ANTIOXIDANT ACTIVITY OF *CALOTROPIS GIGANTEA*

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Summary

Free radicals are implicated for more than 80 diseases including diabetes mellitus, arthritis, cancer, ageing etc. In treatment of these diseases, antioxidant therapy has gained an utmost importance. Current research is now directed towards finding naturally occurring antioxidants of plant origin. In Indian system of medicine *Calotropis gigantea* is an important medicinal plant and its flowers have been used to cure jaundice, inflammation, ulcer and asthma like diseases. To understand the mechanism of pharmacological actions, the in vitro antioxidant activities viz. hydroxyl, nitric oxide, hydrogen peroxide radicals and determination of total flavonoids in aqueous extract of *C. gigantea* was investigated in the present study. In all the tests, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. The antioxidant property may be related to the flavonoids and terpenoids present in the extract. These results clearly indicate that *C. gigantea* is effective against free radical mediated diseases.

Keywords - *Calotropis gigantea,* Antioxidant activity, radical scavenging.

Introduction

Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals. (1) Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism. (2) The most common reactive oxygen species (ROS) include superoxide ($0_2^{\cdot}$) anion, hydrogen peroxide ($H_20_2^{\cdot}$), peroxyl ($ROO^{\cdot}$) radicals, and reactive hydroxyl (OH.) radicals. The nitrogen derived free radicals are nitric oxide ($NO^{\cdot}$) and peroxynitrite anion ($ONOO^{\cdot}$). ROS have been implicated in over a hundreds of diseases states which range from arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome. (3) In treatment of these diseases, antioxidant therapy has gained an immense importance. Current research is now directed towards finding naturally occurring antioxidants of plant origin. Antioxidants have been reported to prevent oxidative damage by free radical and ROS, and may prevent the occurrence of disease, cancer and aging. It can interfere with the oxidation process by reacting with free radicals, chelating and also by acting as oxygen scavengers. (4, 5) Plant and plant products are being used as a source of medicine since long. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability. (6) Flavonoids and phenolic compounds are widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging, anti-inflammatory and anticarcinogenic activities. (7) Novel natural antioxidants from some plants have been extensively studied in the past few years for their antioxidant and free radical scavenging properties. *C. gigantea* R.Br. (Asclepiadaceae) is a shrub or a small tree with 8-10 feet height. It is a genus of about six species, among which *C. gigantea* is the species which is commonly grown in waste land throughout India. (8) The hydroalcoholic extract of flowers is reported to possess hepatoprotective activity. (9) The aerial part of the *C. gigantea* has been proved for its antidiarrhoeal property in castor oil induced diarrhea. (10) Recently the alcoholic extract of flowers has been documented to possess analgesic property. (11) It is used as main ingredient in various hepatoprotective preparations. (12) Traditionally the *C. gigantea* flowers are used to cure jaundice, inflammation, ulcer and asthma like diseases. (13) Aqueous extract of *C. gigantea* flowers (CGFA) contains terpenoids and flavonoids.

Therefore, the objectives of the present study were to investigate the in vitro antioxidant activity of *C. gigantea* aqueous extract through the free radical scavenging activities such as hydroxyl, nitric oxide, and hydrogen peroxide radical scavenging.

Materials and methods

Plant material

The flowers of *C. gigantea* were collected from pune region were authenticated by Dr.Shirolkar from Botanical survey of India, (BSI/WC/Tech./2008/CGDKIPL1) Pune. The shade dried flowers of *C. gigantea* of about 500 g were subjected for size reduction to coarse powder. The powder was defatted with petroleum ether (60-80°C) and then extracted with water using soxhlet apparatus for about 32 h. The CGFA were concentrated under vaccum to get the residues.

