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Evaluation of *in vitro* Antioxidant Activity of *Canthium coromandelicum*

S. Shankar and S. Thiripura Salini

Department of Chemistry, A.V.V.M. Sri Pushpam College, Poondi, Thanjavur, Tamil Nadu, India

Corresponding Author: S. Thiripura Salini, Department of Chemistry, A.V.V.M. Sri Pushpam College, Poondi, Thanjavur, Tamil Nadu, India Tel: +91 9894606764

ABSTRACT

The present study describes the antioxidant activity of *Canthium coromandelicum* for its medicinal values. Antioxidant compounds play an important role as a health defending factor and are called as free radical scavengers. Antioxidant activity or free radical scavenging activity of *Canthium coromandelicum* was evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, nitric oxide radical, superoxide anion radical, reducing power and total antioxidant activity of this plant extract. In all the testing, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. These results clearly indicate that *Canthium coromandelicum* is effective against free radical mediated diseases.

Key words: *Canthium coromandelicum*, free radicals, antioxidant activity, DPPH

INTRODUCTION

An enormous variety of plants have been studied for new source of natural anti oxidants, especially phenolic and flavanoid compounds derived from plants were proved to be potent anti oxidant and free radicals scavengers. Several medicinal plants have been extensively used in the Indian traditional system of medicine (Ayurveda) for the treatment of number of diseases. Some of these plants have shown potent anti oxidant activity (Mohan *et al.*, 2012). The potential of higher plants as sources for new drugs is thus still largely unexplored (Hamburger and Hostettmann, 1991). Medicinal plants are great importance to the health of individual and communities. The medicinal value of these plants lies in some chemical substance that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Okeke and Elekwa, 2003). In recent years there is an upsurge in the areas related to newer developments in prevention of disease especially the role of free radicals and antioxidants. Oxygen is an element obligatory for life, living system have evolved to survive in the presence of molecular oxygen and for most biological systems. Oxidative properties of oxygen play a vital role in diverse biological phenomena. Oxygen has double-edged properties, being essential for life; it can also aggravate the damage within the cell by oxidative events through free radicals (Shinde *et al.*, 2006). The free radicals produced by human body such as superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals causes many diseases like cancer, liver diseases rheumatoid arthritis, ageing etc (Halliwell and Gutteridge, 1999). Current research is now directed towards finding naturally occurring antioxidant of plant origin. *Canthium coromandelicum* is an important medicinal plant, various

phytochemical, antimicrobial and wound healing studies have already been carried out with *canthium coromandelicum* leaf extract (Mohan *et al.*, 2014). So, the present study was subjected to determine the antioxidant activity of leaves of *Canthium coromandelicum*.

MATERIALS AND METHODS

Plant materials: The fully mature *Canthium coromandelicum* leaves were collected from Thanjavur, Tamil Nadu, India.

Preparation of alcoholic extract: The leaves of *Canthium coromandelicum* were first washed well and dust was removed from the leaves. Leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 70% ethanol for 48 h. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used. The different concentrations (20, 40, 60 and 80 $\mu\text{g mL}^{-1}$, respectively) of plant extract were used in this study.

DPPH radical-scavenging activity: DPPH radical-scavenging activity was determined by the method of Shimada *et al.* (1992). Briefly, a 2 mL aliquot of DPPH methanol solution (25 mg mL^{-1}) was added to 0.5 mL sample solution at different concentrations (20, 40, 60 and 80 $\mu\text{g mL}^{-1}$, respectively). The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity. The scavenging activity of sample was expressed as 50% effective concentration (EC_{50}) which represented the concentration of sample having 50% of DPPH radical scavenging effect:

$$\text{Radical scavenging activity (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

where, A_c is the absorbance of the control (ascorbic acid) and A_s is the absorbance of reaction mixture (in the presence of sample). All tests were run in triplicates ($n = 3$) and the average values were calculated.

Nitric oxide scavenging activity assay: Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964). 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. Two milliliter of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of *Canthium coromandelicum* at various concentrations and the mixture incubated at 25°C for 150 min. From the incubated mixture 0.5 mL was taken out and added into 1.0 mL sulfanilic acid reagent (30 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:

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

where, A_0 was the absorbance of the control (blank) and A_1 was the absorbance in the presence of the *Canthium coromandelicum*.

