeCl<sub>3</sub> (100µM), EDTA (104µM), H<sub>2</sub>O<sub>2</sub> (1mM) and L-ascorbic acid. These were prepared immediately before using in distilled water. The mixtures were incubated for 1hour at 37°C, followed by addition of 1.0ml of 1% (w/v) thiobarbituric acid (TBA) in 0.05M NaOH and 1.0ml of 2.8%(w/v) trichloroacetic acid(TCA). The resulting mixture was heated for 15min at 100°C. After cooling on ice, absorbance was measured at 532 nm. The inhibition of 2-deoxyribose degradation expressed in percentage was calculated using the equation:

$$\% \text{ inhibition} = 100\% \times \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}}$$

IC<sub>50</sub> value represented the concentration of the extract that caused 50% inhibition of 2-deoxyribosedeградation. All determinations were carried out in triplicate.

**Superoxide Radical Scavenging Assay:** The method of Martinez *et al.* [30] was used to determine superoxide dismutase in superoxide radical- scavenging assay. The assay based on the capacity of the extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). Each 3ml reaction mixture contained 0.05M phosphate – buffered saline (PBS) (pH 7.8), 13mM methionine, 2µM riboflavin, 100µM EDTA, NBT (75µM) and 1.0ml of test sample solutions (10-250 µg/ml). The tubes were kept in front of a fluorescent light for 20 minutes and absorbance was read at 560 nm. The entire reaction assembly was enclosed in a box lined with aluminium foil. Identical tubes containing reaction mixture were kept in the dark and served as blanks. The percentage inhibition of superoxide generation was estimated by comparing the absorbance of the control and those of the reaction mixture containing test sample using the equation:

$$\% \text{ inhibition} = 100\% \times \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}}$$

**In vitro** Nitric Oxide Radical (NO) Scavenging Assay Nitric oxide generated from sodium nitroprusside (SNP) was measured according to the method of [31]. The reaction mixture (5.0ml) containing SNP (5 mM) in phosphate buffered saline (pH 7.3), with plant extract at different concentrations, was incubated at 25°C for 180min in front of a visible polychromatic light source. The NO radical thus generated interacted with oxygen to produce the nitric ion (NO<sub>2</sub><sup>-</sup>) which was assayed at 30 min intervals by mixing 1 ml of incubation mixture with an equal amount of Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% naphthyl-ethylene-dihydrochloride). The absorbance of the chromophore (purple azo dye) formed during the diazotisation of nitrite ions with sulphanilamide and subsequent coupling with naphthyl-ethylene-diamine-dihydrochloride was measured at 546 nm. Each experiment was carried out in triplicates.

## RESULTS

### Effect of Radical Scavenging Activity of MELC Leaves on DPPH:

The ability of the extract to scavenge DPPH radicals was investigated at various concentrations of the extract. The addition of MELC to the DPPH solution caused a rapid decrease in absorbance at 518 nm indicating the good scavenging capacity of the extract as shown in Table 1, the extract possessed substantial dose-dependent antioxidant activity and the activity was compared to that of L-ascorbic acid, which was used as a control antioxidant. The percentage inhibition of extract of *Lantana camara* leaves was concentration dependent with an effective concentration at fifty percent (EC<sub>50</sub>) of 27.56±0.02µg/ml compared to that of standard (ascorbic acid) with EC<sub>50</sub> of 11.07±0.03µg/ml.

### Scavenging Activity of Methanol Extracts of *Lantana camara* Leaves (MELC) on Hydroxyl Radical (OH<sub>•</sub>):

The radical scavenging potential of the extract was further assessed by investigating its ability to scavenge •OH radical using a Fe<sup>3+</sup> dependent hydroxyl radical generation assay. The effect of *Lantana camara* extract on •OH radicals generated by Fe<sup>3+</sup> ions was measured by determining the degree of deoxyribose degradation, an indicator of thiobarbituric acid-malonaldehyde (TBA-MDA) adduct formation. The extract was observed to inhibit hydroxyl radical-induced deoxyribose degradation, EC<sub>50</sub> (22.18 ± 0.02) compared to that of the standard (α-tocopherol) EC<sub>50</sub> (18.60 ± 0.02) (Table 2).

