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In vitro antioxidant and DNA damage inhibition activity of aqueous extract of *Lantana camara* L. (Verbenaceae) leaves

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ABSTRACT

Objective: To investigate the *in vitro* antioxidant and DNA damage inhibition potential of aqueous extract of *Lantana camara* leaves. **Methods:** Antioxidant activity of the aqueous extract of *L. camara* was estimated by 2, 2-diphenyl-1-picrylhydrazyl radical scavenging assay, metal chelating activity and reducing power assay. DNA damage inhibition was performed by photolysing H_2O_2 by UV radiation in the presence of pBR322 and extract. Estimation of phenolic content was carried out by Folin-Ciocalteau assay. **Results:** Extract exhibited high antioxidant activity in DPPH radical scavenng assay (IC_{50}= 42.66 μg/ml), metal chelating activity (IC_{50}= 1036.4 μg/ml) and reducing power assay. Extract also exhibited the complete protection of pBR322 plasmid DNA during DNA damage inhibition assay. Extract showed high phenolic content which justified the antioxidant and DNA damage inhibition properties of the plant. **Conclusions:** These observations emphasize that aqueous extract of *L. camara* possess high antioxidant and DNA damage inhibition potential, thus, the plant can be used to develop natural antioxidant compounds for therapeutic use.

1. Introduction

Oxidation is a natural cellular metabolic process, lead to the formation of free radicals commonly known as reactive oxygen species (ROS) or reactive nitrogen species (RNS), such as Superoxide, Hydroxyl, Hydrogen peroxide, Peroxyl radical, Ozone, Nitric oxide, Peroxynitrite, Peroxynitrous acid, Nitrogen dioxide free radicals. Now it is scientifically proved that oxidative stress imposed by ROS and/or RNS can damage cellular membranes, proteins, fats and nucleic acid. Nature has endowed each cell with adequate protective mechanisms against any harmful effects of free radicals commonly known as antioxidant defense mechanism. Failure of antioxidant defense mechanism to counter the free radicals plays an important role in many chronic and degenerative diseases, such as heart disease, cancer, diabetes mellitus, neurodegenerative diseases, Parkinson's disease, Alzheimer's disease and ageing [1].

Antioxidants are the compounds with ability to neutralize free radicals, therefore prevent free radical mediated oxidative damage in cell. Antioxidant neutralize the free radicals by interfere with the oxidation process by reacting with free radicals, chelating activity, catalytic activity and oxygen scavenging activity [2, 3]. Recently there has been a growing interest in the study of traditional plants for pharmaceutical applications because of its low toxicity and economic viability. In past, various plant phytochemicals viz phenolic compounds, flavonoids and tannins reported to possess significant antioxidant activity against a wide variety of free radicals [4-6]. These active compounds can be isolated and developed as natural drugs for prevention and treatment of free radical related disorders.

L. camara (Verbenaceae) is an ornamental garden plant commonly known as Lantana. *L. camara* is a gregarious, erect, half climbing and hairy aromatic shrub. It grows up 1.2 meter height and branches are growing all four sided with recurved prickles. Leaves elliptic about 3 inches long and 1.5 inches wide pointed at the tip and rounded in the base and toothed in the margins. Flowers are pink, orange, yellow, white, lilac in color and color usually changes with the age. Seeds germinate very easily throughout the year. In the last decade, this plant has been extensively studied for its medicinal potential by using advanced scientific techniques and reported to possess anthelmintic activity [7], antitermitic [8], wound healing activity [9], Anti-leukemia

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activity [10], larvicidal activity [11], antioxidant activity [12], antibacterial activity [13], antiproliferative activity [4], anti-inflammatory activity [15], antiulcerogenic activity [16], anticancer activity [17], hemolytic activity [18] antimutagenic activity 19], antitumor activity [20] and antihyperglycaemic activity [21]. Above cited literature represents *L. camara* as an important source of novel pharmaceutically important compounds and a future candidate for the drug discovery.

The focus of this study was to evaluate the aqueous extracts of *L. camara* leaves for total phenolic content, DPPH radical scavenging activity, metal chelating activity and reducing power activity by in vitro methods.

