World Journal of Science and Research. 1(2): 37-42 (2017)

ISSN: 2455 2208

Available online at http://www.harmanpublications.com

World Journal of Science and Research

Research Article Chemistry

ANTIOXIDNT ACTIVITY OF *Lanata camera* LEAVES EXTRACT - AN IN VITRO STUDY

G. Pukalarasan and C. Kathiravan*

Department of Chemistry, Rajahs Serfoji Government College (Autonomous), Thanjavur, Tamil Nadu, S. India
,

*Corresponding author

ABSTRACT

Antioxidant activity of methanolic extract of *Lanata camera* whole plant were carried out for proving its utility in free radical mediated diseases including diabetic, cardiovascular, cancer etc. The methanolic extract was screened for in vitro antioxidant activity by nitric oxide radical scavenging, oxygen radical scavenging such as DPPH scavenging, superoxide anion radical scavenging, total antioxidant, metal chelation, hydroxyl and nitric oxide scavenging activity at different concentrations. Throughout the studies extract showed marked antioxidant activity. The antioxidant activity of the extract may be due to the phytochemicals present in it. The antioxidant activity was found to be concentration dependent and may be attributed to the presence of bioflavonoids content in the of *Lanata camera* . Overall, the plant extract is a source of natural antioxidants which might be helpful in preventing the progress of various oxidative stress mediated diseases including aging.

***Citation*:** G. Pukalarasan1 and C. Kathiravan (2017). Antioxidant activity of *Lanata camera* leaves extract. *World Journal of Science and Research*. 1 (2): 37-42.

Article Info:

Received on 15th June 2017
Accepted on 15th August. 2017
Online August 2017

Keywords:
Antioxidant activity, *Lanata camera*, Radical scavenging, Reactive oxygen speceis

Corresponding author
Dr. C. Kathiravan, Assistant Professor, Department of Chemistry, Rajahs Serfoji Government College (Autonomous), Thanjavur Tamil Nadu, S. India

INTRODUCTION

The adverse effects of oxidative stress on human health have become a serious issue. Under stress, our bodies produce more reactive oxygen species (ROS) (e.g., superoxide anion radicals, hydroxyl radicals and hydrogen peroxide) than enzymatic antioxidants (e.g., superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase) and non-enzymatic antioxidants (e.g., ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione, carotenoids, and flavonoids). This imbalance leads to cell damage (Bhatia et al., 2003; Peuchant et al., 2004) and health problems (Steer et al., 2002). A lack of antioxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases, including cardiovascular diseases, cancers, neurodegenerative diseases, Alzheimer's disease and inflammatory

diseases (Velavan, 2011; Alma et al, 2003). Natural and synthetic antioxidants are beneficial to free radical mediated diseases. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have been widely used as antioxidants in the food industry and may be responsible for liver damage and carcinogenesis (Grice, 1988) For this reason, interest in the use of natural antioxidants has increased.

The World Health Organization (WHO) has estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components (Winston, 1999). Plant and its products are rich sources of a phytochemicals and have been found to possess a variety of biological activities including antioxidant potential (Velavan *et al*, 2007; Velavan, 2015). The majority of the active antioxidant constituents are flavonoids, isoflavones, flavones, anthrocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, beta-carotene, and tocopherol are known to possess antioxidant potential (Prior, 2003). With this background and abundant source of unique active components harbored in plants. The chosen medicinal plant namely as *Lanata camera* (Tamil: Unni chedi). *Lanata camera* is considered as stomachic, aperients and antiseptic. Flow and tender shoots are diaphoretic and given in fevers. Hence, the free radical scavenging activity of *Lanata camera* was not evaluated. Therefore, the present study were to investigate the free radical scavenging activity of *Lanata camera* through the free radical scavenging such as DPPH scavenging, superoxide anion radical scavenging, total antioxidant, metal chelation and iron reducing power activity.

