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Hydroxy radical and DPPH scavenging activity of crude protein extract of *Leucas linifolia:* A folk medicinal plant

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ABSTRACT

Leucas linifolia belongs to the family Lamiaceae and is a well know traditional medicinal plant in India. It is an herbaceous annual weed. In ayurveda, it is used for the treatment of cough, cold, fever, loss of appetite, skin disease, head ache, jaundice, Snake bite and scorpion sting. It has shown hepato protective activity against d-galactosamine and CCl_4 induced hepatotoxicity in rats. Methanol extract of the plant has shown wound healing property. Antibacterial, antipyretic and antitussive activity of the plant have been reported which confirms its traditional medicinal usage from the time of ayurveda. In the present study, we have shown the antioxidant activity of the ammonium sulphate precipitated protein extract of Leucas linifolia. Crude protein extract of Leucas linifolia (CPLL) extract showed In vitro antioxidant activity in both hydroxyl radical scavenging and as well as in DPPH scavenging activity in Dose dependant manner . At 500µg/ml concentration of CPLL, it showed 78 and 63% inhibition, with an IC50 value of 150 and 175µg/ml in hydroxyl radical and DPPH assay respectively. As CPLL extract, scavenge hydroxyl radical more than the DPPH radical, it might have DNA protectant activity also. To conclude, CPLL showed a good antioxidant activity and might be an alternate to synthetic antioxidants available in the market.

Keywords: Leucas, Antioxidant, Protein extract, Hydroxyl radical DPPH.

INTRODUCTION

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism. Various metabolic processes, UV radiations, smoke etc trigger the production of free radicals [1]. Reactive oxygen species (ROS) includes superoxide anions (O2. -), hydroxyl radical (.OH), singlet oxygen, hydrogen peroxide (H_2O_2), ferric ion, nitric oxide (NO) etc. Excessive production of free radicals leads to Oxidative stress. The diseases associated with the ROS mainly depend on the balance of the pro-oxidant and the antioxidant concentration in the body. Pro-oxidant conditions dominate either due to the increased generation of the free radicals or due to the excessive oxidative stress of the depletion of the dietary antioxidant [2]. Free radicals have been implicated in causation of ailments such as cancer, inflammation, diabetes, liver cirrhosis, cardio vascular disease, Alzheimer's, Aging and acquired immunodeficiency syndrome. [3,4, 5, 6]. Reactive oxygen species (ROS) inactivate enzymes and damage important cellular components causing tissue injury through covalent binding and lipid per oxidation [7], The increased production of toxic oxygen derivatives is considered a universal feature of stress conditions. Plants and other organisms have evolved a wide range of mechanisms to contend with this problem, with a variety of antioxidant molecules and enzyme [8]. Recent evidences suggested the involvement of

oxidative stress in the pathogenesis of various diseases and have attracted much attention of the scientists and general public on the role of antioxidants in the maintenance of human health and prevention and treatment of diseases [9]. Living organisms have developed a complex antioxidant network to counteract reactive species that are detrimental to human life. Enzymatic antioxidants such as Catalase, superoxide dismutase and non-enzymatic antioxidants, such as albumin, GSH, ascorbic acid, α-tocopherol, b-carotene, uric acid, bilirubin, and flavonoids constitute an important aspect of the network [10]. Synthetic antioxidants such as butylhydroxyanisole (BHA) or butylhydroxytoluene (BHT) has been used by many food industries but has concern on its side effects on humans. So the use of traditional medicine is spreading worldwide, and plants still present a large source of natural antioxidants that might serve as leads for the development of novel drugs. Antioxidants such as α-tocopherol, ascorbic acid, carotenoids, amino acids, peptides, proteins, flavonoids and other phenolic compounds plays a significant role as physiological and dietary antioxidants, thereby augmenting the body's natural resistance to oxidative damage [11, 12]. Such natural antioxidant substances are believed to play a potential role in interfering with the oxidation process by reacting with free radicals, chelating catalytic metals and scavenging oxygen in biological systems [13]. External supply of antioxidants may overcome the effect of free radicals on the body, and in turn can prevent the occurrence of many diseases[14].

