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IN–VITRO ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF *LANNEA COROMANDELICA* (Hout.) Merill

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Free radicals are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes including other micromolecules, resulting in cellular damage. Cell damage caused by free radicals appears to be a major contributor to aging and degenerative disease such as cancer, cardiovascular diseases, cataract, liver diseases, diabetes mellitus, inflammation, renal failure, etc. Naturally there is a dynamic equilibrium between the free radicals produced in the body and antioxidants that scavenge them to protect the body against deleterious effects. The amount of antioxidants present under normal physiological conditions may be insufficient to neutralize free radicals generated. Therefore, it is obvious to enrich our diet with antioxidants to protect against harmful diseases. Hence there has been an increased interest in the food industry and in preventive medicine in the development of "Natural antioxidant" from plant material. Considering the significance of antioxidant activity, crude extract from leaves of *Lannea coromandelica* (Hout.) Merill belonging to Family Anacardiaceae was prepared in methanol and evaluated for its radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Assay, Nitric Oxide Radical Inhibition Assay, Reducing Power Assay and H_2O_2 Radical Scavenging Assay. The antioxidant activity of methanolic extract was studied in comparison with the standard ascorbic acid. The extract showed significant free radical scavenging activity as compared to ascorbic acid. The antioxidant activity observed in the present investigation might be due the presence of phenolics and flavonoids.

KEY WORDS: DPPH Assay, H_2O_2 Assay, *Lannea coromandelica,* Nitric Oxide Assay, Reducing Power Assay

Lannea coromandelica (Hout.) Merill belongs to Family Anacardiaceae. It is a deciduous tropical tree distributed throughout India, Bangladesh and few other tropical countries. Traditionally boiled leaves are applied in sprains and bruises, local swellings, pains of body and in elephantiasis (Wealth of India 1988) Five dihydroflavonols were isolated and identified from the stem barks of Lannea coromandelica (Tofzzal and Santoshi 2000). The bark is useful in cuts, wounds, bruises, ulcers, opthalmia, gout, ulcerative stomatitis, odontalgia, sprains, diarrhea and dysentery. The fruit paste of Lannea coromandelica is therapeutically used for bone fractures by tribes in Eastern Ghats of Andhra Pradesh (Ratnam and Venkata 2000). The pharmacological properties of extract of Lannea coromandelica stem barks were screened for anti-inflammatory (Singh and Singh 2005), hypotensive (Islam *et al.*2002), and cytotoxicity effects (Rahman *et al.*2008). Various phytochemicals have been isolated from Lannea spp., including quercetin-3-O-arabinoside and ellagic acid (Subramanian and Nair 1971), 6,6-dimethyl-[2,3:7,6]- pyrano-8-(γ,γ-dimethylallyl) avanone (Sultana and Ilyas 1986), rutin and quercetin (Sulochana and sastry 1968), and lanceolatin-B and 7,2'-dimethoxy-4',5 '- methylenedioxy avone (Sultana and Ilyas 1986) from the leaves and flowers; phlobatannin and leucocyanidin (Nair *et al.* 1963), β-sitosterol, physcion and physcion anthranol B from the bark (Rahman *et al.*2008); and a ferulic acid ester from the roots (Govindachari *et al.*1971). In the present study we investigated radical scavenging potential of methanolic extract of leaves of *Lannea coromandelica.*

Oxygen consumption inherent in cell growth leads to the generation of a series of reactive oxygen species (ROS). They are continuously produced by the body's normal use of oxygen such as respiration and some cell-mediated

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immune functions. ROS include free radicals such as superoxide anion radicals (O2•−), hydroxyl radicals (OH•) and non-free radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen ($1O_2$) (Gulcin 2006). ROS are continuously produced during normal physiologic events and can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. ROS are also capable of damaging crucial biomolecules such as nucleic acids, lipids, proteins and carbohydrates and may cause DNA damage that can lead to mutations. If ROS are not effectively scavenged by cellular constituents, they lead to disease conditions. ROS have been implicated in more than 100 diseases (Halliwell 1990). Antioxidants can protect the human body from free radicals and ROS effects. They retard the progress of many chronic diseases as well as lipid peroxidation (Gulcin and Buykokurogu 2002). Hence, a need for identifying alternative natural and safe sources of food antioxidants has been created, and the search for natural antioxidants, especially of plant origin, has notably increased in recent years. Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods. At the present time, the most commonly used antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate and tert-butyl hydroquinone. However, BHA and BHT have been suspected of being responsible for liver damage and carcinogenesis (Branen *et al.* 1990). Therefore, there is a growing interest in natural and safer antioxidants (Gulcin *et al.* 2006).