Chemicals

Ferric chloride ($FeCl_3$), 1, 10 phenonthroline, Sodium thiosulphate (NaS_2O_3), Sulphuric acid (H_2SO_4), Potassium iodide (KI), Sodium nitroprusside, Naphthyl ethylenediamine dihydrochloride, Aluminum chloride, potassium acetate, Hydrogen peroxide (H_2O_2) and Ascorbic acid (Vitamin-C) were purchased from Vijay Chemicals, Pune. All other chemicals and solvents used were of analytical grade available commercially.

In-vitro antioxidant activity

Hydroxyl Radical Scavenging Activity Assay

The scavenging activity for hydroxyl radicals was measured with Fenton reaction. (14) Reaction mixture contained 60 µl of 1.0mM $FeCl_3$, 90 µl of 1mM 1,10-phenanthroline, 2.4 ml of 0.2 M phosphate buffer (pH 7.8), 150 µl of 0.17 M H_2O_2, and 1.5 ml of CGFA at various concentrations. Addition of H_2O_2 started the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture at 560 nm was measured with a spectrophotometer. The hydroxyl radicals scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_0 - A_1) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (blank, without CGFA) and A1 was the absorbance in the presence of the CGFA.

Nitric Oxide Scavenging Activity Assay

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat. (15) Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of CGFA at various concentrations and the mixture incubated at 25^{o}C for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1 % w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_0 - A_1) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (blank, without CGFA) and A_1 was the absorbance in the presence of the CGFA.

Hydrogen Peroxide Scavenging Activity Assay

Hydrogen peroxide scavenging activity of the extract was estimated by replacement titration. (16) Aliquot of 1.0 ml of 0.1 mM H_2O_2 and 1.0 ml of various concentrations of CGFA were mixed, followed by 2 drops of 3 % ammonium molybdate, 10 ml of 2 M H_2SO_4 and 7.0 ml of 1.8 M KI. The mixed solution was titrated with 5.09 mM NaS_2O_3 until yellow color disappeared. Percentage of scavenging of hydrogen peroxide was calculated as:

$$\% \text{ Inhibition} = (V_0 - V_1) / V_0 \times 100$$

Where V_0 was the volume of NaS_2O_3 solution used to titrate the control sample in the presence of hydrogen peroxide (without CGFA), V_1 was the volume of NaS_2O_3 solution used in the presence of the CGFA.

Determination of Total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination. (17)1 ml of CGFA was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with

UV/Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol. The concentration of flavonoids was expressed in terms of mg/100 ml of CEFA.

Statistical analysis: Statistical analysis of difference between groups was evaluated by one-way ANOVA followed by Dunnett's test. Data for the *in vitro* antioxidant activity was expressed as Mean ± SD from three separate observations.

Results and discussion

The phytochemical analysis of CGFA revealed the presence of flavonoids which was found to be 10.6 ± 0.03 mg/100 ml. Flavonoids are used for the prevention and cure of various diseases which are mainly associated with free radicals. (18)

Hydroxyl Radical Scavenging Activity Assay

Hydroxyl radical is very reactive and can be generated in biological cells through the Fenton reaction. (Figure 1) shows the concentration dependent scavenging activities of CGFA extract exhibited against hydroxyl radicals generated in a Fenton reaction system. The potential scavenging abilities of phenolic substances might be due to the active hydrogen donor ability of hydroxyl substitution. Similarly, high molecular weight and the proximity of many aromatic rings and hydroxyl groups are more important for the free radical scavenging by specific functional groups. (19)

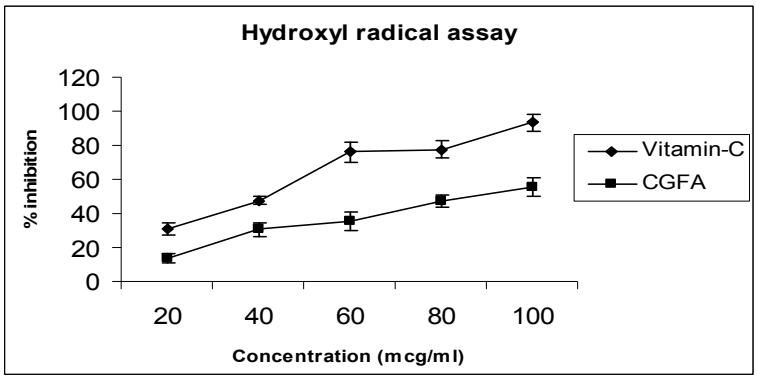

Figure 1: Hydroxyl radical scavenging activity of CGFA at different concentrations. Each value represents means ± SD (n=3).