Superoxide anion scavenging activity assay: The scavenging activity of the *Canthium coromandelicum* towards superoxide anion radicals was measured by the method of Liu *et al.* (1997). Superoxide anions were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system through the reaction of PMS, NADH and oxygen. It was assayed by the reduction of nitroblue tetrazolium (NBT). In these experiments the superoxide anion was generated in 3 mL of tris-HCl buffer (100 mmol, pH 7.4) containing 0.75 mL of NBT (300 μmol) solution, 0.75 mL of NADH (936 μmol) solution and 0.3 mL of different concentrations of the *Canthium coromandelicum*. The reaction was initiated by adding 0.75 mL of PMS (120 μmol) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured in spectrophotometer. The superoxide anion scavenging activity was calculated according to the following equation:

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

where, A_0 was the absorbance of the control (blank) and A_1 was the absorbance in the presence of the *Canthium coromandelicum*.

Reducing power assay: The Fe^{3+} reducing power of the *Canthium coromandelicum* was determined by the method of Oyaizu (1986) with slight modifications. The *Canthium coromandelicum* (0.75 mL) at various concentrations was mixed with 0.75 mL of phosphate buffer (0.2 mole, pH 6.6) and 0.75 mL of potassium hexacyanoferrate [$\text{K}_3\text{Fe}(\text{CN})_6$] (1%, w/v), followed by incubating at 50°C in a water bath for 20 min. The reaction was stopped by adding 0.75 mL of Trichloro Acetic Acid (TCA) solution (10%) and then centrifuged at 3000 rpm for 10 min. The 1.5 mL of the supernatant was mixed with 1.5 mL of distilled water and 0.1 mL of ferric chloride (FeCl_3) solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power.

Determination of total antioxidant capacity: The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.* (1999). The assay is based on the reduction of Mo(VI)-Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. 0.3 mL extract was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Methanol (0.3 mL) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

Statistical analysis: Tests were carried out in triplicate for 3-5 separate experiments. The free radical scavenging activity of *Canthium coromandelicum* (expressed in percentage %).

RESULTS AND DISCUSSION

Present study was carried out on the plant sample revealed the presence of natural antioxidants. The antioxidant activity was measured by using DPPH assay, superoxide anion scavenging activity assay, nitric oxide scavenging assay, reducing power assay and total antioxidant assay. The antioxidant activity of *Canthium coromandelicum* was found to be significantly high.

DPPH Radical scavenging activity: DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron, donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The antioxidant activity of *Canthium coromandelicum* is shown in Fig. 1. The *Canthium coromandelicum* exhibited a significant dose dependent inhibition of DPPH activity.

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 effective molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities (Miller *et al.*, 1993). *Canthium coromandelicum* also moderately inhibited nitric oxide in dose dependent manner shown in Fig. 2.

Superoxide anion radical scavenging activity: The superoxide anion radical scavenging activity *Canthium coromandelicum* assayed by the PMS-NADH system is shown in Fig. 3. The superoxide scavenging activity of *Canthium coromandelicum* was increased markedly with the increase of concentrations. These results suggested that *Canthium coromandelicum* had superior superoxide radical scavenging effect.

Iron reducing power activity: Figure 4 depicts the reductive effect of *Canthium coromandelicum*. Similar to the antioxidant activity, the reducing power of

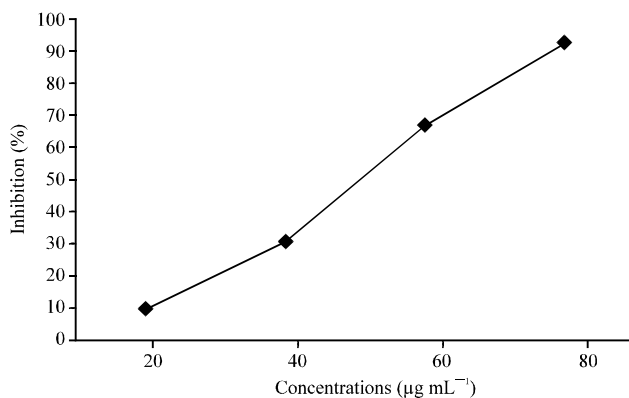


Fig. 1: DPPH radical scavenging activity of ethanolic extract of *Canthium coromandelicum* at different concentrations

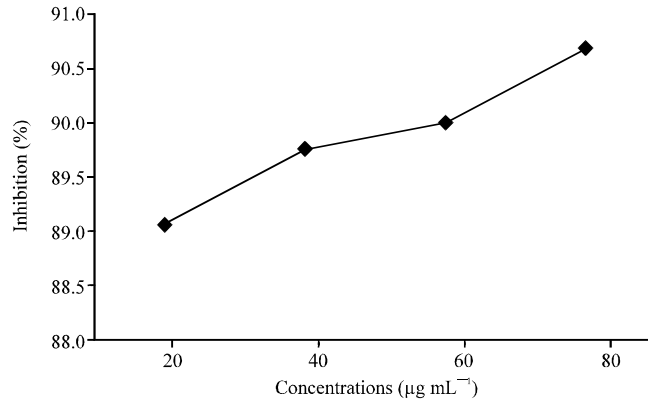


Fig. 2: Nitric oxides scavenging activity of ethanolic extract of *Canthium coromandelicum* at different concentrations