MATERIALS AND METHODS

Fresh leaves of *Lannea coromandelica* collected from Kandivali area of Mumbai were authenticated from Department of Life science, Ruia College, Mumbai. The leaves were shade dried. Dried leaves were ground to coarse powder, extracted with methanol, which was further evaporated to dryness to obtain alcoholic extract. The antioxidant activity of the plant extracts and standard were assessed on the basis of the radical scavenging effect of the stable DPPH free radical according to method of Blois MS (1958). The reducing power of the MELC was determined according to the method of Oyaizu (1986). The ability of the extract to scavenge hydrogen peroxide was determined according to the method of Ruch, Cheng and Klaunig (1989). Nitric oxide was generated from nitroprusside and measured by the Griess reaction (Marcocci, 1994).

RESULTS AND DISCUSSION

Free radical scavenging activity

DPPH. is a free radical compound and has been widely used to test the free radical-scavenging ability of various samples (Sakanaka *et al.* 2005). The assay is based on the reduction of DPPH radical in methanol which causes an absorbance drop at 517 nm. DPPH is known to abstract labile hydrogen (Matsubara *et al.* 1991). DPPH being a stable free radical can accept an electron or hydrogen radical to become stable diamagnetic molecule. Due to its odd electron, the methanolic solution of DPPH shows a strong absorption band at 517 nm; reduction of the DPPH radicals can be observed by the decrease in absorbance at 517 nm. DPPH radicals react with suitable reducing agents and then electrons become paired off and the solution loses color stoichometrically with the number of electron taken up. Such reactivity has been widely used to test the ability of compounds/ plant extracts to act as a free radical scavengers. Antioxidant molecules can quench DPPH free radicals and convert them to a colorless/bleached product (i.e. 2,2-diphenyl-1-hydarzine, or a substituted analogous hydrazine), resulting in a decrease in

absorbance at the 517 nm band (Yamaguchi *et al.* 1998). Hence, the more rapidly the absorbance decreases, the more potent the antioxidant activity of extract in terms of hydrogen atom-donating capacity. Figure 1 shows the dose-response curve of DPPH radical scavenging activity of the MELC, compared with ascorbic acid, as standard. The MELC extract scavenged 42.50% of DPPH radical at concentration of 25 µg and ascorbic acid scavenged 55.4% at same concentration i.e. 25 µg. The EC50 for MELC extract was found to be 29.41 µg and 22.56 µg for ascorbic acid respectively.

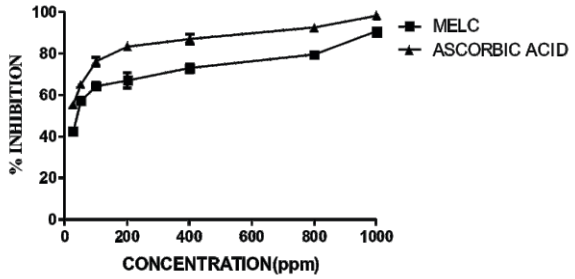

Figure.1. Free radical scavenging activity of MELC (n=3)

Reducing power assay

The reducing power of the extracts was measured by the direct electron donation in the reduction of $[Fe\ (CN)_6]^{3-}$ to $[Fe(CN)_6]^{4-}$. The product was visualized by addition of free Fe^{3+} ions after the reduction reaction, by forming the intense Prussian blue colour complex, $(Fe^{3+})4\ [Fe^{2+}(CN^-)_6]_3$, and quantified by absorbance measurement at 700 nm. The reducing capacity of MELC might serve as a significant indicator of its potential antioxidant activity. A direct correlation between antioxidant activity and reducing power of certain plant extracts has been observed. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordan 1998). Figure 2 shows the reductive capabilities of the plant extract compared to ascorbic acid. The reducing power of MELC was very potent and the power of the extract was increased with quantity of sample. The plant extract could reduce the most Fe_3+ ions, which had a lesser reductive activity than the standard of ascorbic acid. Increased absorbance of the reaction indicated increased