2. Material and Methods

2.1. Plant material

L. camara was collected from the natural population growing in the industrial area Ranipet (12.9275°N 79.3302 Λ E), Vellore, Tamil Nadu, India, during August 2010. The plant was carried to the Molecular and Microbiology Research Laboratory, VIT University, Vellore. A herbarium was maintained in our laboratory for the future reference (Herbarium no.: LC/VIT/MMRL/17.08.2010-6).

2.2. Processing of plant

The leaves of *L. camara* were collected and washed thoroughly in tap water followed by distilled water. The leaves were shade dried at room temperature. Dried leaves were uniformly powdered using mechanical grinder to make fine powder. The leaves powder (50 gm) was soaked in sterilized distilled water (10% w/v) and loaded on a shaker at a speed of 120 rpm for 24 hour at room temperature. Mixtures were filtered by using filter cloth followed by Whatman number 1 filter paper. The filtrate was dried in a water bath. Dried extract was collected in air tight container and stored at 4℃ up for further use [22].

2.3. DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The DPPH radical scavenging activity was performed according to the standard protocols reported earlier [23]. Two millilitres of plant extracts (10, 20, 40, 60, 80 and 100 μg/ml in distilled water) was mixed with 1 ml of DPPH solution (0.2 mM/ml in methanol) and mixed thoroughly. The mixture was incubated in dark at 20℃ for 40 min. Absorbance was measured at 517 nm using UV-Vis spectrophotometer with methanol as blank. The percentage scavenging of DPPH by the extracts was calculated according to the following formula:

% DPPH Radical scavenging= [(Ac − At) / Ac] × 100

Here,

Ac is the absorbance of the control (DPPH)

At is the absorbance of test sample.

2.4. Metal chelating activity

Metal chelating activity of aqueous extracts of *L. camara* leaves was performed according to the standard protocols reported earlier [24]. 2 ml of crude extract (125, 250, 500 and 1000 μg/ml) was mixed with 100 μl of 2 mM FeCl2 and 400 μl of 5 mM ferrozine solutions and allowed to react for 10 minutes at room temperature. The absorbance at 562 nm of the resulting solutions were measured and recorded. Mixture of FeCl2 and ferrozine was used as control. Each experiment was performed in triplicates at each concentration.

The percentage inhibition of the ferrous ion was calculated by comparing the results of the test with those of the control using the formula.

Percentage metal chelating activity = [1 − (At / Ac)] × 100

Here,

At is the absorbance of test sample.

Ac is the absorbance of the control.

2.5. Reducing power assay

Various concentrations (125, 250, 500 and 1000 μg/m) of *L. camara* aqueous extracts (1 ml) were mixed with sodium phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 2.5 ml of 1 % K3Fe (CN)6. The mixture was incubated at 50 C for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v) was added and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml deionised water and 1 ml of 0.1% of ferric chloride, and the absorbance was measured at 700 nm; higher absorbance indicates higher reducing power. The assays were carried out in triplicate and the results are expressed as mean values ± standard deviations [25].

2.6. DNA damage inhibition efficiency

DNA damage inhibition by aqueous extracts of *L. camara* leaves was tested by photolysing H_2O_2 by UV radiation in the presence of pBR322 plasmid DNA and performing agarose gel electrophoresis with the irradiated DNA [26]. A total of 1 μl aliquots of pBR322 (200 μg/ml) were taken in two microcentrifuge tubes. A quantity of 50 μg of aqueous extracts was added to one tube. The other tube was left untreated as the irradiated controls. An amount of 4 μl of 3% H_2O_2 was added to all the tubes, which were then placed directly on the surface of a UV transilluminator (300 nm) for 10 min at room temperature. After irradiation, 4 μl of tracking dye (0.25% bromophenol blue, 0.25% xylene cyanol FF and 30% glycerol) was added. The samples in all the tubes were analyzed by gel electrophoresis on a 1% agarose gel (containing ethidium bromide) in TBE buffer (pH 8). Untreated non-irradiated pBR322 plasmid (C) was run along with untreated UV-irradiated plasmid DNA (R) and aqueous extract-treated UV-irradiated sample (S).

2.7. Estimation of total phenolic content

Total phenolic content of the aqueous extract of *L. camara* leaf was determined using the Folin-Ciocalteau reagent method [27]. The crude aqueous extract was diluted in methanol to obtain different concentrations (125, 250, 500 and 1000 μg). 50 μl of each extract was mixed with 2.5 ml of Folin- Ciocalteau reagent (1/10 dilution in purified water) and 2 ml of 7.5% Na_2CO_3 (w/v in purified water). The mixture was incubated at 45℃ for 15 min. The absorbance was measured at 765 nm. Na_2CO_3 solution (2 ml of 7.5% Na_2CO_3 in 2.55 ml of distilled water) was used as blank. The results were expressed as gallic acid equivalence in μg.