MATERIALS AND METHODS

Chemicals

Nitro blue tetrazolium (NBT), ethylene diamine tetra acetic acid (EDTA), sodium nitroprusside (SNP), trichloro acetic acid (TCA), thio barbituric acid (TBA), potassium hexa cyano ferrate [$K_3Fe(CN)_6$], and L-ascorbic acid were purchased from Sisco Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

Plant materials

The fully mature *Lanata camera* leaves were collected in Janury 2015 from Tamil University campus, Thanjavur District, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Dr. S. John Britto, The Director, the Rabinat Herbarium and centre for molecular systematic, St. Joseph's college Trichy-Tamilnadu. India. A Voucher specimen (RCS001) has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of alcoholic extract

The collected *Lanata camera* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with different extracts (Ethanol, Petroleum ether, methanol and aqueous) for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The Lanata camera leaves extract (LCLE) was stored in refrigerator until used. Doses such as 20, 40, 60 and 80µg/ml were chosen for *in vitro* antioxidant activity.

In vitro antioxidant activity

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.* (1999). The scavenging activity of the *Lanata camera* towards superoxide anion radicals was measured by the method of Liu et al. (1997). The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis et al. (1994). The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu (1986). The scavenging activity for hydroxyl radicals was measured with Fenton reaction by the method of Yu *et al*., (2004). Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964).

Statistical analysis: Tests were carried out in triplicate for 3–5 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically estimated using a nonlinear regression algorithm.

RESULTS AND DISCUSSION

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals fruits and herbs and each works differently. The phytochemical screening of *Lanata camera* reported that the presence of flavonoids, terpenoids, steroids, tannin, saponins, glycosides, phlopatannins, carbohydrate, triterpenoids, protein, alkaloids and anthroquinones (Sanjeeb Kalita,, 2012).

DPPH Assay

DPPH radical scavenging activity of *Lanata camera* extract and standard as ascorbic acid are presented in Fig 1. The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants (Nuutila *et al.,* 2003). Recently, the use of the DPPH• reaction has been widely diffused among food technologists and researchers, for the evaluation of free radical scavenging activity on extracts from plant, food material or on single compounds. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 2, 2-diphenyl-1-picryl hydrazine is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole. The proton transfer reaction of the DPPH• free radical by a scavenger (A-H) causes a decrease in absorbance at 517 nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH• is thought to be due to their hydrogen donating ability (Sindhu and Abraham, 2006). The half inhibition concentration (IC_{50}) of plant extract and ascorbic acid were 48.51µg ml^{-1} and 41.81 µg ml^{-1} respectively**.** The plant extract exhibited a significant dose dependent inhibition of DPPH activity. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay activity is near to standard as ascorbic acid.

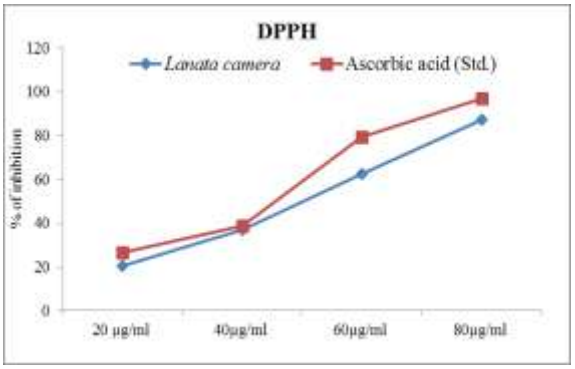

Fig. 1. DPPH radical scavenging activity of *Lanata camera*

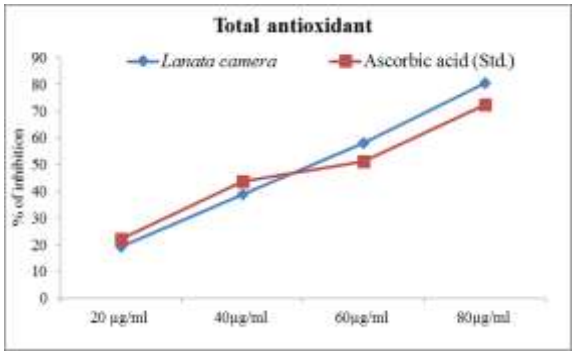

Fig. 2.Total antioxidant assay of *Lanata camera*

Total antioxidant activity

The yield of the methanol extract of the plant extract and its total antioxidant capacity are given in Fig. 2. Total antioxidant capacity of *Lanata camera* extract is expressed as the number of equivalents of ascorbic acid. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extract (Prieto et al., 1999). Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the plant extract. The half inhibition concentration (IC_{50}) of plant extract and ascorbic acid were 50.80µg ml^{-1} and 45.48 µg ml^{-1} respectively**.**