Recently, the use of natural protein extracts or purified proteins as antioxidants has been identified. Protein isolated from soya, mushroom, maize zein, potato, yam, chickpeas and white beans were reported to have antioxidant activity [15, 16, 17, 18, 19]. The proteins owe their antioxidant activity due to its constituent amino acids. For example, the antioxidant activities of tyrosine, phenylalanine and tryptophan are due to their ability to donate protons to free radicals whereas lysine, arginine, aspartic acid and glutamic acid, exercise antioxidant activity by chelating metal ions. histidine may behave both as a radical-scavenger and also as a metal-chelator due to its imidazole ring [19].

Leucas linifolia (Alternative names: *Leucas lavandulaefolia* Rees, *Leonurus indicus*, *Phlomis linifolia*), belongs to the family Lamiaceae and is a well know traditional medicinal plant in India. It is an herbaceous annual weed [20]. In ayurveda, it is used against many health disorders such as cough, cold, fever, loss of appetite, skin disease, head ache, jaundice, snake bite and scorpion sting [21]. It has shown hepatoprotective activity against d-galactosamine and Carbon tetra chloride induced hepatotoxicity in rats [22, 23, 24, 25]. Methanol extract of the plant has shown wound healing property. [26]. Antibacterial [27], antipyretic [28] and antitussive activity [29] of the plant have been reported which confirms its traditional medicinal usage from the time of ayurveda. Linifolioside, an isopimarane rhamnoglucoside was isolated from *Leucas linifolia.* [30]. Phytochemical studies reveal the plant is rich in Linifolioside and Flavonoids [31]. In the present study, we have shown the antioxidant activity of the protein extract isolated from the *Leucas linifolia.*

MATERIALS AND METHODS

Chemicals

2-Deoxy-D-ribose, butylated hydroxyanisole (BHA), linoleic acid (99%), 2, 2-Diphenyl-1-picryl-hydrazyl (DPPH_), ∝-tocopherol and Bovine serum albumin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), EDTA, ascorbic acid, Ammonium sulfate (for biochemistry), polyvinyl polypyrrolidone (PVPP), acetone, ferrous chloride and ferric chloride were obtained from Merck (Darmstadt). All other reagents were of analytical grade.

Plant material

Whole plant of *Leucas linifolia* in full bloom was collected in the month of August and September, 2011 at Kakana Kote Forest Area, (H.D Kote Taluk), Mysore district of Karnataka, India. Plant was identified by Dr.G.Panduranga Murthy, Scientist and Head, CSRF, Shridevi institute of Engineering & Technology, Tumkur, India. The Voucher specimen was identified after collection using a standard flora and the Plant materials was deposited in the R&D Section. The collected plant material was washed with distilled water and dried at room temperature. The dried herb with roots, leaves and flowers were manually ground to a fine powder and stored in Air tight sealed plastic cover at room temperature.

Preparation of Protein extract
To remove phenolic compounds and lipids, acetone powders were used as sources of protein extracts. For the preparation of acetone powders, 100 g dry powdered plant was homogenized in a blender for 3-5 min with 200 ml of chilled acetone. The slurry obtained was filtered under vacuum through a Buchner funnel containing a Whatman No: 1 filter paper and the residue remaining on the filter paper were collected. The homogenization with 300 ml acetone and filtration were then repeated twice for the filtrate, Left out Acetone was either evaporated by keeping in Rotary Vacuum evaporator or just by keeping overnight at room temperature. Acetone powder was stored at -20 ^{0}C until next use. Water-soluble crude protein extract, was extracted by the method given by [32] with a few minor modifications. Briefly, 20 g of acetone powder, 0.5 g of insoluble PVPP (used to absorb possible residual phenolic compounds), 0.1% polyethylene amine (to precipitate nucleotides) and 180 ml of distilled water were mixed and extracted with a magnetic stirrer for 2 hr at 4 ^{0}C. The extract was then filtered through Whatmann no 1 filter paper, centrifuged at 12,000 rpm for 30 min at 4 ^{0}C and the resultant cake was discarded the supernatant was brought to 80 % saturation of ammonium sulphate in step wise manner. The mixture was then constantly stirred slowly for 2 hr at 4 ^{0}C and the precipitate obtained by centrifugation at 12,000 rpm for 30 min at 4 ^{0}C was dissolved in minimum amount of double distilled water. The extract was then dialyzed for 72 hr at 4 ^{0}C against double distilled water. Extract was again centrifuged at 12,000 rpm for 30 min at 4 ^{0}C. To remove traces of chlorophyll and other coloring compounds in the Water-soluble crude protein extract of *L .linifolia,* it was subjected to Activated charcoal column (5 X 1.2 cm, flow rate 0.5 ml/5 min) eluted with 100 % distilled water and the eluted sample was concentrated by lyophilization and stored at -20 ^{0}C until its further use. Protein estimation was done by Bradford's method [33]. Crude protein extract obtained was named as CPLL (Crude Protein extract of *Leucas linifolia*).

