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RESEARCH ARTICLE

Frap Assay and Nitric Oxide Free Radical Scavenging Activity of Aerial Parts of Ethanolic Extract of Cordia Obliqua

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Abstract

Objective: Oxidative stress resulting from accumulation of reactive oxygen species has been associated with disease. Research study was undertaken to investigate and evaluate the *in vitro* antioxidant activities such as FRAP and Nitric oxide of ethanolic extract of *cordia obliqua*. Methods: The ethanolic extract of *cordia obliqua* was examined by Nitric oxide radical scavenging activity and Ferric Reducing Antioxidant Power Assay with reference standard ascorbate respectively through *in vitro* models. Results: Ethanolic extract of *cordia obliqua* showed significant free radical scavenging activity than that of standard. Conclusion: *In vitro* study indicates that these plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: *FRAP, Nitric oxide, Antioxidant, Scavenging Flavonoids, Reactive Oxygen Species.*

Introduction

Oxidative stress resulting from accumulation of reactive oxygen species has been associated with disease. The search for natural antioxidants of plant origin is necessitated by the side effects associated with synthetic antioxidants currently available. FRAP assay stands for Ferric Reducing Antioxidant Power Assay. The assay measures the antioxidant potential in samples through the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by antioxidants present in the samples. The total antioxidant activity can be measured by the ferric reducing antioxidant power assay (FRAP).

The flavonoids and phenolic acids are present in the medicinal plant exhibit strong antioxidant activity which is depending on their potential to form the complex with metal atoms, particularly iron and copper. This method is based on the principle of increase in the absorbance of the reaction mixtures, the absorbance increases the antioxidant activity increases. Nitric oxide (NO) is an important bioregulatory molecule, which has a number of physiological effects including control of blood pressure, neural signal transduction, platelet function, antimicrobial, and antitumor activity. Low concentrations of NO are sufficient, in most cases, to effect these beneficial functions. However, during infections and inflammations, formation of NO is elevated and may bring about some undesired deleterious effects [1-3] like renal dysfunction, tumor growth, etc. Nitric oxide (NO*) is an effective chain-breaking antioxidant in free radical-mediated lipid oxidation (LPO). It reacts rapidly with peroxyl radicals as a sacrificial chain-terminating antioxidant.

The goal of this work was to determine the minimum threshold concentration of NO* required to inhibit Fe2+ -induced cellular lipid peroxidation. Antioxidants however, can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions [4-6]. Recently, there has been a great increase of interest in natural antioxidant phytochemicals of plant origin since they are viewed as promising therapeutic drugs for free radical pathologies and also found to be useful in the food industries as nutraceuticals due to their good antioxidant potentials and impact on the status of human

health [7-9]. *Cordia obliqua Willd*. [10-12] also known as Clammy cherry, is a flowering plant species in the genus Cordia belonging to the family Boraginaceae. The scope of this work was to evaluate *in-vitro* antioxidant activity of ethanolic extract of *Cordia obliqua*.by FRAP and Nitric oxide radical scavenging activity.

Materials and Methods

Collection, Identification, Extraction and Isolation of *Cordia oblique*

Cordia obliqua was collected from B. Maduvangarai, Chidambaram Taluk, Cuddalore District, Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India, Palayamkottai. The leaves of *Cordia obliqua* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered plant materials were stored in an air-tight container.

The above powdered components were continuously extracted with ethanol in a Soxhlet apparatus using a 24 hours continuous hot percolation method. The extract was concentrated on a rotary evaporator and subjected to freeze drying in a lyophilizer until a dry powder was obtained [13-15].

Evaluation of Antioxidant Activity

Ferric Reducing Antioxidant Power Assay

A modified method of Benzie and Strain (1996) [16] was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-S-triazine) solution in 40 mMHCl and 20 mM$Fecl_3$. $6H_2O$. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $Fecl_3$.$6H_2O$. The temperature of the solution was raised to 37ºC before using.

Plant extracts (0.15 ml) were allowed to react with 2.85 ml of FRAP solution for 30 min in the dark condition. Readings of the colored product (Ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $Feso_4$. Results are expressed in µM (Fe (II) /g dry mass and compared with that of ascorbic acid.

Nitric Oxide Radical Scavenging Activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat (1964)[17].The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM) and 0.5 ml of phosphate buffer saline (1M) was incubated at 250C for 150 mins.

After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological P^H spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Illosvery reaction at 540 nm.

Results and Discussion

FRAP Assay and Nitric Oxide Radical Scavenging Activity

The assay measures the antioxidant potential in samples through the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by antioxidants present in the samples. The reducing properties associated with the presence of compounds exert their action by breaking the free radical chain through donating a hydrogen atom.

The total antioxidant activity can be measured by the ferric reducing antioxidant power assay (FRAP). The maximum reducing ability at 1000µg/ml for plant extract and ascorbate was found to be 64.89 ± 0.01% and 55.23±0.03% respectively. Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes.

The ethanolic extract of *Cordia obliqua* exhibited a scavenging activity of 64.38±0.01% at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 ± 0.01% at 1000 µg/ml.

Table 1: FRAP assay and Nitric oxide radical scavenging activity of ethanolic extract of *Cordia obliqua*

S.No	Concentration	Percentage of activity		
		FRAP assay	Nitric oxide scavenging activity	**Standard (Ascorbate)**
1	125 µg/ml	32.68±0.06	24.27±0.06	26.87 ± 0.08
2	250 µg/ml	48.69±0.03	28.65±0.03	30.30 ± 0.05
3	500 µg/ml	59.34±0.02	46.73±0.01	60.64 ± 0.02
4	1000 µg/ml	64.89±0.01	64.38±0.01	55.23 ± 0.01

*All values are expressed as mean ± SEM for three determinations

Conclusion

Antioxidants can be explained as reductants, and inactivators of oxidants. Some previous studies have also reported that the reducing power may serve as a significant indicator of potential antioxidant activity. Antioxidative activity has been proposed to be related to reducing power. Therefore, the antioxidant potential of ethanolic extract of *Cordia obliqua* was estimated for their ability to reduce TPTZ–Fe (III) complex to TPTZ–Fe (II). The ferric reducing ability of the extracts revealed that all of them gave good FRAP activity and the extract have antioxidant activity by scavenging the nitric oxide free radical. It is very much helpful for investigation of new drugs for various free radical generation diseases by identifying the compound isolation process.

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