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In-vitro* Antioxidant Activity, Total Phenolic and Total Flavonoid Contents of Flower Extract of *Calotropis gigantea

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ABSTRACT

Recently antioxidant properties of plants have been found to be correlated with the defense of oxidative stress. In this respect flavonoids and other phenolic compounds have gained considerable attention. *Calotropis gigantea* has been widely used for its various pharmacological activities including cancer studies. In this study, the evaluation of antioxidant activity of methanolic extract of the flowers of *C. gigantea* has been done by *in vitro* method. The antioxidant activity of flower extract was evaluated by determining lipid peroxidation method, total phenolics contents was determined according to the Folin-Ciocalteu method and total flavonoid content by aluminium chloride colorimetric method. The results of the study showed that the methanolic extract of flowers of *C. gigantea* possesses antioxidant activity and it can be known by the protection of the cells. The total phenolic and flavonoid content was determined and expressed in term of gallic acid equivalent and quercetin equivalent, respectively. The results of the present study clearly showed that the flower extract of *C. gigantea* demonstrated antioxidant activities and it may act as potential antioxidant sources for human biological system.

Key words: Milkweed, lipid peroxidation, TFC, TPC, malondialdehyde

INTRODUCTION

Calotropis gigantea (Asclepiadaceae) is distributed throughout India. It is popularly known as Erukku or Erukkan chedi in Tamil. *Calotropis gigantea* is commonly known as milkweed or swallow-wort, is a common wasteland weed. *Calotropis gigantea* belongs to Asclepiadaceae or milkweed family which includes 280 genera and 2,000 species of world-wide distribution but most abundant in the sub-tropics and tropics, and rare in cold countries. *Calotropis gigantea* is used as a traditional medicinal plant with unique properties (Oudhia and Tripathi, 1999). Traditionally *C. gigantea* is used alone or with other medicine to treat common disease such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting and diarrhea (Caius, 1986). According to ayurveda, dried whole plant is a good tonic, expectorant and anthelmintic. The flowers are bitter, digestive, astringent, anthelmintic and tonic (Agharkar, 1991). Root bark of *C. gigantea* exhibits febrifuge, anthelmintic, depurative, expectorant, laxative and used in the treatment of asthma, bronchitis and dyspepsia. The leaves are useful in the treatment of paralysis, arthralgia, swellings, hepatoprotective activity, anticancer activity, antifertility and anti-inflammatory activity. Cardiac glycosides from *C. gigantea* exhibit anticancer properties (Bhat and Sharma, 2013).

Antioxidants are compounds which possess the ability to protect cells from the damage caused by unstable molecules known as free radicals. Free radicals have been implicated as mediators of many diseases, including cancer, atherosclerosis and heart diseases (Tirzitis and Bartosz, 2010; Al-Dabbas *et al.*, 2006; Tsao and Deng, 2004; Hayet *et al.*, 2008). Antioxidants have the capacity in preventing or slowing the oxidation reactions and have been recognized for their potential in promoting health and lowering the risk for cancer, hypertension and heart disease (Souri *et al.*, 2008; Suryanti *et al.*, 2015). Lipid Peroxidation (LP) is oxidative deterioration of polyunsaturated lipids and it involves ROS and transition metal ions. It is a molecular mechanism of cell injury leading to generation of peroxides as lipid hydroperoxides which can decompose to yield a wide range of cytotoxic products most of which are aldehydes, as exemplified by malondialdehyde (MDA), 4-hydroxynonenal etc (Luo *et al.*, 1997). The stimulation of LP as a consequence of tissue injury can sometimes make significant contribution to worsening of injury. Many drugs and medicinal substances like adriamycin, menadione, paraquat, alloxan etc., have capacity to produce peroxides. Lipid peroxidation induction capacity of drugs may be related to their toxic potential adriamycin induced cardiotoxicity is mediated through free-radical mediated process. Thus evaluation of antioxidant as suppressors of drug induced LP provides a scope to select free-radical scavengers which on co-administration *in vivo*, in case of reduced endogenous antioxidant defense may reduce toxic effects of drugs used for therapeutic purpose (De *et al.*, 2000).

The present study deals with lipid peroxidation induced by a drug ceftizoxime sodium (CZX), a third generation cephalosporin antibiotic. An *in vitro* evaluation of *C. gigantea* which is a component of endogenous antioxidant defense mechanism, as inhibitors drug induced lipid peroxidation.