Nitric oxide radical scavenging activity

Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumor activities. (20) CGFA extract moderately inhibited nitric oxide in dose dependent manner (Figure 2).

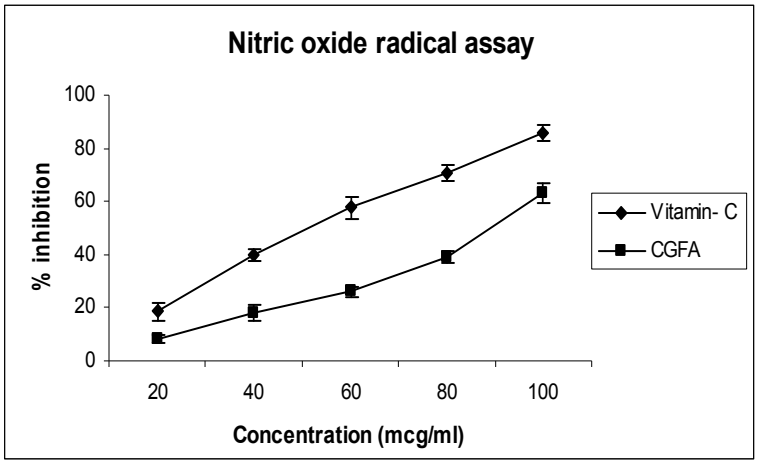

Figure 2: Nitric oxide scavenging activity of CGFA at different concentrations. Each value represents means ± SD (n=3).

Hydrogen peroxide scavenging activity

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. (21) It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. As shown in Figure 3, CGFA extract demonstrated hydrogen peroxide scavenging activity in a concentration dependent manner.

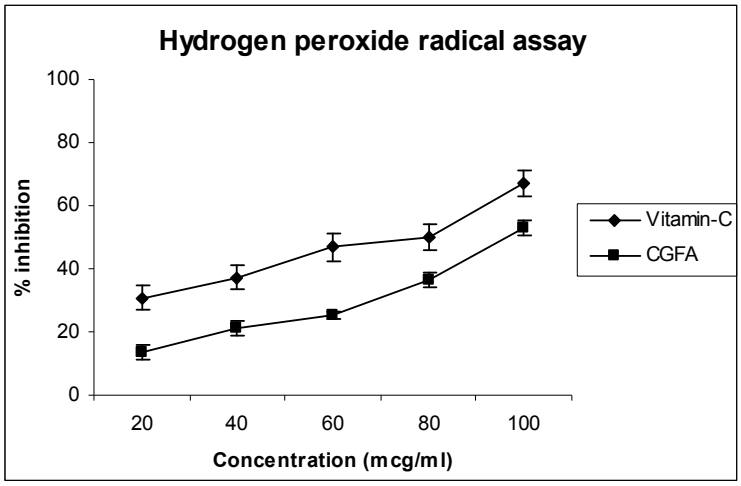

Figure 3: H_2O_2 scavenging activity of CGFA at different concentrations. Each value represents means ± SD (n=3).

In conclusion, the results of the present study show that the CGFA which contains highest amount of flavonoids and terpenoids exhibits the greatest antioxidant activity as compared to CGFA. The possible mechanism is due to the scavenging of free radicals such as hydroxyl, hydrogen peroxide and nitric oxide radical, which participate in various pathophysiologies of diseases including ageing. CGFA also exert reducing power activity. Overall, the plant extract is a source of natural antioxidants that can be important in disease prevention, health preservation and promotion of longevity promoter.

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