Table 1: Percentage Inhibition of DPPH Radical by Methanol Extracts of *Lantana camara* Leaves (MELC)

Concentration (µg/ml)	Percentage (%) Inhibition
2.5	32.49±0.01
5	40.61±0.03
10	70.00±0.03
20	50.79±0.02
40	58.38±0.01
80	68.02±0.02
EC <sub>50</sub> (µg/ml)	27.56±0.02 (MELC) 11.07±0.03 (ascorbic acid standard)

Values are represented as mean ± SEM (n=3)

Table 2: Hydroxyl Radical Scavenging Activity of MELC

Concentrations (µg/ml)	Percentage (%) Inhibition
2.5	30.24±0.02
5	38.05±0.05
10	43.90±0.04
20	54.15±0.02
40	69.27 ±0.03
80	79.02± 0.02
EC <sub>50</sub> (µg/ml)	22.18±0.02 (MELC) standard's EC <sub>50</sub> 18.60 ± 0.02

Values are represented as mean ± SEM (n=3)

Table 3: Superoxide Radical Scavenging Activity of MELC

Concentrations (µg/ml)	Percentage (%) Inhibition
2.5	40.82 ± 0.04
5	43.83 ± 0.02
10	48.58 ±0.02
20	52.06 ± 0.03
40	54.27 ± 0.05
80	58.54 ± 0.01
EC <sub>50</sub> (µg/ml)	27.94 ± 0.03(MELC) standard's EC <sub>50</sub> 62.47 ± 0.02

Values are represented as mean ± SEM (n=3)

### Scavenging Capacity of Methanol Extracts of *Lantana camara* Leaves (MELC) on Superoxide Radical:

Table 3 showed the radical scavenging activity of different concentrations of the extract against superoxide radical. The superoxide anion radical was inhibited in concentration dependent manner. The extract had a significant O<sub>2</sub><sup>-</sup> anion radical scavenging ability EC<sub>50</sub> of 27.94 ± 0.03 µg/ml compared to ascorbic acid EC<sub>50</sub> of 62.47 ± 0.02 µg/ml. The lower EC<sub>50</sub> of the extract implies that the extract was more effective in scavenging superoxide radicals than the standard.

### Effect of Methanol Extract of *Lantana camara* Leaves (MELC) on Nitric Oxide (NO) Radical:

Sodium nitro-prusside (SNP) in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions (NO<sub>2</sub><sup>-</sup>). At the first 90 minutes there was no significant reduction in nitrite

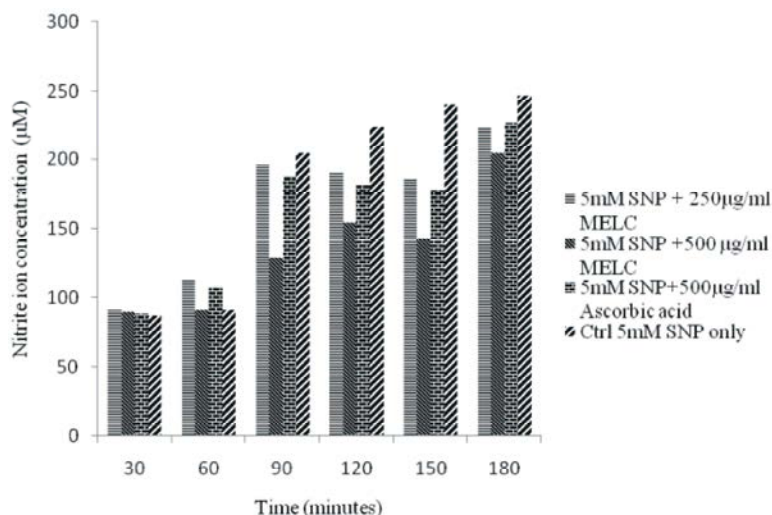


Fig. 1: Nitric Oxide Radical Scavenging Activity of MELC

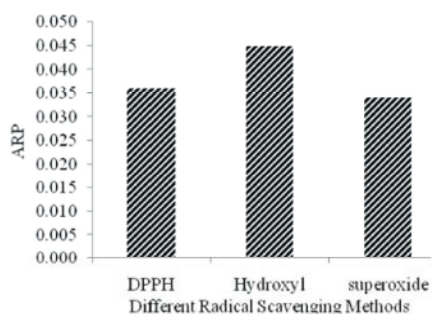


Fig. 2: Anti Radical Power (ARP) of the MELC against the various Free Radicals

Table 4: Antioxidant Vitamins and Total Phenolic Contents of Methanol Extract of *Lantana Camara* Leaves