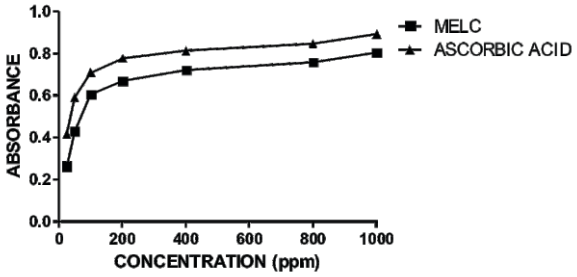

Figure.2. Reducing power assay of MELC (n=3)

reducing power.

Scavenging of hydrogen peroxide

Hydrogen peroxide itself is not very reactive, but can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells (Wichi 1988). Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water. The MELC was capable of scavenging hydrogen peroxide in a concentration-dependent manner. Figure 3 shows that MELC shows less scavenging activity (H_2O_2) than that of Ascorbic acid. The EC50 value for scavenging of for MELC was 28.06 µg while

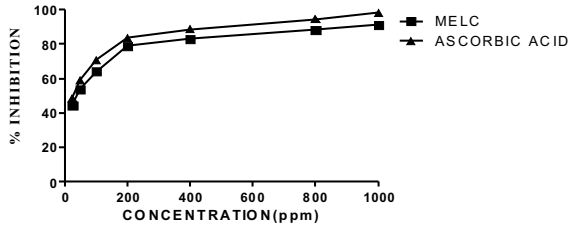

Figure.3. Hydrogen peroxide activity of MELC (n=3)

EC50 value for ascorbic acid was 25.73µg.

Nitric oxide scavenging activity

Nitric oxide is an important radical in biological systems, which serves many important biological functions as an intra and intercellular messenger. As a free radical, it is oxidized, reduced or complexed with other biomolecules, depending on microenvironment (Moncada *et al.* 1991). These reactive nitrogen species are implicated in inflammation, cancer and other pathological conditions. The reactivity of .NO is enhanced by oxygen (O_2) through the conversion in reactive intermediates including nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3) and peroxynitrite (ONOO-). Hence, the extract was evaluated for their ability to scavenge the NO radical. Nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite (Sreejayan and Rao 1997). Figure 4 shows moderately good nitric oxide scavenging activity between 25 and 1000 µg/ml. The percentages of inhibition were increased with increasing concentration of the extracts. EC50value for scavenging of nitric oxide for MELC was 242.71 µg while EC50 value for ascorbic acid was 208 µg. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological condition.

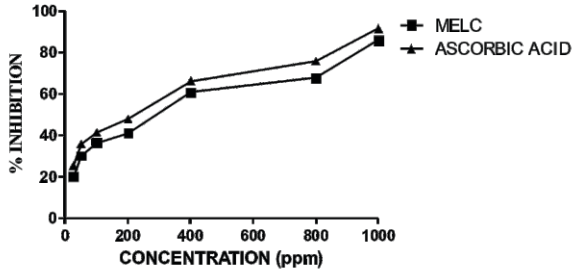

Figure.4. Nitric oxide scavenging activity of MELC (n=3)

CONCLUSION

On the basis of the results obtained in the present study, it is concluded that methanolic extract of *Lannea coromandelica* leaves, exhibits high antioxidant and free radical scavenging activities. It also chelates iron and has reducing power. These in vitro assays indicate that this plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. However, the components responsible for the antioxidative activity are currently unclear. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract. Furthermore, the *in vivo* antioxidant activity of this extract needs to be assessed prior to clinical use.

REFERENCES

Blois MS 1958 Antioxidant determinations by the use of stable free radical. *Nature* **181** 1199-1200.

Branen AL, Davidson PM & Salminen S 1990 *Food Additives* New York.

Gordon MH 1998. *The mechanism of antioxidant action in vitro. Food antioxidants.* Elsevier Applied Science, London.

Govindachari TR, Parthasarathy PC, Desai HK & Mohamed PA 1971 Cluytyl ferulate, a new long chain ester from *Gmelina arborea* and *Lannea grandis*. *Indian Journal of Chemistry* **9** 1027.

Gulcin I 2006 Antioxidant and antiradical activities of l-carnitine. *Life Sci.* **78** 803–811.

