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Free Radical Scavenging Activity of *Leucas indica* Flowers and Leaves

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Present work was undertaken to study antioxidant free radical scavenging activity of leaves and flowers extracts of *Leucas indica*. Crude methanolic extracts was screened for *in vitro* antioxidant free radical scavenging activity by diphenyl picryl hydrazyl, nitric oxide radical scavenging and superoxide radical scavenging assay methods. Results showed that IC_{50} value of methanol extracts of leaves in diphenyl picryl hydrazyl, nitric oxide scavenging and superoxide radical scavenging assays are 241.11, 282.97 and 250.45 µg/mL. Similarly IC_{50} value of methanol extracts of flowers in diphenyl picryl hydrazyl, nitric oxide scavenging and superoxide radical scavenging assays are 0.19, 2.05 and 2.15 µg/mL, respectively. Phytochemical screening revealed that flavonoids, alkaloids and phenolic compound are present in methanol extracts of leaves and flowers and may be responsible for the antioxidant activity. The result showed that *Leucas indica* flowers are more potential free radical scavenging activity than leaves.

Key Words: *Leucas indica*, Diphenyl picryl hydrazyl, Nitric oxide, Superoxide radical scavenging assay.

INTRODUCTION

The aim of the present work was to evaluate the antioxidant potential of *L. indica* flowers and leaves extracts using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging, nitric oxide radical scavenging and superoxide radical scavenging assays to support the IC_{50} (inhibitory concentration) of this plant. Although numerous studies have shown the medicinal values of *L. indica*, there still remains ample scope for further research.

Leucas indica [family- (Lamiacae)] is an annual herb found throughout India as a weed in cultivated fields, wastelands and roadsides. The flowers are given with honey to treat cough and cold in children. The leaves are applied to the bites of serpents, poisonous insects and scorpion sting. *L. indica* leaves are also used as insecticides and mosquito repellent in rural area[1]. The plant extract with honey is a good remedy for stomach pain and indigestion.

Reactive oxygen species, which include the oxygen free radicals; superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$) and some non-radical hydrogen peroxide (H_2O_2) derivatives of oxygen are normally produced in living organisms with the potential of reacting with almost all types of molecules in living cells[2]. The harmful effects of free radicals are neutralized by the enzymatic antioxidant defenses including the superoxide dismutase, glutathione peroxidase (GPx) and catalase (CAT). However, over production of the reactive oxygen species arising from either mitochondrial electron transport chain, excessive stimulation of NAD(P)H, or exposure to environmental pollutants, cigarette smoke, ultraviolet rays, some parasitic infections, radiation and toxic chemicals results in oxidative stress- a phenomenal disturbance in the equilibrium status of pro-oxidant/antioxidants reactions in living systems, which mediates damage to cell structures, including lipids and membranes, proteins and DNA[3].

Recently, there has been an increased interest in oxygen containing free-radicals in biological systems and their implied roles as causative agents in the etiology of a variety of chronic and ageing diseases, including heart disease, stroke, arteriosclerosis, diabetes mellitus, cancer, malaria, rheumatoid arthritis, neurodegenerative diseases (Alzheimer's and Parkinson's diseases) and AIDS[4]. Hence, therapy using free radical scavengers (antioxidants) has potential to prevent, delay or ameliorate many of these disorders[5]. Numerous natural antioxidants have already been isolated from different varieties of plant material such as leafy vegetables, fruits, seeds, cereals and algae[6]. They have been shown to have reactive oxygen species scavenging and lipid peroxidation preventive effects[7]. The protection can be explained by the capacity of the antioxidants phenolics, flavonoids and polypropanoids in the plants and plant products to scavenge free radicals, due its proton donating ability.

Plant phenolics are commonly found in both edible and non-edible plants and have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers. In addition, they have a metal chelation potential[8]. The phenolic compounds are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food[9].

EXPERIMENTAL

1,1-Diphenyl-2-picryl hydrazyl (DPPH), gallic acid, curcuminoids, sodium nitroprusside, sulphanilamide, *N*-(1-naphthyl)ethylenediamine, orthophosphoric acid, di-potassium hydrogen phosphate anhydrous, potassium di-hydrogen orthophosphate, sodium chloride, reduced nicotinamide adenine dinucleotide sodium salt monohydrate (NADH), phenazine methosulphate (PMS), nitroblue tetrazolium chloride (NBT), sodium dihydrogen orthophosphate dehydrate, di-sodium hydrogen phosphate dehydrate, methanol from Rankem, S.D. Fine Chem., Synthite, Sigma, India.