Vitamins	Concentrations
A	0.50 mg/100g
E	1.64mg/100g
C	6.585mg/100g
Total Phenolics	2.36±0.01 GAE

ions showing little scavenging activity of nitric oxide by both the extract and ascorbic acid standard. At 90 - 180 minutes, 250, 500 µg/ml of the extract and 500 µg/ml of ascorbic acid scavenged the nitric oxide which is shown as a reduction in the concentration of nitrite ions. The overall scavenging activity of the extract on nitric oxide radical showed that the extract at 500 µg/ml was most potent in scavenging nitric oxide radical compared to ascorbic acid standard at 500 µg/ml with respect to other concentrations of the extract as shown in Fig. 1.

**Comparison of the Anti-Radical-Power (ARP) of the Extract against DPPH., Superoxide Radical and Hydroxyl**

**Radical Scavenging Activity:** Fig. 2 shows the anti-radical power exhibited by the extract against hydroxyl radical, DPPH and superoxide. The extract showed highest ARP against hydroxyl radical, followed by DPPH radical and finally superoxide radical. Anti-Radical Power (ARP) is the inverse of the effective concentration (EC<sub>50</sub>) and it shows the strength of the extracts in reducing the free radicals (DPPH, O<sub>2</sub><sup>-</sup> and OH). The higher the ARP the more powerful the extract was in reducing the free radical with respect to the method used.

**Antioxidant Vitamins and Total Phenolic Contents of Methanol Extracts of *Lantana Camara* Leaves (MELC):**

Table 4 shows that vitamin A (fat soluble antioxidant vitamin) has the lowest concentration (0.50 mg/100g) compared to those of vitamins C and E with 6.5 and 1.6mg/100g respectively. The total phenolic compound detected was 2.36 GAE (gallic acid equivalent).

**DISCUSSION**

Free radicals are involved in many disorders such as neurodegenerative diseases, cancer and rheumatoid arthritis. Antioxidants, through their scavenging power are useful for the management of these ailments. In the present study, leaves of *Lantana camara*, traditionally used for the treatment of various disorders, were studied for their free radical scavenging activities on such radicals as DPPH, superoxide, hydroxyl and nitric oxide radicals. Natural antioxidants which could be secondary metabolites make plants to be beneficial medicinally [32]. In the present study, the extract was found to be effective

