

Research Article

Antioxidant Activity of Leaves of an Important Medicinal Plant *Ormocarpum cochinchinense* (Lour.) Merr.

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Abstract

Plant based or plant-derived drugs occupy 30% of the modern system of medicine. Several plants possess a variety of biologically active compounds. Among them *Ormocarpum cochinchinense*, belonging to the family Fabaceae is an important traditional and unexplored bone-setting plant in terms of its medicinal values. The methanolic extract of the leaf was used to isolate the active principles by column chromatography. A total of 12 fractions were collected sequentially and their antioxidant potential was analyzed using DPPH assay. The results of the present study revealed that the sixth fraction separated from column chromatography possesses a good antioxidant property up to a level of 73.4%.

Keywords: Antioxidant potential, DPPH assay, Column chromatography, *Ormocarpum cochinchinense*, methanolic leaf extracts.

INTRODUCTION

The ancient Indian system of medicine is mainly plant based (*material medica*) making the use of most of our native plants. The oxidation induced by reactive oxygen species (ROS) can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate the development of many disorders, such as cancer, liver injury and cardiovascular diseases (Liao and Yin, 2000). Consumption of fruits and vegetables has proven to substantially reduce the risk of cardiovascular diseases, cancers (Gerber *et al.*, 2002) and neurodegenerative diseases including Parkinson's and Alzheimer's diseases (Ames *et al.*, 1993; Dimatteo and Esposito, 2003). Although the body possesses defense

mechanisms, enzymes and antioxidant nutrients which arrest the damaging properties of ROS (Sies 1993; Halliwell *et al.*, 1995), continuous exposure to chemical and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them and cause irreversible oxidative damage (Tseng *et al.*, 1997). Antioxidant agents are helpful in minimizing the risk of such oxidative damages occurring in cell.

Several antioxidant agents including chemical and biological materials are available for usage now-a-days. Synthetic antioxidant compounds which are used commercially in food processing industries known to have side-effects (Branien, 1975; Ito *et al.*, 1983). Recently restriction in the use of some synthetic antioxidants is

being imposed because of their carcinogenicity (ShyamaGowri and Vasantha, 2010). This has attracted a great deal of research interest in the identification and exploitation of natural antioxidants. Subsequently, a worldwide trend towards the use of natural phytochemicals present in berry crops, tea, herbs, beans, fruits and vegetable has increased (Lee and Shibamoto, 2000).

Ormocarpum cochinchinense is a rare valuable medicinal shrub belonging to the family Fabaceae (Shanthi, 2008) and commonly known as Elumbotti or Kattumoringai (Gamble, 1935). It is distributed throughout tropical and Southern Africa, Madagascar, Southern Asia, Northern Australia and the islands of the Pacific Ocean (Bean, 2006). *O. cochinchinense* is a widespread species and it occurs in the Eastern regions of the Indian Subcontinent (Wealth of India, 2001). In South India, it was reported from Deccan, Tamil Nadu (Gamble, 1935), Orissa and Eastern Ghats (Pullaiah and SriRamamurthi, 2001).

The roots are considered to be tonic and stimulant and are used in treatment of lumbago (Ambasta, 2000; Wealth of India, 2001). An application prepared by rubbing the root bark in oil is used in the treatment of paralysis (Wealth of India, 2001; Nadkarni, 2001; Chopra *et al.*, 2002). The plant is considered to be a fish poison and probably contains rotenone or related compounds (Anonymous, 1948). The leaves are eaten fresh or prepared into a medicated candy (lehiyam) and consumed. It is also used in the cure of chest pain. The leaves are included in formulations used for setting bone fractures and for nervous pain (Adyarpoonga, 2007; Maria John *et al.*, 2011; Dinesh Kumar *et al.*, 2013). The young leaves are used as a vegetable and flavor sago in Yam Islands (Bean, 2006).

The present study focuses on assessing the total antioxidant capacity (TAC) using the modern technique, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay in the shade dried leaves of *O. cochinchinense* by separating different fractions through silica gel column chromatography.