Preparation of plant extract: *Leucas indica* leaves and flowers were cut into pieces, dried and then ground to powdered form, which was then kept in an air-tight plastic bag until use. The leaves and flowers were extracted at 60 ºC in methanol for 6 h using ultra sonication. The extracts were then filtered through whatmann filter paper and the filtrate was concentrated with a vacuum rotary evaporator under low pressure.

Thumba (*Leucas indica*) as identified and authenticated by Dr. P.N Sudha, department of chemistry, Thiruvallur University and was collected in Jan 2012.

Evaluation of antioxidant activity

DPPH radical scavenging assay: The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples. It was found that the radical-scavenging activities of all the extracts increased with increasing concentration. Free radical scavenging potential of extract was determined by DPPH assay[10]. 7.9 mg of DPPH was accurately weighed and dissolved in 100 mL methanol to obtain 200 µM solution of DPPH. Different concentrations of extracts were prepared. To 2 mL methanol solution of DPPH, 2 mL of sample solution was added. The mixture was incubated in dark at room temp for 0.5 h. The degree of free radical scavenging activity in presence of different concentration of extracts and their absorbance were measured calorimetrically at 517 nm.

The degree of free radical scavenging activity was expressed as:

$$\% \text{ Inhibition} = \{(A_{control} - A_{sample})/ A_{control}\} \times 100$$

where A control = absorbance of DPPH alone; A sample = absorbance of DPPH along with different concentrations of extracts.

IC_{50} was calculated from equation of line obtained by plotting a graph of concentration *versus* % inhibition.

Nitric oxide radial scavenging assay: The procedure is based on the principle that, sodium nitropruside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. The extracts *L. indica* screened for nitric oxide radical scavenging activity[11]. 1 mL of sodium nitropruside (10 mM) in 0.5 M phosphate buffer (pH 7.4) was mixed with 3.0 mL of the different concentrations of the sample dissolved in methanol and incubated at 25 ºC for 15 min. Above samples were reacted with Griess reagent (1 % sulphanilamide in 5 % H_3PO_4 and 0.1 % *N*-(1-naphthyl) ethylenediamine dihydrochloride in water). The absorbance of the chromophore formed during the diazotization of nitrate with sulphanilamide and subsequent coupling with *N*-(1-napthyl) ethylenediamine was read at 546 nm. The same reaction mixture without extract of plant but with equivalent amount of 0.5 M phosphate buffer served as control. Curcuminoids used as positive control. The antioxidant activity of the extracts was expressed as IC_{50}. It was calculated from equation of line obtained by plotting a graph of concentration (µg/mL) *versus* % inhibition.

Superoxide anion radical scavenging assay: The NADH /PMS/NBT system was used to determine the superoxide anion scavenging activities of the compounds[12,13]. The scavenging activity of superoxide anion was determined by the method of Yen and Chen[14]. The reaction mixture consists of 1 mL of different concentrations plant extract, 1 mL of phenazine methosulphate (60 µM) prepared in phosphate buffer (0.1 M pH 7.4) and 1 mL of NADH (phosphate buffer) was incubated at 25 ºC for 5 min, the absorbance was read at 560 nm against blank samples.

RESULTS AND DISCUSSION

The phytochemical screening of plant extract showed the positive reaction for flavonoids, steroids, *etc*. DPPH, nitric oxide and superoxide radical scavenging methods exhibited inhibition of free radicals by *Leucas indica* leaves and flowers extracts. Free radical is known as a major contributor to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defense mechanism[15].

Methanolic extracts of leaves and flowers, which were taken up for the research work showed significant activity. In DPPH method the methanolic extract of the flowers exhibited low IC_{50} values as compared to the leaves extracts. The IC_{50} value for flowers and leaves was found to be 0.19 µg/mL and 241.11 µg/mL respectively. Gallic acid was used as a standard in DPPH method which showed IC_{50} value of 2.10 µg/mL (Table-1). The result of DPPH scavenging activity assay in this study indicates that the plant was potently active. This suggests that the plant extract contain compounds that are capable to scavenge free radical which is responsible for radical's reactivity (Fig. 1).