scavenger against DPPH radical. It was observed that the more the decolourization of DPPH, the more its reducing ability [33, 34], suggesting that the extract is capable of donating a hydrogen atom to the stable free radical (DPPH). Quantitative DPPH radical scavenging assay also revealed the ability of the extract to donate hydrogen atom to the stable free radical, the activity increasing in a concentration dependent manner. The  $EC_{50}$  value for DPPH scavenging activity of the extract was found to be  $27.56 \pm 0.02 \mu\text{g/ml}$  compared to a known strong antioxidant (Ascorbic acid) with a value of  $11.07 \pm 0.02 \mu\text{g/ml}$ , thereby suggesting a moderate scavenging activity of the extract. Hydroxyl radical is a potent extremely reactive oxidising radical that reacts with most bio-molecules in diffusion-controlled rates [35]. This radical has the capacity to join nucleotides in DNA and as well cause strand breakage, which contributes to condition such carcinogenesis, mutagenesis and cytotoxicity. The extract showed hydroxyl radical scavenging activity with an effective concentration at fifty percent ( $EC_{50}$ ) of  $22.18 \pm 0.02 \mu\text{g/ml}$  compared to that of the standard  $\alpha$ -tocopherol's  $EC_{50}$  of  $18.60 \pm 0.01 \mu\text{g/ml}$  thereby suggesting that the extract is a potentially high radical scavenger. The extract was found to inhibit the degradation of 2- deoxy-ribose generated from the  $Fe^{3+}$ -dependent system. The extract could be acting as a chelator of the  $Fe^{3+}$  ions in the system, thereby preventing them from complexing with the deoxyribose, or donating hydrogen atoms and accelerating the conversion of  $H_2O_2$  to  $H_2O$  [36]. The observed ability of the extract to scavenge or inhibit the  $\cdot OH$  radical indicates that the extract could significantly inhibit lipid peroxidation since  $\cdot OH$  radical is highly implicated in peroxidation reaction. The extract exhibited scavenging ability for superoxide anion radicals generated from the photochemical reduction of the nitro-blue tetrazolium (NBT) resulting in the formation of blue formazan solution. The plant extract inhibited the formation of reduced nitro-blue tetrazolium (NBT) in a concentration-dependent manner and had a significant  $O_2^{\cdot -}$  anion radical scavenging ability of  $EC_{50}$  of  $27.94 \pm 0.03 \mu\text{g/ml}$  compared to ascorbic acid with  $EC_{50}$  of  $62.47 \pm 0.02 \mu\text{g/ml}$ . Flavonoids and catechins have been reported to be effective scavengers of superoxide anion radical [37]. The presence of flavonoids in the extract, therefore could be responsible for the extract's antioxidant potency. The presence of tannin was reported to inhibit the generation of superoxide [38]. This further could confirm the antioxidant property of the extract, thereby making it an agent that could ameliorate the deleterious effect of free radicals (reactive oxygen species) which are sometimes generated during natural metabolic activities. Despite the possible

beneficial effects of nitric oxide ( $NO^{\cdot}$ ), its contribution to oxidative damage is increasingly becoming evident since  $NO^{\cdot}$  can react with superoxide ion to form peroxynitrite anion, which is a potential strong oxidant that can decompose to produce  $\cdot OH$  and  $NO_2$  [39]. The extract exhibited strong  $NO^{\cdot}$  radical scavenging activity leading to the reduction of the nitrite ion concentration in the assay medium, a possible protective effect against oxidative damage. The  $NO^{\cdot}$  scavenging capacity was concentration dependent with  $500 \mu\text{g/ml}$  of the extract scavenging most efficiently as shown in Fig. 1 in the result. It has also been observed that antioxidant activity of plant extracts is not limited to phenolics. Activity may also come from the presence of other antioxidant secondary metabolites such as volatile oils, carotenoids and vitamins [40]. The presence of these vitamins in the plant may suggest their possible role in curbing the incidence of oxidative stress in humans [22]. Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids [17]. It is capable of neutralizing reactive oxygen species (ROS) in the aqueous phase before lipid peroxidation is initiated. Vitamin E, a major lipid-soluble antioxidant, is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation [25]. Beta carotene and other carotenoids are also believed to provide antioxidant protection to lipid-rich tissues [20]. Anti-Radical Power (ARP) is the inverse of the effective concentration ( $EC_{50}$ ) and it showed the strength of the extract in reducing the free radicals ( $\cdot OH$ , DPPH $\cdot$  and  $O_2^{\cdot -}$ ). The higher the ARP the more powerful the extract was in reducing the free radical with respect to the method used. *Lantana camara* leaf extract contained vitamins A, C and E, with Vitamin C having the highest concentration. The presence of these vitamins and phenolic compounds could make *Lantana camara* leaf a good source of antioxidant.

## CONCLUSION

*Lantana camara* exhibited antioxidant property within the test systems used. Since this study used *in vitro* approach, there is therefore a compelling need to check the *in vivo* antioxidant efficacy of the plant for a better biochemical knowledge and utilization of the plant.