2.8. Statistical Analysis

All tests were conducted in triplicate. Data are reported as means ± standard deviation (SD). Results were analyzed statically by using Microsoft Excel 2007 (Roselle, IL, USA).

3. Results

The results of DPPH radical scavenging activity of aqueous extract of *L. camara* leaves are reported in Figure 1. The extract exhibited high DPPH radical scavenging activity with an IC50 value 42.66 μg/ml. The DPPH radical scavenging activity was found to be increasing as dose increases. The metal chelating activity of *L. camara* leaves extract is reported in Figure 2. The results are expressed as percentage metal chelating activity. Extract exhibited dose dependant metal chelating activity with an IC_{50} value 1036.4 μg/ml. The results for ferric reducing power activity of *L. camara* leaves extract is reported in Figure 3. Aqueous extract showed high reducing power potential and followed dose dependant increase in the reducing power activity. The outcome of DNA damage inhibition activity assay is summarized in Figure 4. The extract treatment exhibited complete protection of plasmid DNA at a dose of 50 μg. Total phenolic content analysis revealed the high quantity of phenolic compounds in the extract. Total phenolic content of is expressed as gallic acid equivalence (GAE) in μg and reported in Figure 5.

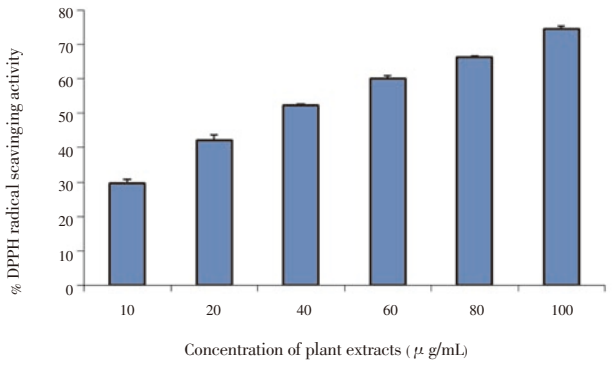

Figure 1. DPPH radical scavenging activity of aqueous extract of *L. camara* leaves.
Results are expressed as percentage inhibition of DPPH, All values represent the mean ± standard deviation (n = 3).

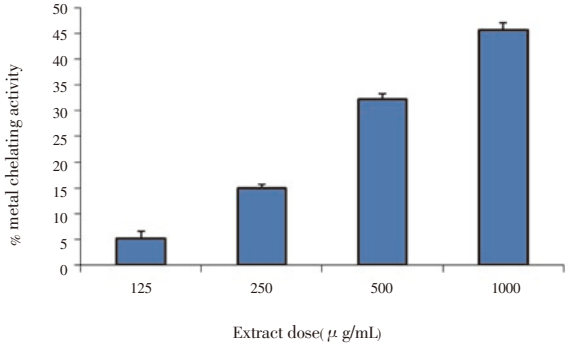

Figure 2. Metal chelating activity of aqueous extract of *L. camara* leaves.
Results are expressed as percentage metal chelating activity. All values represent the mean ± standard deviation (n = 3).

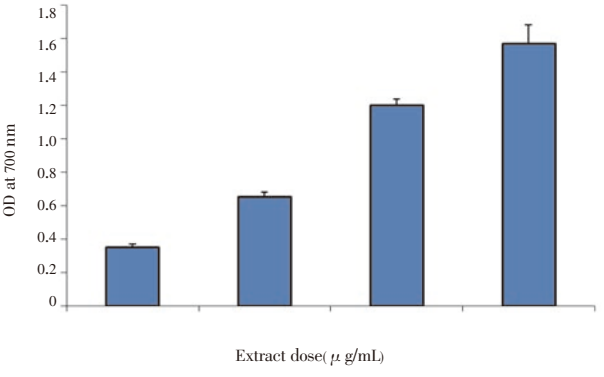

Figure 3. Reducing power activity of aqueous extract of *L. camara* leaves.
All values represent the mean ± standard deviation (*n* = 3).

