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*Full Length Research Paper*

# ANTI-OXIDANT PROPERTY OF ETHANOLIC EXTRACT OF *CATUNAREGAM SPINOSA THUNB*

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## ABSTRACT

*Oxidative stress may be one of the factors which play a role in the development of chronic and degenerative diseases, such as cancer, heart disease, and neuronal degeneration. Fruits, nuts, and vegetables have long been considered high in antioxidants. Total antioxidant capacity (TAC) levels are affected by a wide array of factors, such as cultivar, growing conditions, harvesting, food processing and preparation, sampling, and analytical procedures. The ethanolic leaf extract of Catunaregam spinosa Thunb was analyzed for its antioxidant capacity using the DPPH and ferric ion reducing antioxidant power (FRAP) methods. The Catunaregum spinosa leaves showed the highest antioxidant property for DPPH and FRAP assays.*

***Key words:*** *Oxidative stress, Total antioxidant capacity, DPPH, FRAP, C.spinosa*

## INTRODUCTION

Free radicals which have one or more unpaired electrons are produced in normal or pathological cell metabolism. Reactive oxygen species (ROS) react easily with free radicals to become radicals themselves. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals ($O_2$**.-)** and hydroxyl radicals (OH**.),** as well as non-free radical species ($H_2O_2$)and the singled oxygen ($1O_2$) [1, 2, 3, 4]. Also, excessive generation of ROS, induced by various stimuli and which exceed the antioxidant capacity of the organism, leads to a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity, and cancer [5, 6, 7].

Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides [3, 8, 9, 10]. Therefore, ROS can cause lipid peroxidation in foods, leading to their deterioration. In addition, these ROS can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxidation. The peroxidation products and their secondary oxidation products such as malondialdehyde and 4-hydroxyinonenal can react with biological substrates such as protein, amines, and deoxyribonucleic acid [7, 11]. As a result of this, much attention has been focused on the use of antioxidants, especially natural antioxidants to inhibit lipid peroxidation and to protect from damage due to free radicals. A great number of aromatic and other medicinal plants contain chemical compounds that exhibit antioxidant properties. Sources of natural antioxidants are primarily, plant phenolics that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks [12, 13].

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive

oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's).

*Catunaregam spinosa* (Rubiaceae) is also called as Mountain Pomegranate. It is a perennial, native rare, thorny shrub, found in moist deciduous forests.

## MATERIALS AND METHODS

### Plant material

A survey was conducted to collect the plants such as *Catunaregum spinosa* in various parts of Karnataka. Botanical species were collected from the forests of Nanjangud area, Mysore district, Karnataka.

### Extract Preparation

The plants collected were shade dried and finely powdered. The powdered plant material was subjected to Soxhlet extraction with ethanol (20g +250ml). The extract obtained was subjected for simple distillation process for the removal of excess ethanol.

### Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2, 4, 6-tripyridyl-s-triazine (TPTZ), potassium ferri cyanide; ascorbic acid and FeCl3 were purchased from Sigma Chemical Co. Sodium carbonate, acetate buffer, HCl and other were from Merck Chemical Supplies. All the chemicals used including the solvents, were of analytical grade.

### DPPH radical scavenging assay

The effect of the extracts on DPPH radical was estimated using the method of Liyana-Pathiranan and Shahidi [14]. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract in ethanol containing 0.02–0.1 mg of the extract. The reaction mixture left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = [(Abs control – Abs sample)]/ (Abs control)] x 100

where

Abs control is the absorbance of DPPH radical + methanol;

Abs sample is the absorbance of DPPH radical + sample extract /standard.

*Reducing ability (FRAP assay)*

The determination of the total antioxidant activity (FRAP assay) in the extract is a modified method of Benzie and Strain [15]. The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3 \cdot 6H_2O$. The temperature of the solution was raised to 37 °C before use. Plant extracts (150 µL) were allowed to react with 2850 µl of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tri-pyridyl-triazine 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. The total antioxidant activity of FRAP is calculated by the following equation: FRAP value of Sample (µM) = (Change in absorbance of sample from 0 to 4 minute /Change in absorbance of standard from 0 to 4 minute) X FRAP value of standard (1000 µM)

**RESULT AND DISCUSSIONS**

The antioxidant activity of various plant extracts can be determined accurately, conveniently, and rapidly using DPPH. The trend in antioxidant activity obtained by using the DPPH method is comparable to trends found using other methods reported in the literature. The reaction time of four hours and a temperature of 35□C facilitate the extraction and reaction of antioxidant compounds with DPPH. Antioxidant activity measured using DPPH accounts partially for the bound and insoluble antioxidants. At low pH, reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has an intense blue color) can be monitored by measuring the change in absorption at 593nm. The reaction is non specific, in that any half reaction that has lower redox potential, under reaction conditions, than that of ferric ferrous half reaction, will drive the ferrous (Fe III to Fe II) ion formation. The change in absorbance is therefore, directly related to the combined or "total" reducing power of the electron donating antioxidants present in the reaction mixture. The *Catunaregum spinosa* leaves showed the highest antioxidant property for DPPH and FRAP assays than the standard reference ascorbic acid.

The botanical species collected were separated into fruits and leaves. After shade drying the crude extracts were prepared using ethanol separately. The *Catunaregum spinosa* leaves showed the highest antioxidant property for DPPH (Table 1 & 2) and FRAP assays (Table 3). The Figure 1 & 2 represents the DPPH activity of C.spinosa and ascorbic acid graphically where in the IC50 value is found to be 85% and 37% respectively.

