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In Vitro Antioxidant Activity of Methanolic Leaves and Flowers Extracts of *Lippia Alba*

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Abstract: *In-vitro* antioxidant activity of methanolic leaves and flowers extract of *Lippia alba* was determined by DPPH free radical scavanging assay. The Reducing power of extracts was also determined. Ascorbic acid was used as standard and positive control for both the analysis.All the analysis was made with the use of UV-Visible Spectrophotometer (Systronics 117, Japan). The methanolic leaves and flowers extracts of *Lippia alba* had shown very significant DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging activity compared to standard antioxidant. The DPPH radical scavenging activity of the extract was increased with the increasing concentration. In DPPH free radical scavanging assay IC50 value of leaves and flowers extracts of *Lippia alba* was foun to be 34.4 µg/mL. The results concluded that the extracts have a potential source of antioxidants of natural origin.

Key words: Antioxidant, *Lippia alba*, Free radical, DPPH.

INTRODUCTION

In living systems, free radicals are generated as part of the body's normal metabolic process, and the free radical chain reactions are usually pro-duced in the mitochondrial respiratory chain, liver mixed function oxidases, through xanthine oxi-dase activity, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics.In addition, chemical mobilization of fat stores under various conditions such as lactation, exercise, fever, infection and even fasting, can result inincreased radical activity and damage. Free radi-calsor oxidative injury now appears the funda-mental mechanism underlying a number of human neurologic and other disorders. Oxygen free radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long-term complication of diabetes[1,8-9,16].

Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties[2,6-11,15].

Plants are potent biochemical factories and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plants based natural constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e. any part of the plant may contain active components[2]. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct (Wink, 1999). Antioxidant-based dugs/formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer have appeared during the last 3 decades [4-6,9,10].

This has attracted a great deal of research interest in natural antioxidants. Subsequently, a worldwide trend towards the use of natural phytochemicals present in berry crops, tea, herbs, oilseeds, beans, fruits, and vegetables have increased. Several herbs and spices have been reported to exhibit antioxidant activity, including rosemary, sage, thyme, nutmeg, turmeric, white pepper, chili pepper, ginger, and several Chinese medicinal plants extracts. The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, 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

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antioxidant potential. The systematic record of the relative antioxidant activity in selected Iranian medicinal plant species extracts was recorded by Pourmorad et al in 2006. With this background and abundant source of unique active components harbored in plants, the present study was taken up on the medicinal plant namely Lippia alba belongs to the family verbenaceae[1,2,6-8,13].

The plant Lippia alba (aromatic shrub) locally known as Motmotya belongs to the family Vebenaceae. The plant is extensively grown in all over the Bangladesh, is widely used all over South and Central America for different purposes[4,6]. Different parts of this plant are used in the indigeneous systems of mrdicine for the treatment of a variety of human aliments. Africa,Mexico,South and Central America.The targeted plant has many uses in traditional medicine.The majority of Lippia species have been used traditionally for the treatment of Stomach ailments, car-diovascular troubles , coughs, colds and asthma, tranquilising remedy, prevention of gastritis etc[10,11-13]

MATERIALS AND METHODS

Plant Materials: The fresh leaves of *Lippia alba* were collected from Natore city of Rajshahi district of Bangladesh in March 2008 and identified by Dr.M.A Razzaque Shah, Tissu Culture Specialist, BRAC Plant Biotechnology Laboratory, Dhaka Bangladesgh.

Preparation of Extracts: Fresh leaves and flowers of *Lippia alba* were cut into small pieces, dried in the sun for seven days.and finally in an oven below 60^0 .The dried plant materials (1 kg) was ground into fine powder and exhaustively extracted with methanol. The extract was concentrated to a dark greenwish residue. This crude extract was used for further investigation for potential antioxidant properties[1,4].

Antioxidant Assay: The antioxidant activity of Plant extracts were determined by different *in vitro* methods such as, the DPPH free radical scavenging assay and reducing power methods. All the assays were carried out in triplicate and average values were considered.

DPPH Radical Scavenging Activity[15-17]: The free radical scavenging capacity of the methanolic extracts of *Lippia alba* was deter-mined using DPPH. DPPH solution (0.004% w/v) was prepared in 95% methanol. Methanol extract of *Lippia alba* was mixed with 95% methanol to prepare the stock solution (10 mg/100mL). The concentration of this *Lippia alba* methanolic extracts solution was 10 mg /100 ml or 100µg/ml. From stock solution 2ml, 4ml, 6ml, 8ml & 10ml of this solution were taken in five test tubes & by serial dilution with same solvent was made the final volume of each test tube up to 10 ml whose concentration was then 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml & 100µg/ml respectively.Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing *Lippia alba* methanolic *extracts* (20 µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, 100µg/ml) and after 10 min, the absorbance was taken at 517 nm using a spectrophotometer (HACH 4000 DU UV–visible spectrophotometer). Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (10 mg/100mLor 100µg/ml) of methanolic extracts of *Lippia alba*. Control sample was pre-pared containing the same volume without any extract and reference ascorbic acid. 95% methanol was used as blank. % Scavenging of the DPPH free radical was measured using the following equation-

% DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance Of control)] x 100

Assay of Reducing Power[2,15]**:** This was carried out as described previously (Yildrim et al 2001; Lu and Foo). 1 ml of plant extract solution (final concentration 100-500 mg/l) was mixed with 2.5 ml phosphate buffer(0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [K3Fe(CN6)] (10g/l), then mixture was incubated at 50 degree C for 20 minutes. Two and one-half, 2.5 ml of trichloroacetic acid (100g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml Fecl3 (1g/l) and absorbance measured at 700nm in UV-Visible Spectrophotometer (Systronics UV-Visible Spectophotometer 117, INDIA). Ascorbic acid was used as standard and phosphate buffer used as blank solution. The absorbance of the final reaction mixture of two parallel experiments was expressex as mean ± standard deviation. Increased absorbance of the reaction mixture indicates stronger reducing power.