Antioxidant activity of CPLL
a) Hydroxyl radical scavenging activity
Deoxyribose assay was used to determine the hydroxyl radical scavenging activity in an aqueous medium [34]. The reaction mixture containing $FeCl_3$ (100 µM), EDTA (104 µM), H_2O_2 (1 mM) and 2-deoxy- D-ribose (2.8 mM) were mixed with or without CPLL at various concentrations (10-250 µg) in 1 ml final reaction volume made with potassium phosphate buffer (20 mM, pH 7.4) and incubated for 1 hr at 37 ^{0}C. The mixture was heated at 95 ^{0}C in water bath for 15 min followed by the addition of 1 ml each of TCA (2.8%) and TBA (0.5% TBA in 0.025 M NaOH containing 0.02% BHA). Finally the reaction mixture was cooled on ice and centrifuged at 5000 rpm for 15 min. Absorbance of supernatant was measured at 532 nm. All readings were corrected for any interference from brown color of the extract or antioxidant by including appropriate controls. The negative control without any antioxidant or CPLL was considered 100% deoxyribose oxidation. The % hydroxyl radical scavenging activity of test sample was determined accordingly in comparison with negative control. Ascorbic acid was taken as the positive control.

b) 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity
DPPH radical scavenging activity was assessed according to the method of Shimada et al. [35]. The CPLL at various concentrations ranging from 10 to 250 µg/ml was mixed in 1 ml of freshly prepared 0.5 mM DPPH ethanolic solution and 2 ml of 0.1 M acetate buffer, pH 5.5. The resulting solutions were then incubated at 37 ^{0}C for 30 min and measured calorimetrically at 517 nm. Ascorbic acid was used as positive control under the same assay conditions. Negative control was without any inhibitor or CPLL. Lower absorbance at 517 nm represents higher DPPH scavenging activity. The % DPPH radical scavenging activity of CPLL was calculated from the decrease in absorbance at 517 nm in comparison with negative control. Experiment was done in triplicates. DPPH radical's concentration was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = Ao - A1 / Ao \times 100$$

Where Ao was the absorbance of the control and A1 was the absorbance in the presence of the sample (Crude Protein extract of *Leucas linifolia*).

RESULTS AND DISCUSSION

In the present statues, there is a strong need for an effective antioxidants from natural sources as alternatives to synthetic antioxidant in order to prevent the free radicals implicated diseases like cancer, cardiovascular diseases, Age related macular degeneration, atherosclerosis, etc [36]. The extracts and essential oils of many plants have been

investigated for their antioxidant activity In the present study, we have isolated the protein from the whole plant extract by step wise method. First, the acetone powder of the plant was prepared by precipitating the protein by acetone and redissolved in distilled water. Polyphenolics and nucleotides present in the acetone powder extract were removed by PVPP and polyethylene amine treatment. Than the extract was subjected to ammonium sulphate precipitation (80 %). The precipitate was dissolved in distilled water, traces of chlorophyll were removed by activated charcoal column and the final extract obtained was called as Crude Protein *Leucas linifolia* (CPLL) extract. The extract was checked for its antioxidant activity in two model systems i.e. in Deoxy ribose and DPPH method.

CPLL extract showed In vitro antioxidant activity in both hydroxyl radical scavenging and DPPH scavenging activity in Dose dependant manner. At 500 µg/ml concentration of CPLL, it showed 78 and 63 % inhibition in Hydroxy radical and DPPH assay respectively. IC 50 value of CPLL was 150 and 175 µg/ml in hydroxyl radical and DPPH assay respectively. Figure 1 and 2 shows the antioxidant activity of CPLL against hydroxyl radical and DPPH radical. CPLL extract was observed to scavenge Hydroxy radical more than the DPPH radical.