Nitric oxide is a reactive free radical produced by phagocytes and endothelial cells, to yield more reactive species such as peroxynitrite which can be decomposed to form OH radical. The level of nitric oxide was significantly reduced in this study by the crude extract. In nitric oxide radical scavenging method the methanolic extract of the flowers exhibited low IC_{50} values as compared to the leaves extracts. The IC_{50} value for flowers and leaves was found to be 2.05 µg/mL and 282.97 µg/mL

TABLE-1
IC_{50} VALUES OF METHANOLIC EXTRACT OF *Leucas indica* LEAVES AND FLOWERS BY DPPH, NITRIC OXIDE AND SUPEROXIDE ANION RADICAL SCAVENGING ASSAY

Name of free radical scavenging assay	IC_{50} values (µg/mL)			
	Methanolic extract (*Leucas indica* leaves)	Methanolic extract (*Leucas indica* flowers)	Gallic acid (standard)	Curcuminoids (standard)
DPPH free radical scavenging assay	241.11	0.19	2.10	-
Nitric oxide radical scavenging assay	282.97	2.05	-	21.17
Superoxide anion radical scavenging assay	250.45	2.15	35.60	-

respectively. Curcuminoids was used as a standard in nitric oxide method which showed IC_{50} value of 21.17 µg/mL (Table-1). Results showed that methanol extract of *Leucas indica* flowers shows better antioxidant activity. It is observed that, phenolic compounds are responsible for antioxidant activity[16,17] (Fig. 2).

Superoxide anion radical is one of the strongest reactive oxygen species among the free radicals that could be generated; it also has the ability to change to other harmful reactive oxygen species and free radicals within the living cells[18]. In superoxide radical scavenging method the methanolic extract of the flowers exhibited low IC_{50} values as compared to the leaves extracts. The IC_{50} value for flowers and leaves was found to be 2.15 µg/mL and 250.45 µg/mL respectively. Gallic acid was used as a standard in superoxide method which showed IC_{50} value of 35.60 µg/mL (Table-1).The scavenging activity of this radical by the plant extracts compared with the standard suggests that the plant *Leucas indica* flowers are greater potent scavenger of superoxide radical than standard gallic acid (Fig. 3).

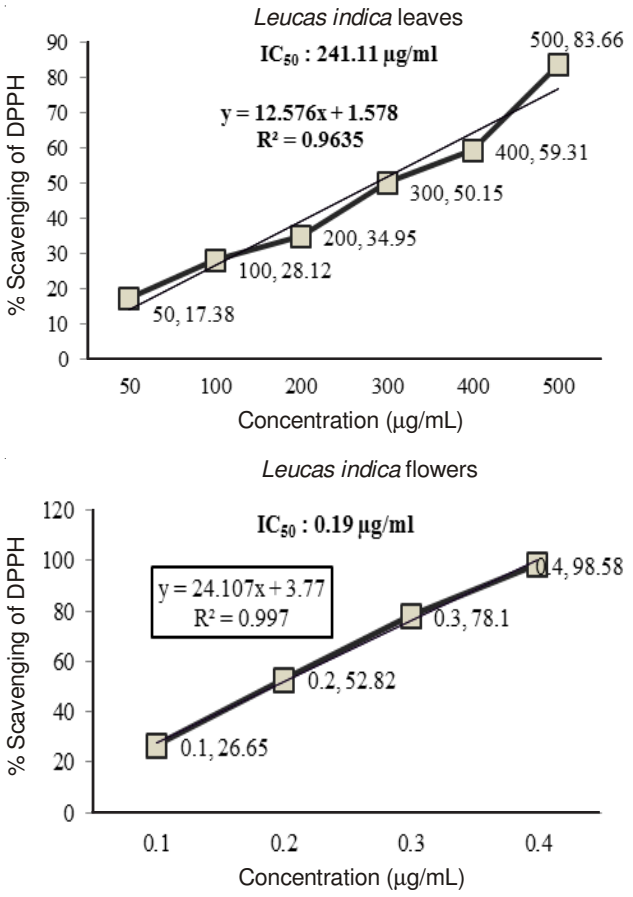


Fig. 1. DPPH radical scavenging activity of the methanolic extracts of *Leucas indica* leaves and flowers