Superoxide anion radical scavenging activity

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, is very harmful to the cellular components in a biological system (Korycka-Dahl & Richardson, 1978). The superoxide anion radical scavenging activity of the extract from Lanata camera assayed by the PMS-NADH system was shown in Fig 3. The superoxide scavenging activity of *Lanata camera* was increased

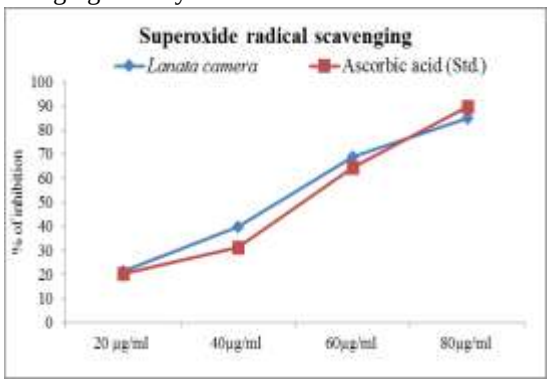

Fig. 3.Super oxide scavenging activity of *Lanata camera*

markedly with the increase of concentrations. The half inhibition concentration (IC_{50}) of *Lanata camera* was 48.89µg ml^{-1} and ascorbic acid were 46.60µg ml^{-1} respectively**.** These results suggested that *Lanata camera* had notably superior superoxide radical scavenging effects.

The ferrous ion chelating activity

Ferrozine can make complexes with ferrous ions. In the presence of chelating agents, complex (red colored) formation is interrupted and as a result, the red color of the complex is decreased. Thus, the chelating effect of the coexisting chelator can be determined by measuring the rate of color reduction. The formation of the ferrozine– Fe^{2+} complex is interrupted in the presence of aqueous extract of *Lanata camera* , indicating that have chelating activity with an IC_{50} of 51.36µg ml^{-1} and ascorbic acid was 45.91µg ml^{-1} respectively (Fig. 4). Ferrous iron can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals (Halliwell, 1991; Fridovich, 1995). Metal chelating activity can contribute in reducing the concentration of the catalyzing transition metal in lipid peroxidation. Furthermore, chelating agents that form s bonds with a metal are effective as secondary antioxidants because they reduce the redox potential, and thereby stabilize the oxidized form of the metal ion (Gordon, 1990). Thus, *Lanata camera* demonstrate a marked capacity for iron binding, suggesting their ability as a peroxidation protector that relates to the iron binding capacity

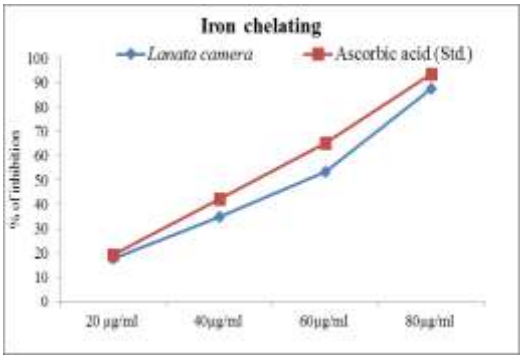

Fig. 4. Ferrous iron chelating activity of *Lanata camera*

Hydroxyl radical scavenging activity of *Lanata camera*

Hydroxyl radical scavenging activity of ethanolic extract was measured by 1, 10 phenanthroline-Fe^{2+} complex oxidation method. Fe^{2+} was formed when ferrous sulphate added to hydrogen peroxide. This formed ferrous ion reacts with 1, 10 phenanthroline and forms 1, 10 phenanthroline Fe^{2+} complex which is acts as indicator in oxidation reduction reaction. Simultaneously the hydroxyl radical formed from the H_2O_2-Fe^{2+} reaction mixture oxidize Phenanthroline - Fe^{2+} into Phenanthroline – $Fe3^{+}$ complex. Presence of free radical scavenger in the extract reduces the oxidation reaction accompanied with reduction in the absorbance which can be measured quantitatively at 560 nm (Olabinri et al., 2010). Hydroxyl radical scavenging activity of *Lanata camera* increased with increasing dosage (Fig 5). The half inhibition concentration (IC_{50}) of *Lanata camera* was 46.10µg/ml-1 and ascorbic acid were 35.26 µg/ml-1 respectively..