MATERIALS AND METHODS

Plant material: Flowers of *C. gigantea* (Fig. 1) were collected from the outfield of Tiruchirappalli, India.



Fig. 1: Flowers of *Calotropis gigantea*

Preparation of the extract: The collected flowers were air dried under shade for 14 days and pulverized. The pulverized leaves (400 g) were extracted by maceration with 1 L of 80% methanol for 24 h at 4°C. The *C. gigantea* flowers extract solution was filtered through Whatman filter paper No. 1 and freeze dried. The extract was weighed, percent yield calculated and stored in an air tight container at 4°C.

In vitro studies: The *in vitro* antioxidant capability of *C. gigantea* flowers extract was evaluated with the estimation of total phenolic content, total flavonoid content and lipid peroxidation activity.

Estimation of total phenol content: An amount of total phenolic content in the extract was determined using a series of gallic acid as standard solutions (0.05-0.35 mg mL⁻¹) as described by Slinkard and Singleton (1977). Each extract solution (0.1 mL) was mixed with 2 mL of a 2% (w/v) sodium carbonate solution and vortexed vigorously. After 3 min, 0.1 mL of 50% Folin-Ciocalteu's phenol reagent was added and incubate for 30 min at room temperature and then absorbance was measured at 760 nm. All samples were analyzed in triplicates. Total phenolic contents of extracts were expressed as milligram gallic acid equivalent (GAE)/g dry weight. All samples were analyzed in triplicates.

Estimation of total flavonoid content: The aluminium chloride colorimetric assay was used for total flavonoids determination, as described by Zhishen *et al.* (1999). Results were expressed as milligram of quercetin equivalent/g extract.

Lipid peroxidation: Fresh blood sample was collected from goat and it was used as the lipid source. Blood being the transporting tissue may be considered as close stimulator of more complex biological system. Goat blood was selected because of its easy availability and close similarity to human blood. Blood sample was mixed with equal volume of sterilized Alsever solution (containing 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride) and stored at 4°C and used within 5 h. Different portions of the blood were treated with drug (CZX) and or antioxidant from *C. gigantea*. A portion of blood not treated with drug or antioxidant served as control. The CZX was treated as solution in saline and the effective concentration was 40 mg. The antioxidant also was treated as solutions in saline in effective concentrations 10, 25, 50, 75 and 100 µg of *C. gigantea* extract. Lipid peroxidation of blood samples was measured in terms of malondialdehyde (MDA) content following the thiobarbituric acid (TBA) method (Tarladgis *et al.*, 1964; Prior, 2003). Different sets of experiments were performed for each drug-antioxidant part and it was repeated. In CZX measurement of MDA content of blood samples were done at 3, 6, 8 and 24 h of incubation. The mean MDA content of 0 h of the control sample served as the reference for comparison in all cases. The method of measurement of MDA content involved precipitation of the protein part of the blood by treating with 10% trichloroacetic acid (TCA) solution and centrifugation at 3000 rpm for 30 min followed by filtration of the supernatant. The filtrate was then treated with 0.002 M TBA solution and boiled for 30 min. The resultant mixture was cooled to room temperature and its absorbance was estimated at 530 nm against TBA blank by using EC digital spectrophotometer GS5700 B. The standard curve was prepared using tetraethoxy propane and TBA according to the method and the corresponding best fit equation was found out using the method of least squares. The percent changes in MDA content of different samples were calculated with respect to the corresponding control 0 h and change in MDA level was considered as an indicator of the extend of LP.

Statistical analysis: Significant differences of the data among the parameters were calculated by performing ANOVA test with the help of SPSS and means were compared by Least Significant Difference (LSD). The values $p < 0.05$ were regarded as significant and the values $p < 0.01$ were considered as highly significant.

RESULTS AND DISCUSSION

Plant extraction: The percentage yield of *C. gigantea* flowers extract (w/w) was 18.56%.