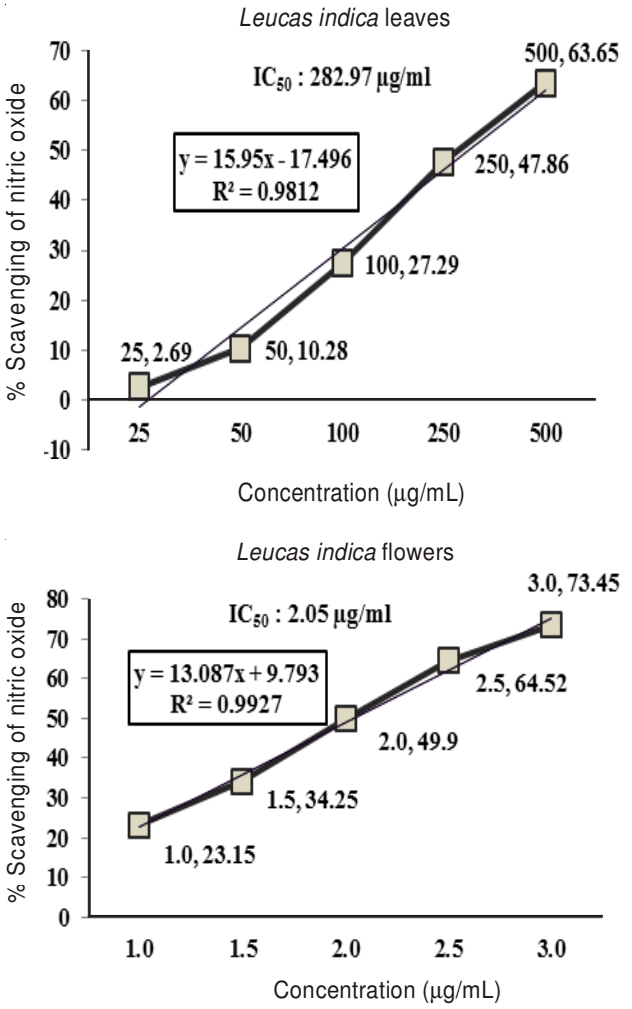
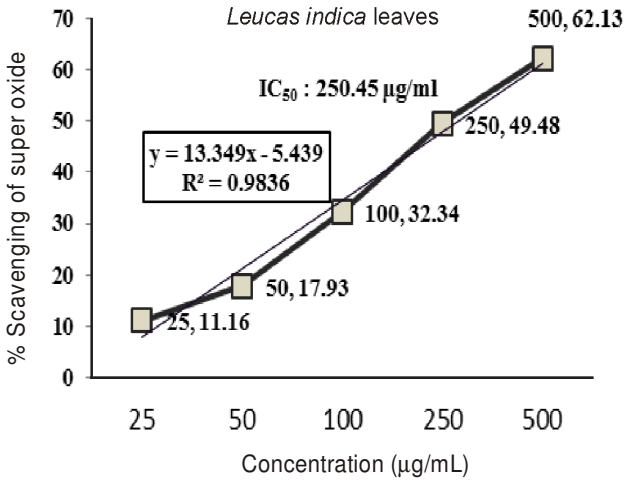


Fig. 2. Nitric oxide radical scavenging activity of the methanolic extracts of *Leucas indica* leaves and flowers



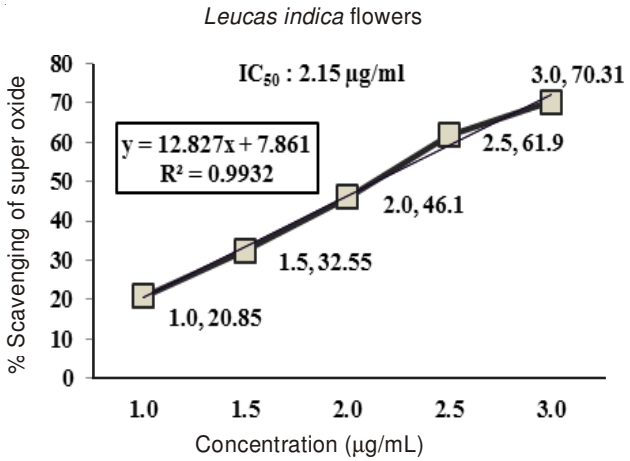


Fig. 3. Superoxide radical scavenging activity of the methanolic extracts of *Leucas indica* leaves and flowers

Conclusion

The present study suggests that methanolic extract of Leucas indica flowers possess more powerful antioxidant activity than leaves due to the presence of flavonoids, phenolics and proanthocyanidins. Plant extracts and plant-derived antioxidant compounds potentiate body's antioxidant defense, they are antioxidants of choice because of their lower toxicity and side effects over the synthetic ones. Also, they are relatively cheaper and are easily accessible. Further studies regarding the isolation and characterization of the active principles responsible for these activities is currently under progress in our research.

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