MATERIAL AND METHODS

Plant material

The disease free leaves of *O. cochinchinense* were collected from Chengalpet, Kancheepuram District of Tamil Nadu, India, after proper identification, the herbarium specimens were prepared and deposited in the

Department of Botany, Madras Christian College, Chennai – 600059. The leaves were shade-dried and fine powdered by using pulverizer and maintained in an air tight container at 4°C and used for the further study.

Extraction and separation by column chromatography

The fine shade-dried powder of leaves was extracted with 70% of ethanol and then evaporated using Rotoevaporator (Buchi, Switzerland). The residue of the extract was mixed with n-butanol and water (2:1) and the upper n-butanol and the lower water layer were separated and evaporated under vacuum, then the extract was dissolved in methanol. After filtration, the methanol solution was concentrated under vacuum and used for column chromatography (Shimizu *et al.*, 1997). About 10 g of silica gel (100–200 mesh) were washed 3 times with methanol and dissolved in 20 mL of distilled water. The slurry of silica gel was carefully poured into the column without air bubbles. The extract of leaves (100 mg/mL) was placed carefully on the upper surface of the column and was used as the stationary phase. Water and methanol (8:2) was used as the mobile phase. Eluant was slowly passed through the column and the fractions were not colored, therefore equal sized fractions were collected sequentially and stored at 4°C.

Estimation of free-radical inhibiting property of antioxidants

The estimation of free radical inhibiting property was performed by the method of Brand-Williams *et al.* (1995) and also followed by many workers (Ozkan *et al.*, 2004; El-Sayed *et al.*, 2012). About 100 µL of each fraction derived from leaf extracts were mixed with 2.7 mL of methanol and 200 µL of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. Subsequently at 5 minute intervals, the maximum absorption of the solution was measured using the UV double beam spectra scan (Chemito, India) at 517 nm. The antioxidant activity of each sample was compared with known synthetic standard of (0.16%) of butylated hydroxy toluene (BHT).

% inhibition was calculated using the following formula.

$$\frac{\text{Optical Density (Control)} - \text{Optical Density (Sample)}}{\text{Optical Density (Control)}} \times 100$$

RESULTS AND DISCUSSION

A total of twelve fractions were collected from the column and used for antioxidant assay (Table 1). After calculating the OD value of 12 fractions, the percentage of free radical scavenging activity was calculated; this is shown in the Table 2. The percentage antioxidant potential of control (BHT) was also calculated. Among 12 fractions, the 6th fraction showed a higher percentage of activity (73.4%) followed by 11th fraction (66.6%), 9th fraction (58.9%) and 12th fraction (55.8%) respectively. Fraction 1 showed less percentage of activity with 29.8 percent (Figure 1). The DPPH method is very rapid and it can be helpful in the investigation of novel antioxidants for quick estimation (Blois, 1958). The antioxidant activity of Black tea, Green tea (*Camellia sinensis*), Ginger (*Gingiber officinale*), Pepper (*Piper nigrum*) were analyzed and reported by Nooman *et al.* (2008) and the results indicated that all the methanolic extracts of these plants exhibited antioxidant activity significantly by DPPH method. The various natural products such as nuts, aromatic plants and essential oils and their antioxidant properties were reviewed in detail by Maestri *et al.* (2006).

Table 1: Sequential fractions by silica gel column chromatography

Fraction	Time (min)	Fraction	Time (min)
Fraction 1	0-5	Fraction 7	30-35
Fraction 2	5-10	Fraction 8	35-40
Fraction 3	10-15	Fraction 9	40-45
Fraction 4	15-20	Fraction 10	45-50
Fraction 5	20-25	Fraction 11	50-55
Fraction 6	25-30	Fraction 12	55-60