In vitro antioxidant determination: Mostly antioxidant activities of plant sources are due to the presence of flavonoids, phenolic-type compounds. Most of the tannins and flavonoids are phenolic compounds may be responsible for antioxidant properties of many plants (Larson, 1988). Flavonoids are capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups and so they are potent antioxidants (Cao *et al.*, 1997). But the present study did not try to establish any correlation between flavonoid, phenolic content and biological activity. Total phenol content of the extract was 19.86 ± 0.02 (mg GAE g^{-1}) and total flavonoid was 5.10 ± 0.60 (mg quercetin g^{-1}) (Table 1). The antioxidant activity was investigated by measuring the MDA content after the precipitation and filtration of protein part. The methanolic extract of the flowers of *C. gigantea* had profound antioxidant activity. The extract is capable of inhibiting the infer percentage of release enhances with the increase in concentration. At higher concentration i.e., at 100 μg the extract was found to be protecting the cells around 77%. Antioxidant activity of methanolic extract of *C. gigantea* against induced lipid peroxidation shown in Table 2 and Fig. 2.

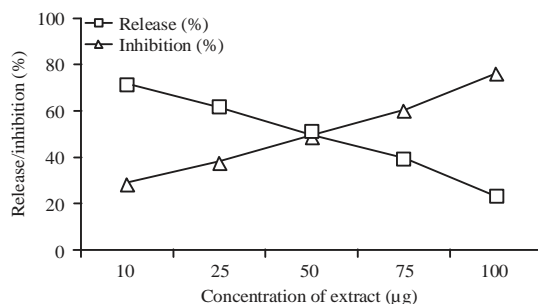


Fig. 2: Antioxidant activity of methanolic extract of *Calotropis gigantea* against induced lipid peroxidation

Table 1: Results of quantitative estimation of total phenol and flavonoid content

Phytochemicals	Results
Total phenolics	19.86 ± 0.02 mg gallic acid equivalents g^{-1} extract
Flavonoids	5.10 ± 0.60 mg quercetin equivalents g^{-1} extract

Table 2: Antioxidant activity of methanolic extract of *Calotropis gigantea* against induced lipid peroxidation

Dose (μg)	Release (%)	Inhibition (%)
10	71.42	28.58
25	61.84	38.16
50	50.64	49.36
75	39.32	60.68
100	23.20	76.80

Previous studies report the presence of phytochemicals like cardenolides, flavonoids, terpenes, pregnanes, nonprotein amino acid and cardiac glycoside as major constituents in *C. gigantea* may acknowledge the antioxidant property of this plant (Ali and Gupta, 1999; Elakkiya and Prasanna, 2012; Amit *et al.*, 2010; Khasawneh *et al.*, 2011; Kumar *et al.*, 2010; Wang *et al.*, 2008). The chemical constitute and secondary metabolites of different parts of *C. gigantea* may be responsible for antioxidant potential (Khairnar *et al.*, 2012). This is in agreement with the present study. The TPC and TFC have been proved to be responsible for the antioxidant activity of the plants (Liu *et al.*, 2008; Pietta, 2000). The results of the evaluation of antioxidant activity study showed that the extract is capable of protecting the cells by decreasing the percentage of release. It has been reported that reactive oxygen species contribute to various path physiological 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 plant derived agents may help to reduce it. Determination of the natural antioxidant compounds of plant extracts will help to develop new drugs for antioxidant therapy (Trease and Evans, 1989). The plant may be considered as good sources of natural antioxidants for medical uses. Research on relationship between antioxidants and prevention of some diseases, such as cardiovascular disease and cancer has been increasing sharply in recent years (Kubola and Siriamornpun, 2008). However, synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been restricted due to their carcinogenic effect (Sun and Fukuhara, 1997; Hirose *et al.*, 1998). Therefore, investigations of antioxidants are focused on naturally occurring substances, especially plant phytochemicals. Fruits, vegetables and many medicinal plants have antioxidant components, especially phenolic compounds and their consumption has contributed to prevention of destructive processes caused by oxidative stress (Kaur and Kapoor, 2001; Vinson *et al.*, 2001).

CONCLUSION

The methanolic extract of the flowers of *C. gigantea* has got profound antioxidant activity. The extract is capable of inhibiting the infer percentage of release enhances with the increase in concentration. At higher concentration i.e., at 100 µg the extract was found to be protecting the cells around 77%. The results of the evaluation of antioxidant activity study showed that the extract is capable of protecting the cells by decreasing the percentage of release. Determination of the natural antioxidant compounds of plant extracts will help to develop new drugs for antioxidant therapy. The plant may be considered as good sources of natural antioxidants for medical uses.

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