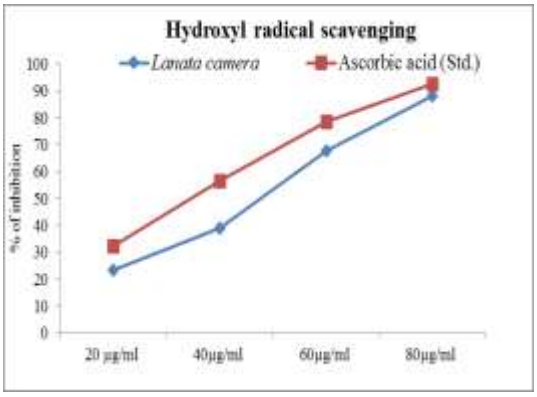

Fig.5 . Hydroxyl radical scavenging activity of *Lanata camera*

Nitric oxide scavenging activity of *Lanata camera*

Nitric oxide (NO•) released from sodium nitroprusside (SNP) has a strong NO^{+} character which can alter the structure and function of many cellular components. The extract of *Lanata camera* exhibited good NO• scavenging activity leading to the reduction of the nitrite concentration in the assay medium. The NO• scavenging capacity was concentration dependent with 80µg/ml scavenging most efficiently. The *Lanata camera* in SNP solution significantly inhibited the accumulation of nitrite, a stable oxidation product of NO• liberated from SNP in the reaction medium with time compared to the standard ascorbic acid. The toxicity of NO• increases when it reacts with superoxide to form the peroxynitrite anion (•ONOO-), which is a potential strong oxidant that can decompose to produce •OH and NO_2 (Pacher et al., 2007). The present study shows that *Lanata camera* leaf extract has a potent nitric oxide scavenging activity. The nitric oxide scavenging activity of *Lanata camera* increased with increasing concentrations (Fig 6). The half inhibition concentration (IC_{50}) of *Lanata camera* was 46.10µg/ml-1 and ascorbic acid were 35.26 µg/ml-1 respectively.

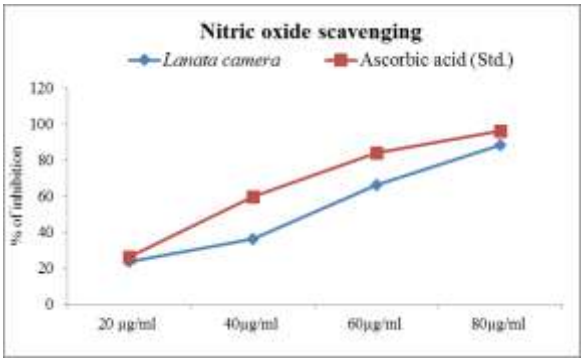

Fig 6 Nitric oxide scavenging activity of *Lanata camera*

CONCLUSION

The results of the present study showed that the extract of *Lanata camera* extract which contains of flavonoids and polyohenols. These phytochemicals are exhibited the greatest antioxidant activity DPPH scavenging, superoxide anion radical scavenging, total antioxidant, metal chelation, hydroxyl radical and nitric oxide scavenging activity which participate in various pathophysiology of diseases including cancer, diabetic, ageing etc. This work has gathered experimental evidence on the *Lanata camera* extract as natural antioxidant for its capacity to scavenge reactive oxygen and nitrogen species and protect cells/organism from oxidative damage and thus could be an effective against oxidative stress. In addition, the *Lanata camera* extract found to contain a noticeable amount of total phenols which plays a major role in controlling antioxidants. Thus, it can be concluded that *Lanata camera* extract can be used as an accessible source of natural antioxidants with consequent health benefits.

REFERENCES:

Alma MH, Mavi A, Yildirim A, Digrak M, Hirata T (2003). Screening chemical composition and antioxidant and antimicrobial activities of the essential oils from Origanum syriacum L. growing in Turkey. Biol. Pharm. Bull. 26: 1725–1729.

Bhatia, S., Shukla, R., Madhu, S.V., Gambhir, J.K. and Prabhu, K.M., 2003, Antioxidant status, lipid peroxidation and NO end products in patients of type 2 diabetes mellitus with nephropathy. Clin Biochem, 36: 557–562.

Dinis TCP, Madeira VMC, Almeidam LM (1994). Action of phenolic derivates (acetoaminophen, salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and peroxyl radicals scavengers. Achieves of Biochemistry and Biophysics, 315: 161-169.