**Table 1:** Absorbance and evaluation of % inhibition of DPPH by the ethanolic leaf extract of C. spinosa

| Conc. of Ascorbic acid (ug/ml) | Absorbance (517 nm) | | | Absorbance of blank solution (nm) | | | % Inhibition |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | Average | 1 | 2 | Average | |
| 1 | 0.651 | 0.648 | 0.649 | 0.664 | 0.670 | 0.667 | 2.6 |
| 5 | 0.621 | 0.624 | 0.622 | | | | 6.7 |
| 10 | 0.525 | 0.530 | 0.527 | | | | 14 |
| 50 | 0.238 | 0.242 | 0.240 | | | | 64 |
| 100 | 0.056 | 0.059 | 0.057 | | | | 91 |
| 500 | 0.041 | 0.043 | 0.042 | | | | 93.7 |

**Table 2:** Absorbance and evaluation of % inhibition of DPPH by ascorbic acid

| Conc. of *Catuneragum spinosa* (ug/ml) | Absorbance (517 nm) | | | Absorbance of blank solution (nm) | | | % Inhibition |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | Average | 1 | 2 | Average | |
| 1 | 0.651 | 0.655 | 0.653 | 0.664 | 0.670 | 0.667 | 2.09 |
| 5 | 0.600 | 0.598 | 0.599 | | | | 10.19 |
| 10 | 0.542 | 0.546 | 0.544 | | | | 18.44 |
| 50 | 0.496 | 0.500 | 0.498 | | | | 25.34 |
| 100 | 0.264 | 0.266 | 0.265 | | | | 60.27 |
| 500 | 0.131 | 0.135 | 0.133 | | | | 80.06 |

**Table 3:** Total FRAP Values

| Test samples | FRAP activity µmol TE/100g (dry weight) |
|---|---|
| Ethanolic leaf extract of *Catunaregum spinosa* | 2.5 |
| Ascorbic acid | 2.0<br>(1.9–2.1) measured range |

**Fig 1**

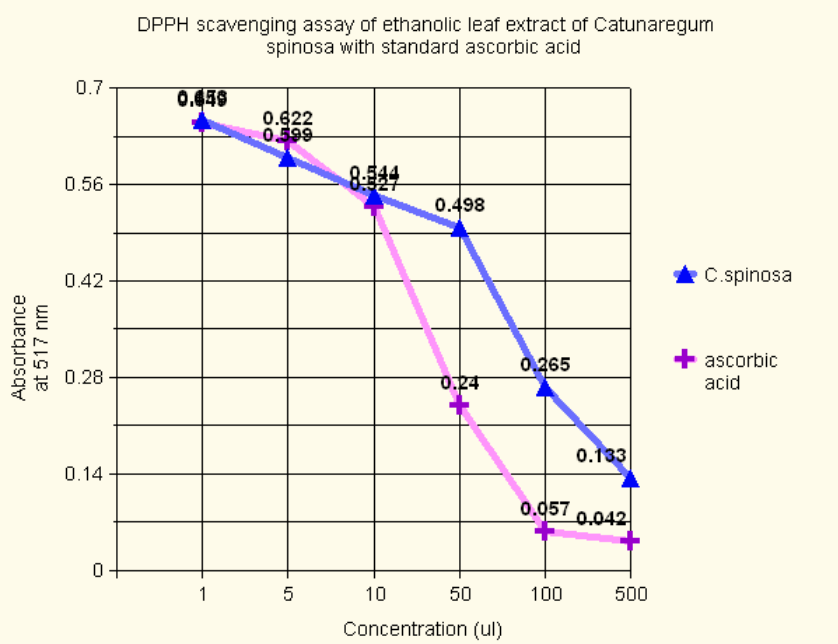
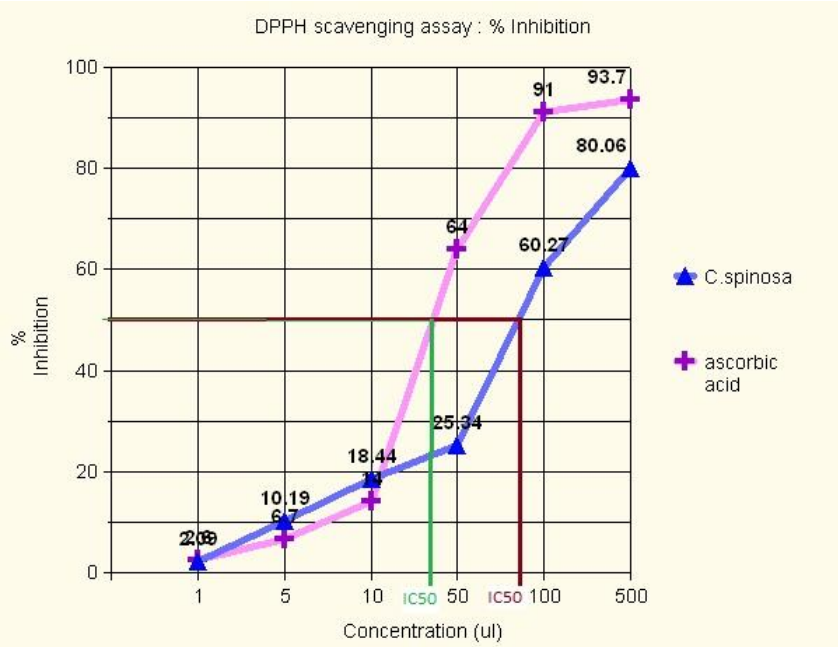



**Fig 2.**




**CONCLUSION**

Relative antioxidant content provides an indication of importance of each of the extracts. Antioxidant

activity will aid in the interpretation of clinical results obtained as various products which are tested in biological models for chronic disease. It is reasonable to expect that high antioxidant activity have greater potential to reduce free radicals in the body than do low antioxidant activity. Thus it is important to know the antioxidant content of the medicinally important plants.

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