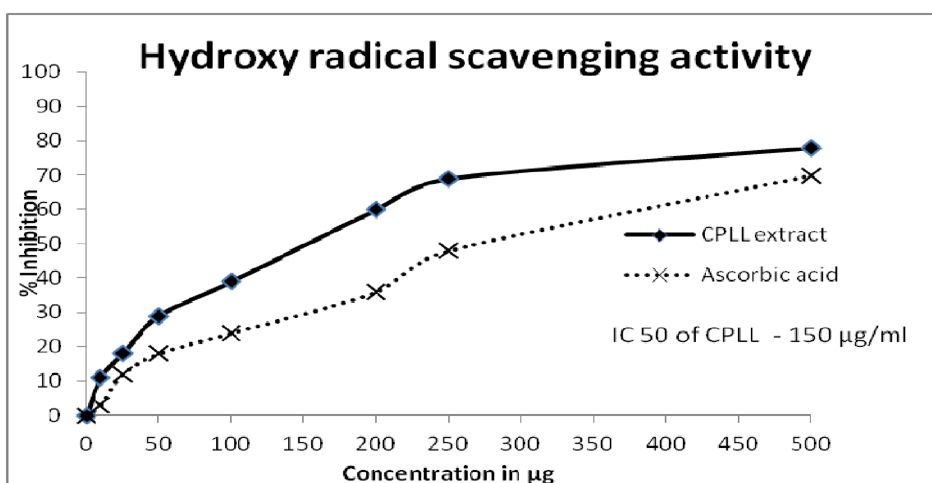

Figure 1: CPLL extract was tested for scavenging the hydroxyl radical in deoxy ribose method. Ascorbic acid was taken as the standard antioxidant. IC-50 value of CPLL was found to be 150 µg/ml. Assay was done in triplicates. Results were mean ± SD where n=3

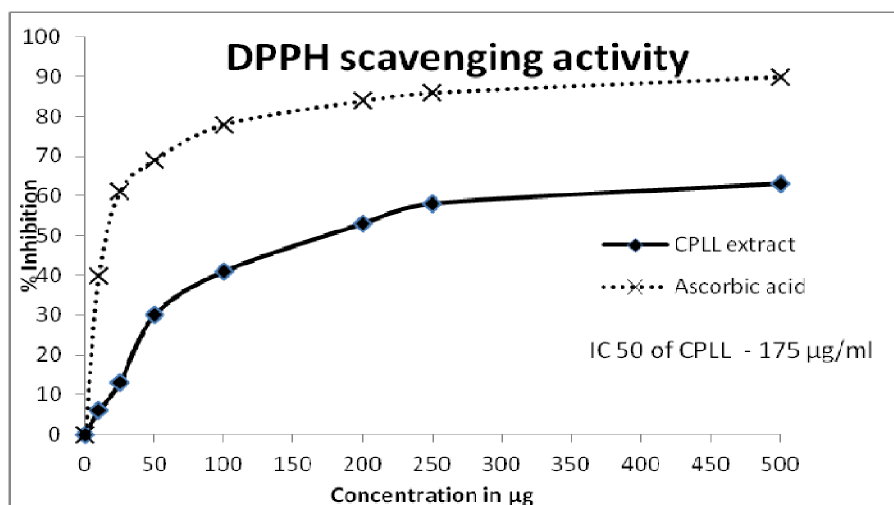

Figure 2: CPLL extract was tested for scavenging OD DPPH radical. Ascorbic acid was taken as the standard antioxidant. Extract was taken from the range of 10 – 500 µg/ml. IC-50 value of CPLL was found to be 175 µg/ml. Assay was done in triplicates. Results were mean ± SD where n=3

CONCLUSION

Crude protein extract of *Leucas linifolia* showed a good antioxidant activity and might be an alternate to synthetic Antioxidants available in the market. Further work is to separate the proteins in the extract and to check for its antioxidant activity individually.