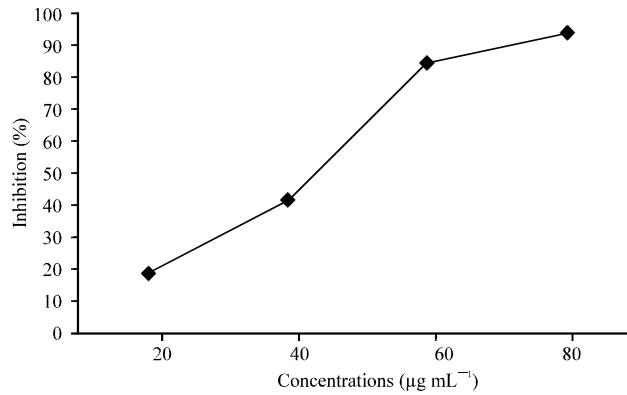


Fig. 3: Superoxide anion radical scavenging activity of ethanolic extract of *Canthium coromandelicum* at different concentrations

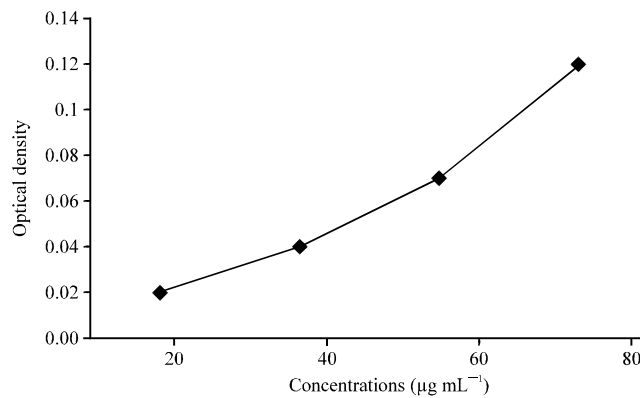


Fig. 4: Reducing power assay of ethanolic extract of *Canthium coromandelicum* at different concentrations

Canthium coromandelicum increased with increasing dosage. All the doses showed significantly higher activities than the control exhibited greater reducing power, indicating that *Canthium coromandelicum* having the greater reducing power.

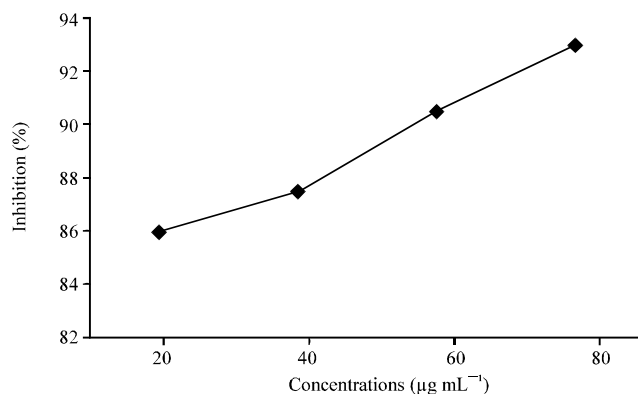


Fig. 5: Total antioxidant assay of ethanolic extract of *Canthium coromandelicum* at different concentrations

Total antioxidant activity: Total antioxidant capacity of *Canthium coromandelicum* are expressed as the number of equivalents of ascorbic acid. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extract (Prieto *et al.*, 1999). Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the plant extract shown in Fig. 5.

CONCLUSION

The plants and its derivatives may considered as good sources of natural antioxidants for medicinal uses such as against cancer, diabetic mellitus, cardiovascular diseases, aging and other diseases related to radical mechanisms. Determination of the natural antioxidant compounds will help to develop new drug candidates for antioxidant therapy. The results of this study clearly indicate that *Canthium coromandileum* leaves has powerful antioxidant capacity against various reactive oxygen species/free radicals under *in vitro* conditions and the capacity was concentration dependent. This study also proved that *Canthium coromandileum* leaves has possessed as a metal chelator and exhibited greater reducing power. Preliminary phytochemical analysis of the leaves of *Canthium coromandileum* revealed presence of flavonoids, tannins, phenolic compounds and glycosides (Mohan *et al.*, 2014). Presence of flavonoids and tannins in extracts of leaves of *Canthium coromandileum* may be responsible for its antioxidant activity. Overall, the *Canthium coromandelicum* leaves are a source of natural antioxidants that can be important in disease prevention and health preservation. However, the *in vivo* safety of *Canthium coromandelicum* needs to be thoroughly investigated in experimental models prior to its possible application as an antioxidant ingredient, either in animal feeds or in human health foods.

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