## REFERENCES

1. Dasture, A.N., 2002. Herbal therapy. Ancient Science of Life, 21(4): 281-286.

2. Divatar, M.C., 2002. Herbal therapy. Plant Drug Evolution, 119: 1-11.
3. Mahdi, A.A., B. Rathore, S.K. Dasand B.N. Paul, 2005. Comparative studies of different organs of *N. arbortristis* in modulation of lipid peroxide levels, anti-oxidant content, cytokines and effect on hepatic and renal marker enzymes in experimental rheumatoid arthritis. *Book of abstract, 3<sup>rd</sup> International Symposium on natural antioxidants molecular mechanisms and health effects.*
4. Cacereves, A., O. Cano, L. Agular and B. Samayoa, 1990. Plants used in Guatemala for the treatment of gastrointestinal disorder. Screening of 84 plants against enterobacteria. *Journal of Ethnopharmacology*, 30(1): 55-73.
5. Muller, F.L., M.S. Lustgarten, Y. Jang, V. Richardson and R.H. Van, 2007. Trends in oxidative aging theories. *Free Radical in Biology and Medicine*, 43(4): 477-503.
6. Villano, D., M.S. Fernandez-Pachon, M.L. Moya, A.M. Troncoso and Garcia-M.C. Parilla, 2007. Radical scavenging ability of phenolic compounds towards DPPH free radical. *Talanta*, 71: 230-235.
7. Chatterjea, M.N. and R. Shinde, 2007. *Free radicals (7<sup>th</sup> Edition) JapeeBrothers Medical Publishers. New Delhi*, pp: 156-629.
8. Ghafourifar, P. and E. Cadenas, 2005. Mitochondria nitric oxide synthase. *Trends in Pharmacological Science*, 26: 190-195.
9. Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Sci.*, 7(9): 405-410.
10. Mohammad, I., I. Zafar, H. Javid, H. Hidayat, A. Manzoor, E. Asma and I.C. Muhammad, 2009. Chemical constituents and antioxidant activity of *Geranium Wallichianum*. *National Product*, 3(4): 193-197.
11. Benzie, I.F., 2003. Evolution of dietary antioxidants. *Comparative Biochemistry and Physiology. Part A. Molecular and Integrative Physiology*, 136(1): 113-126.
12. Oyedapo, O.O., F.C. Sab and J.A. Olagunju, 1999. Bioactivity of fresh leaves of *Lantana camara*. *Biomedical Letters*, 59: 179-183.
13. Schaffer, F.O. and G.R. Buettner, 2001. Redox environment of the cell as viewed through the redox state of glutathione disulphide/glutathione couple. *Free Radical in Biology and Medicine*, 30(11): 1191-1212.
14. Zhou, Y.C. and R.L. Zheng, 1991. Phenolic compounds as an analog as superoxide anion scavengers and antioxidants. *Biochemical Pharmacology*, 2: 1177-1179.
15. Awah, F.M., P.N. Uzoegwu, J.O. Oyugi, J. Rutherford, P. Ifeonu, X. Yao, K.R. Fowke and M.O. Eze, 2010. Free radical scavenging activity and immunomodulatory effect of *Stachytarphetaangustifolia* leaf extract. *Food Chemistry*, 119: 1416-1416.
16. Ozyurt, D., B.D. Ozturk and R. Apak, 2004. Determination of total flavonoid content of *Urticadioica* L. by a new method. *Adnan Menderes University (4<sup>th</sup> Edition)*, pp: 137-140.
17. Barry, A.L., 1998. The role of nitric oxide in hepatic metabolism. *Nutrition*, 14: 376-390.
18. Basu, M. and B. Hazra, 2006. Evaluation of nitric acid scavenging activity, *in vitro* and *in-vivo* of the selected medicinal plants traditionally used in inflammatory diseases. *Phytotherapy Research*, 20: 896-900.
19. Valko, M., M. Izakovic, M. Mazur, C.J. Rhodes and J. Telser, 2004. Role of oxygen radicals in DNA damage and cancer incidence. *Molecular and Cellular Biochemistry*, 266(1-2): 37-56.
20. Begun, S., B.S. Mohammad and S. Siddiqui, 1995. Triterpenoids from the aerial parts of *Lantana camara*. *Journal National Product*, 58: 1570-1574.
21. Beckman, J.S. and W.H. Koppenol, 1996. Nitric oxide, superoxide and peroxynitrite: The good, the bad and the ugly. *American Journal of Physiology-Cell Physiology*, 271: 1424-1437.
22. Day, M.D., Wiley, C. Playford and M.P. Zalucki, 2003. *Lantana- Current Management, C.J Status and Future Prospects. Australian Centre for International Agricultural Research, Canberra*, pp: 18-34.
23. Ross, I.A., 1999. Medicinal plants of the world. Chemical constituents, traditional and modern medical uses. New Jersey, *Journal of Tropical Biomedicine*, 74: 40-56.
24. Pattnaik, S. and B. Pattnaik, 2010. A study of *Lantana camara* aromatic oil as an antibacterial agent. *International Journal of Pharmaceutical Science*, 1: 32-39.
25. Lee, J.C., 2006. Effect of soy bean proteins and Genistein on blood glucose. Antioxidant enzyme activities and Lipid profile in streptozocine-induced diabetes in rats. *Bioc. Pharmacol.*, 33: 2609-2674.