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REFERENCES

[1] B. Halliwell, J.M.C. Gutteridge,(Ed.) *Free radicals in biology and medicine, fourth ed.* Oxford, UK: Clarendon, 2007.
[2] A.K. Tiwari, *Current Sciences*, **2001**, 81, 1179.
[3] J.L. Marx, *Science*, **1987**, 235, 529.
[4] D.A. Joyce, *Adv. Drug Reac. Bull.* **1987**, 127, 476-79.
[5] Shiv kumar, *Advances in Applied Science Research*, **2011**, 2 (1), 129-135.
[6] C.I. Sajeeth, P.K. Manna, R. Manavalan, *Der Pharmacia Sinica*, **2011**, 2 (2), 220-226.
[7] J.G. Geesin, J.S. Gordon, R.A. Berg, *Arch Biochem Biophys*, **1990**, 278, 352.
[8] Priyesh Pankaj, V.B. Narayanasamy, M. Manjunatha Setty, Annie shirwaikar, *Pharmacology online,* **2007**, 2, 226-235.
[9] A. M. Papas, Ed.; *Antioxidant status, diet, nutrition, and health*. Boca Laton, USA: CRC Press; 1999.
[10] D.D.M. Wayner, G.W. Burton, K.U. Ingold, S. Locke, *FEBS Lett.* **1985,** 187, 33–37.
[11] F. Shahidi, *Nahrung*, **2000**, 44, 158.
[12] P. Arulpriya, P. Lalitha, S. Hemalatha, *Der Chemica Sinica,* **2010**, 1 (2), 73-79
[13] B. Halliwell, M.C. Gutteridge, *Biochemical Journal*, **1984,** 219, 1–4.
[14] Wee Sim Choo, Wee Khing Yong, *Advances in Applied Science Research,* **2011**, 2 (3): 418-425.
[15] H. Chen, K. Muramoto, F. Yamauchi, *Journal of Agricultural and Food Chemistry*, **1995**, 43, 574–578.
[16] W. Hou, M. Lee, H. Chen, W. Liang, C. Han, Y. Liu, et al. *Journal of Agricultural and Food Chemistry*, **2001**, 49, 4956–4960.
[17] Y. Liu, C. Han, M. Lee, F. Hsu, W. Hou, *Journal of Agricultural and Food Chemistry*, **2003**, 51, 4389–4393.
[18] D. Rajalakshmi, S. Narasimhan,. Food antioxidants: Sources and methods of evaluation. In D. L. Madhavi, S. S. Deshpande, D. K. Salunke (Eds.), Food antioxidants **1996**, 65–157). New York: Marcel Dekker, Inc.
[19] Iskender Arcan, Ahmet Yemenicioglu, *Food Chemistry*, **2007**, 103 301–312.
[20] Anonymous, the Wealth of India, Raw Materials P.I.D., C.S.I.R., New Delhi. **1962**, vol. 4, 79.
[21] K.R. Kirtikar, B.D. Basu, Indian Medicinal of Plants, International Book Publisher, Dehradun, **1975**, 3, 2016
[22] K.S. Chandrashekar, K.S. Prasanna, *International Journal of Pharma Sciences and Research,* **2010**, 1(2), 101-103.
[23] K.S. Chandrashekar, K.S. Prasanna, A.B. Joshi, *Fitoterapia*, **2007**, 78(6), 440-442.
[24] J. Kotoky, B. Dasgupta, G.K. Sarma, *Fitoterapia*, **2008**, 79(4), 290-292.
[25] S. Shirish Pingale, *Der Pharmacia Sinica,* **2010**, 1 (2), 136-140.
[26] Kakali Saha, Pulok K. Mukherjee, J. Das, M. Pal, B. P. Saha *Journal of Ethnopharmacology*, **1997**, 56(2), 139-144.
[27] K. Saha, P.K. Mukheijee, S.C. Mandal, M. Pal, B.P. Saha, *Indian Drugs,* **1995***,* 2(8), 402-404.
[28] Mukherjee Kakali, B.P. Saha, Mukherjee Pulok, *Phytotherapy research*, **2002**, 16(7), 686-8.
[29] K. Saha, P.K. Mukherjee, T. Murugesan, B.P. Saha, M. Pal, *Journal of ethnopharmacology*, **1997**, 57(2), 89-92.
[30] B. Shashi, Mahato, C. Bikas Pal, *Phytochemistry*, 1986, 25(4), 909-912.
[31] K. Saha, P.K. Mukerjee, M, Pal, M.Saha, *Journal of Medicinal and Aromatic Plant Sciences*, **1997**, 19, 1045-1050.
[32] M.I. Genovese, F.M. Lajolo, *Food Chemistry*, **1998**, 62, 315–323.
[33] M.M. Bradford, *Analytical Biochemistry.* 1976, 72, 248-254.
[34] B. Halliwell, M. Grootveld, J.M.C. Gutteridge, *Methods of Biochemical Sciences*, **1981**, 33, 59-90.

[35] K. Schimada, K. Fujikawa, K. Yahara, T. Nakamura, *Journal of Agricultural and Food Chemistry*, **1992**, 40, 945–948.
[36] O.L. Aruoma, *J Am Oil Chem Soc*. **1998**, 75: 199–212.