Gulcin I, Buyukokurogu ME & Oktay M 2002 In vitro antioxidant properties of melatonin. *J. Pineal Res.* **33** 167–171.

Gulcin I, Mshvildadze V & Gepdiremen A 2006 Antioxidant activity of a triterpenoid

glycoside isolated from the berries of *Hedera colchica*: 3-O-(β-d-glucopyranosyl)-hederagenin, *Phytother. Res.* **20** 130–134.

Halliwell B & Gutteridge JMC 1990 Role of free radicals and catalytic metal ions in human disease: an overview. *Method Enzymol.* **186** 1–85.

Islam MT, Ito T, Sakasai M & Tahara S 2002. Zoosporicidal Activity of Polyflavonoid Tannin Identified in *Lannea coromandelica* Stem Bark against Phytopathogenic Oomycete *Aphanomyces cochlioides*. *J. Agric Food Chem.* **6(23)** 6697-6703.

Marcocci L, Maguire JJ, Droy-Lefaix MT & Packer L 1994 The nitric oxide scavenging properties of *Gingko biloba* ECB 761. *Biochem. Biophy. Res. Commun.* **15** 748-755.

Matsubara T, Fuchimoto S & Iwagaki H 1991 The possible involvement of free radical scavenging properties in the action of cytokines. Res. Commun. *Chem. Pathol. Pharmcol.* **71(2)** 239-42.

Moncada A, Palmer RM & Higgs EA 1991 Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol. Rev.* **43(2)** 109-42.

Nair AGR., Subramanian SS & Sridharan K 1963 Chemical examinations of the flowers of *Lannea coromandelica*. *Current Science* **3** 115-116.

Oyaizu M 1986 Studies on products of browning reaction: Antioxidative activities of browning reaction products prepared from glucosamine. *Eiyogaku Zasshi,* **44** 307-315.

Rahman MS, Begum B, Chowdhury R, Rahman KM & Rashid MA 2008 Preliminary cytotoxicity screening of some medical plants of Bangladesh, Dhaka Univ. *J Pharm Sci.* **7(1)** 47-52

Ratnam KV & Venkata RR 2000 Traditional medicine used by the adivasis of eastern Ghats, Andhra Pradesh – For bone fractures. *Ethnobotanical leaflets* **12** 19-22.

Ruch RJ, Cheng SJ & Klaunig JE 1989 Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from chinese green tea. *Carcinogenesis* **10** 1003-1008.

Sakanaka S, Tachibana Y & Okada Y 2005 Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). *Food Chemistry* **89** 569–575.

Singh S & Singh GB 2005 Antiinflammatory Activity of *Lannea coromandelica* Bark Extract in Rat, *Phytotherapy Research* **8** 311-318.

Sreejayan & Rao MN 1997 Nitric oxide scavenging by curcuminoids. *J Phar Pharmacol.* **49(1)** 105-107.

Subramanian SS & Nair AGR 1971 Angiospermae dicotyledonae Anacar-diaceae, polyphenols of *Lannea coromandelica*. *Phytochemistry* **10** 1939-1940.

Sulochana V & Sastry KNS 1968 Isolation of rutin and quercetin from Odina (*Lannea grandis*) leaves. *Leather Science* **15** 170-171.

Sultana S & Ilyas M 1986a Isolation of a new flavanone and lanceolatin-B from *Lannea acida* (Rich). *Indian Journal of Chemistry* **25B** 416.

Sultana S & Ilyas M 1986b A flavanone from Lannea acida. *Phytochemistry* **25** 963.

The wealth of india, *A dictionary of Indian raw materials and industrial products raw materials.* Vol-**2**:B, Revised edition, 1988. Publication and information directorate, CSIR hillside road, New Delhi.

Tofzzal Islam MD & Satoshi T 2000 Dihydroflavonols from *Lannea coromandelica. Phytochemistry* **54** 901-907

Wichi HP 1988 Enhanced tumour development

by butylated hydroxyanisole (BHA) from the perspective of effect on stomach and oesophageal squamous epithelium. *Food Chem. Toxicol.* **26** 717-723.

Yamaguchi T, Takamura H, Matoba T & Terao J 1998 HPLC method for evaluation of the free radical- scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci. Biotechnol. Biochem.* **62(6)** 1201-4.