RESULTS AND DISCUSSION

DPPH radical scavenging activity of leaves and flowers extracts of lippia alba and ascorbic acid are presented in figure1.

Reducing power ofof leaves and flowers extracts of lippia alba and ascorbic acid are presented in figure2.

In this present study the antioxidant activity of the methanol extracts of the leaves and flowers of *Lippia alba* were investigated by using DPPH scavenging assay and reducing power of the extract. Methanolic

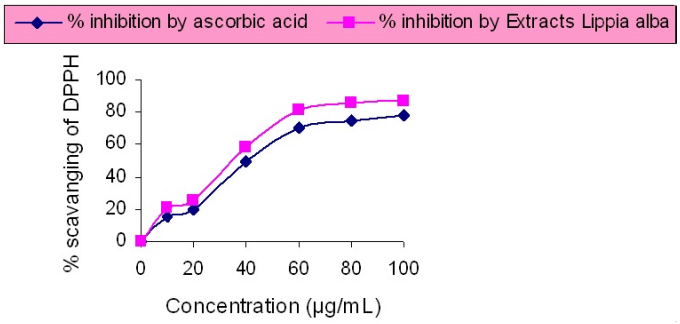

Fig. 1: DPPH radical scavenging activity of methanolic leaves and flowers extracts of *Lippia alba* added to methanolic solution of DPPH and radical scavenging activity was measured as 517 nm as compared to standard Ascorbic acid. Values are the average of triplicate experiments and represented as mean ± standard deviation.

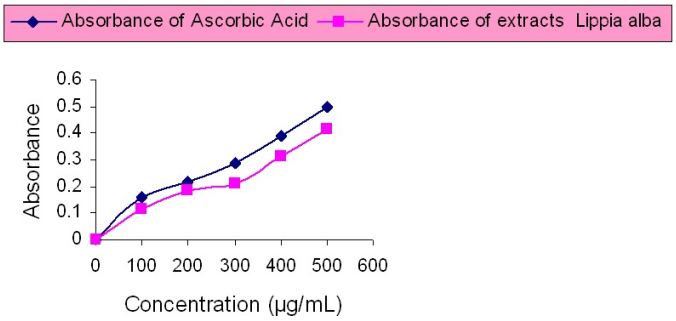

Fig. 2: Reducing power of methanolic leaves and flowers extracts of *Lippia alba* as compared to Ascorbic acid. Values are the average of triplicate experiments and represented as mean ±standard deviation.

leaves and flowers extracts of *Lippia alba* has got profound antioxidant activity. Both methods have proven the effective-ness of the methanol extract compared to the ref-erence standard antioxidant ascorbicacid. The DPPH antioxidant assay is based on the abil-ity of DPPH a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an elec-tron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. Com-parison of the antioxidant activity of the extract and ascorbic acid is shown in Figure 1. The methanol leaves and flowers extract of *Lippia alba* exhibited a signifi-cant dose dependent inhibition of DPPH activity. The IC50 value of The methanol leaves and flowers extract of *Lippia alba and ascorbic acid were found to be at* 34.4 µg/mL and 40.8 µg/mL *respectively*

The reducing ability of a compound generally de-pends on the presence of reductants which have been exhibited antioxidative potentialby breaking the free radical chain, donating a hy-drogen atom. The presence of re-ductants (i.e. antioxidants) in *Lippia alba* leaves and flowers extracts causes the reduction of the Fe 3+ /ferricyanide com-plex to the ferrous form. Therefore, the Fe 2+ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Figure 2 shows the re-ductive capabilities of the *Lippia alba* leaves and flowers extracts compared to ascorbic acid. The reducing power of *Lippia alba* leaves and flowers extracts was very potent and the power of the extract was increased with quantity of sample.

This study has similarity with previous investigation.[4,12-13] This results should be encouraging other researcher to more work on *Lippia alba* including phytochemical and bilogical investigation because different investigators have already been reported their popular used medicinal plant as digestive, respiratory, cardiovascul, infectious troubles and anxiety[4,5,12]. Previous reports of antioxidant activities [1-4,12,15-18] and traditional uses of the plant support the findings of present studies.

Conclusions: It has been reported that reactive oxygen species contribute to various pathophysiological conditions and endogenous defense mechanisms have

evolved to offer protection in these conditions.An increase in the antioxidant reserves of the organism can reduce oxidative stress and some of the plantderived agents may help to reduce it.Determination of the natural antioxidant compounds of plant extracts will help to develop new drug candidates for antioxidant therapy[9,13-14,18,19].The plants may be considered as good sources of natural antioxidants for medicinal uses such as against aging and other diseases related to radical mechanisms[6,13.17,19].Further investigation on the isolation and identification of antioxidant component(s) in the plant may lead to chemical entities with potential for clinical use.

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