Table 2: Antioxidant activity of different fractions

Fractions	Absorbance at 517 nm	Antioxidant Activity (%)
Control	1.456	-
BHT - Standard	0.241	83.4
Fraction – 1	1.021	29.8
Fraction – 2	1.019	30
Fraction – 3	0.964	33.7
Fraction – 4	0.745	48.8
Fraction – 5	0.856	41.2
Fraction – 6	0.386	73.4
Fraction – 7	0.955	34.4
Fraction – 8	0.829	43
Fraction – 9	0.598	58.9
Fraction - 10	0.833	42.7
Fraction-11	0.486	66.6
Fraction - 12	0.643	55.8

Hundreds of natural phenolic compound have been reported to possess high antioxidant properties. Most important commercially available natural antioxidants are tocopherols (Vitamin E), ascorbic acid (Vitamin C) and rosemary extract (Loliger, 1991). The recent findings of Mullick *et al.* (2013) reveal that the leaf and *in vitro* derived callus of *Acalypha indica* also showed significant antioxidant activity.

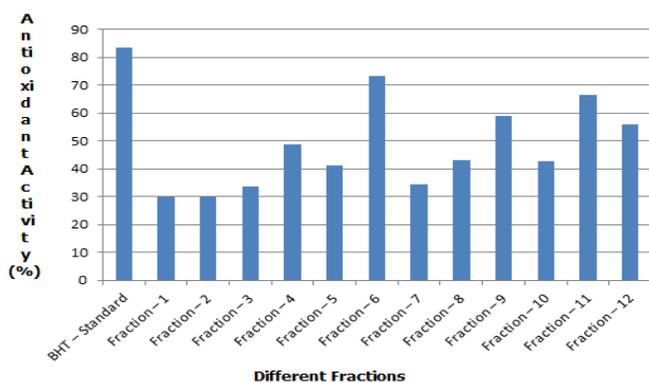


Figure1: Antioxidant activity of different fractions of *O. cochinchinense*

The various plants used as natural antioxidant have been reviewed in detail by Gupta and Sharma (2006). The imbalance between reactive oxygen species and antioxidant defense systems may increase the oxidative burden and lead to the damage of macro molecules such as DNA, carbohydrates and proteins. The model of scavenging of the free radicals is a widely used method to evaluate the antioxidant activity of any given sample in a relatively short time compared with other methods. This method depends on the reduction of purple DPPH radicals by antioxidant agents to a yellow colored diphenyl-picrylhydrazine and the remaining DPPH radicals that show maximum absorption in 517 nm were measured. The decrease of DPPH solution indicates an increase of the DPPH radical scavenging activity (Kumaran and Karunakaran, 2008).

Though antioxidants are found in wild species of *O. cochinchinense*, it was not properly bio-documented and therefore the present investigation is aimed at accomplishing the task from South India. The antioxidant properties of *Tecoma stans* (L.) Juss ex.Kunth (Lopez *et al.*, 2009), *Brassica nigra* L. (Hussein *et al.*, 2010) and *Justicia gendarussa* Burm.F (Bhagya and Chandrasekar, 2013) were also reported. Medicinal plants containing active chemical constituents with high antioxidant property play an important role in the prevention of various degenerative diseases (Selvam *et al.*, 2013).

Natural antioxidants from plants sources are potent and safe due to their harmless nature; many wild herbs have been investigated for their antioxidant properties (Lee *et*

al., 2004). Potential source of antioxidants have been found in leaves, barks and roots of various medicinal plants. Antioxidant activities of wolfberry, cherry, kiwi and cranberry fruits were studied using different fractions obtained by column chromatography (Fan *et al.*, 2010), and in *Eucalyptus globulus* bark (Lila *et al.*, 2012).

CONCLUSION

From the above results, the antioxidant activity of the leaf extract of *O. cochinchinense* and its successive fractions, based on DPPH scavenging activity is attributed to the presence of phenolic compounds as major components and there is a positive correlation between the antioxidant activity and the total phenolics and flavonoids. Phenolic compounds such as flavonoids, phenolic acids and tannins are considered as the major contributor to the antioxidant activity of vegetables, fruits and medicinal plants. The phytochemical and phytobiological characteristics of these extracts will lead to isolation and characterization of the active principle.

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