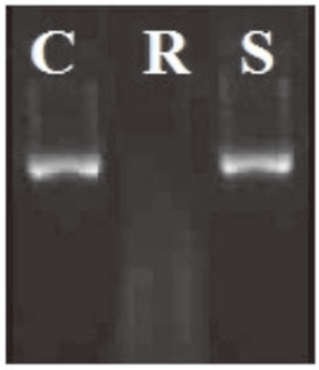

Figure 4 Effect of varying concentrations of *L. camara* leaves aqueous extracts on the protection of plasmid DNA (pBR322) against oxidative damage caused by UV-photolysed H2O2. C=untreated non-irradiated DNA (C), CR=untreated UV-irradiated DNA (R) and aqueous extract treated DNA (S)

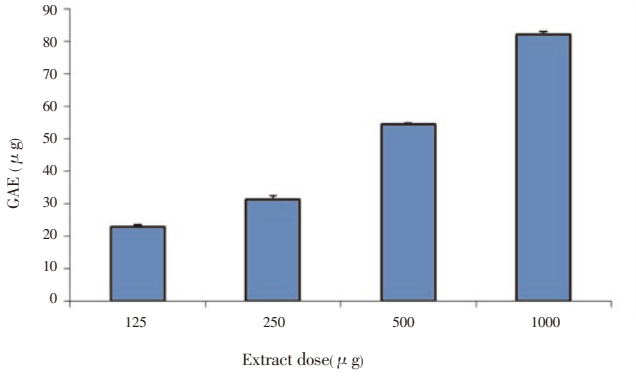

Figure 5 Total phenolic concentration of aqueous extract of *L. camara* leaves.
Results are expressed as gallic acid equivalence (GAE) in μg; All values represent the mean ± standard deviation (n = 3).

4. Discussion

L. camara is a tropical origin plant and widely distributed throughout the world. *L. camara* is an aromatic plant with several traditional medicinal applications in traditional medication system. Selection of *L. camara* for this study was based on its traditional medicinal uses to cure ulcers, cough, mumps, rheumatism, incessant high fever, wounds, malaria, cervical lymph node tuberculosis, influenza, dermatitis, eczema, pruritus, sprains, contusions, tetanus, toothaches and swellings. In this study we have analyzed the antioxidant and DNA protective potential of *L. camara* by various in vitro methods.

In this study aqueous extract of *L. camara* leaves exhibited the significant antioxidant activity in DPPH assay, metal chelating assay and reducing power assay. Extract also exhibited protection of plasmid DNA against free radicals mediated oxidative damage. In our study, extract exhibited the presence of high amount of phenolic compounds, which could be considered as the key reason behind the antioxidant potential of this plant. In previous studies, various solvent extracts of *L. camara* leaves were reported to possess several phenolic compounds such as Gentisic acid, Salicylic acid, β - resoreylic acid, Coumarin ferulic acid, 6 - methyl coumarin, p - hydroxyl benzoic acid, Sabinene, β - caryophyllene, 1,8 cineole, α - humulene etc [28, 29]. Literature suggests that the antioxidant and DNA protective efficacy of the extract may be due to the presence of phenolic compounds. Previously many studies have reported the strong correlation between phenolic content and antioxidant potential of the plants [30, 31], while few other studies have not obtained such correlation [32]. However, phenolic compound are well known and widely accepted antioxidant compounds present in plant. Earlier, leaves of *L. camara* were reported to possess DPPH scavenging activity and reducing power activity. This study also describes the effect of leaf positions on total phenolics, flavonoids and proantho-cyanidins content and antioxidant activities in *L. Camara* [12]. In another study L. camara leaves have been reported to possess DPPH radical scavenging activity with IC50 value 114.63 +6.16 μg/ml [14]. Later, petroleum ether extract of *L. camara* leaves was reported to possess *in vivo* antioxidant activity in Wistar albino rats [16]. Presented study and the above cited literature emphasizes the antioxidant potential of *L. camara* leaves in both *in vitro* and *in vivo* systems. Our study revelled the DNA protective efficacy of *L. camara* leaves; this property could be capitalized to develop some drugs for the prevention/control of free radical induced cancer. However, there is a need of establishing a more systemic study to validate the human consumption of *L. camara* for medicinal use as well as to findout the bioactive principle of the plant.

Conflict of interest statement

We declare that we have no conflict of interest

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