26. Bondet, V., W. Brand-Williams and C. Berset, 1997. Kinetics and mechanisms of antioxidant activity using the DPPH<sup>•</sup> free radical method. *Food Science and Technology*, 30: 609-615.
27. Velioglu, Y.S., G. Mazza, L. Gao and B.D. Oomah, 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *Agriculture and Food Chemistry*, 46: 4113-4117.
28. Gyamfi, M.A., M. Yonamine and Y. Aniya, 1999. Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguine* on experimentally-induced liver injuries. *General Pharmacology*, 32: 661-667.
29. Halliwell, B., J.M.C. Gutteridge and O.I. Aruoma, 1987. The deoxyribose method: Simple "test tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry*, 165: 215-219.
30. Martinez, A.C., E.L. Marcelo, A.O. Marco and M. Moacyr, 2001. Differential responses of superoxide dismutase in freezing resistance *Solanum curtibolum* and freezing sensitive *Solanum tuberosum* subjected to oxidative and water stress. *Plant Science*, 160: 505-515.
31. Bjelakovic, G., D. Nikolova, R.G. Simonetti and C. Glood, 2004. Antioxidant supplements for prevention of gastrointestinal cancer: A systematic review and meta-analysis. *Lancet*, 364: 1219-1228.
32. Herrera, E. and C. Barbas, 2001. Vitamin E: action, metabolism and Perspectives. *Journal of Physiological Biochemistry*, 57(2): 43-56.
33. Pacher, P., J.S. Beckman and L. Liaudet, 2007. Nitric oxide and peroxynitrite: In health and disease. *Physiological Reviews*, 87(1): 315-424.
34. Pervaiz, S. and M. Clement, 2007. Superoxide anion: Oncogenic reactive oxygen species? *International Journal of Biochemistry and Cell Biology*, 39: 1297-1304.
35. Prasad, K., V.A. Laxdal, M. Yu and B.L. Raney, 1996. Evaluation of hydroxyl radical scavenging property of garlic. *Biochemistry and Molecular Biology*, 154: 55-63.
36. Read, M.A., 1995. Flavonoids: naturally occurring anti-inflammatory agents. *American Journal of Pathology*, 147: 235-237.
37. Saxena, R.C., O.P. Dixit and V. Harstan, 1992. Insecticidal action of *Lantana camara* against *Callosobruchus chinensis* (*Coleoptera bruchidae*). *Journal of Stored Product Research*, 53: 230-235.
38. Sharma, S., A. Singh and O.P. Sharma, 1999. An improved procedure for isolation and purification of lantadene A, the bioactive pentacyclitriterpenoid from *Lantana camara* leaves. *Journal of Medicinal Aromatic Plants Science*, 21: 686-688.
39. Toykuni, S., 1999. Reactive oxygen species induced molecular damage and its application in pathology. *Pathology International*, 49: 91-102.
40. Traber, M.G. and J. Atkinson, 2007. Vitamin E, antioxidant and nothing more. *Free Radical in Biology and Medicine*, 43(1): 4-15.