Diplock AT (1997). Will the 'good fairies' please prove to us that vitamin E lessens human degenerative disease? Free Radical Research, 27: 511-532.

Gordon MH (1990). The mechanism of the antioxidant action in vitro. In B. J. F. Hudson, Food Antioxidants, (pp. 1-18). London: Elsevier.

Grice, H.P., 1988, Enhanced tumour development by butylated hydroxyanisole (BHA) from the prospective of effect on forestomach and oesophageal squamous epithelium. Food Chem Toxicol, 26: 717–723.

Halliwell B (1991). Reactive oxygen species in living systems: source, biochemistry, and role in human disease. The American Journal of Medicine, 91: S14-S22.

Halliwell B, Gutteridge JMC (1993). Free Radicals in Biology and Medicine. (pp. 419-422). Oxford: Clarendon.

Harborne JB (1973). Phytochemical methods, London. Chapman and Hall, Ltd. pp. 49-188.

Harborne JB (1984). Phytochemical Methods. A Guide to Modern Technique of Plant Analysis. London: Chapman and Hall.

Korycka-Dahl M, Richardson M (1978). Photogeneration of superoxide anion in serum of bovine milk and in model systems containing riboflavin and aminoacids. Journal of Dairy Science, 61: 400-407.

Liu F, Ooi VEC, Chang ST (1997). Free radical scavenging activity of mushroom polysaccharide extracts. Life Sci. 60: 763-771.

Sanjeeb Kalita, Gaurav Kumar, Loganathan Karthik, Kokati Venkata Bhaskara Rao (2012) A Review on Medicinal Properties of *Lantana camara* Linn.. Research J. Pharm. and Tech. 5(6): 711-717

Nuutila, A. M., Pimia, R. P., Aarni, M., & Caldenty, K. M. O. (2003). Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. Food Chemistry, 81, 485–493.

Okhawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95: 351-358.

Oyaizu M (1986). Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine. Japanese Journal of Nutrition, 44: 307-315.

Peuchant, E., Brun, J., Rigalleau, V., Dubourg, L., Thomas, M. and Daniel, J., 2004, Oxidative and antioxidative status in pregnant women with either gestational or type 1 Diabetes. Clin Biochem, 37: 293–298.

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Speci.c application to the determination of vitamin E. Analytical Biochemistry, 269, 337–341.

Prior RL (2003). Fruit and vegetables in the prevention of cellular oxidative damage. American Journal of Clinical Nutrition. 78 570S-578S.

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidative properties of xanthum on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry, 40, 945–948.

Sindhu M, Abraham TE. (2006) In vitro antioxidant activity and scavenging effects of Cinnamomum verum leaf extract assayed by different methodologies. Food and Chemical Toxicology 44 198–206.

Sofowara A (1993). Medicinal plants and Traditional medicine in Africa.Spectrum Books Ltd, Ibadan, Nigeria. pp. 191-289.

Steer, P., Milligard, J., Sarabi, D.M., Wessby, B. and Kahan, T., 2002, Cardiac and vascular structure and function are related tolipidperoxidation and metabolism. Lipids, 37: 231–236.

Thirumurugan P. Rathi D and Mahadevan K. (2015) In vitro antioxidant activity of *Ficus religiosa* bark extract. World Journal of Science and Research. 1(1): 35-40

Trease GE, Evans WC (1989). Phenols and Phenolic glycosides. In:Textbook of Pharmacognosy. (12th ed.). Balliese, Tindall and Co Publishers, London pp. 343-383.

Velavan S (2011). Free radicals in health and diseases-A Mini Review. Pharmacologyonline Newsletter. 1: 1062-1077.

Velavan S, Nagulendran K, Mahesh R (2007). *In vitro* antioxidant activity of *Asparagus racemosuss* root. Pharmacog. Magaz; 26-33.

Velavan S. (2015) Phytochemical techniques – A Review. World Journal of Science and Research. 1(2): 80-91.

Winston, J.C., 1999, Health-promoting properties of common herbs. Am J Clin Nutr, 70: 491–499.

Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu V (2000). Comparison of antioxidant and antimicrobial activities of Tilia (Tilia argentea Desf Ex DC), Sage (Salvia triloba L.), and Black Tea (Camellia sinensis) extracts. Journal of Agricultural and Food Chemistry, 48: 5030-5034.

Source of support: Nil;
Conflict of interest: None declared