In vitro Antioxidant Properties of Certain Indigenous Medicinal Plants From Western Ghats of India

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Citation

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Abstract

Antioxidant activity (DPPH & reducing power assay), Asteracantha longifolia, Delonix elata, Passiflora edulis

INTRODUCTION

Nature still serves as the man's primary source for the cure of his ailments. The majority of the rich diversity of Indian medicinal plants is yet to be scientifically evaluated for such properties. However, the potential of higher plants as source for new drugs is still largely explored (Oke et al., 2002). The free radicals are chemical species, capable of independent existence; posses an unpaired electron on an orbital. They occur in the form of Superoxide, Hydroxyl radical (OH) and Peroxide ion (HO_2-).They are also known as Reactive Oxygen Species (ROS) or Reactive Oxygen Metabolite (ROM). These radicals are produced by body's normal use of oxygen and are also generated through environmental pollutants, cigarette smoke, automobile exhaust fumes, radiation, air pollution, pesticides, etc (Tiwari, 2001& Aqil et al.,2006). Mammalian cells possess elaborate defense mechanisms for radical detoxification. Key metabolic steps are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), which destroy toxic peroxides. In addition to antioxidant enzymes, nonenzymatic molecules, including thioredoxin, thiols, and disulfide-bonding play important roles in antioxidant defense systems. Some of the compounds are of an exogenous nature and are obtained from food, such as a-tocopherol, b-carotene, and ascorbic acid, and such micronutrient elements as zinc and selenium (Aqil et al .,2006). The medicinal properties of plants have been investigated in the recent scientific developments throughout the world ,due to their potential antioxidant activities, no side effects and economic viability (Auudy et al .,2003).The majority of the active antioxidant compounds flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, b-carotene, and a-tocopherol are known to possess antioxidant potential (Prior RL., 2003, Luo et al., 2004, Kapoor et al., 2002).Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury (Pormouard et al., 2006). This paper reports screening of the antioxidant assay of certain indigenous medicinal plants of Western Ghats of India.

MATERIALS AND METHODS

PLANT MATERIAL

Plant samples of the selected species viz. Asteracantha longifolia, Delonix elata and Passiflora edulis were collected from Coimbatore region of Western Ghats, Tamilnadu. Further identified by DR. Sampath Kumar, Botanical Survey of India (Southern Circle), Coimbatore and voucher specimens were deposited at RVS Pharmaceutical College, Coimbatore. Leaves were separated and dried under shade for three days. Dried leaf samples were ground into a uniform powder using a blender and stored in polythene bags at room temperature.

PREPARATION OF EXTRACTS

25 g of the dried powdered samples from Asteracantha longifolia, Delonix elata and Passiflora edulis species were taken separately in a paper cone and placed into Soxhlet apparatus. 100ml of methanol a polar solvent was taken in the round bottom flask attached to the Soxhlet apparatus. A condenser was attached to this set up .Then the whole set up was placed on a heating mantle. The temperature was set in the range of 25-30o C. Methanol gets vaporized and rises up to the condenser where it condenses back into liquid. This liquid falls into the plant sample in the cone and extracts certain compounds and falls back into the round bottom

flask. This process was continued till all the compounds that can be extracted from the plant by ether gets extracted and finally only clear liquid of ether starts falling into the round bottom flask. The extracts got from the above process was evaporated over night and stored in screw cap vials.

ANTIOXIDANT ASSAY

The antioxidant activity of Plant extracts were determined by different in-vitro methods such as, the DPPH free radical scavenging assay and reducing power methods. The different extracts were dissolved in methanol at the concentration of 2mg/ml. all the assays were carried out in triplicate and average value was considered.

DPPH RADICAL SCAVENGING ACTIVITY:

DPPH scavenging activity was carried out by the method of Blois, (1957).Different concentrations (1000,500,250,125,62.5 and 31.2 mg/ml) of A.longifolia,P.edulis and Delonix elata extracts (Methanol) were dissolved in DMSO (dimethyl sulfoxide) and taken in test tubes in triplicates . Then 5 ml of 0.1mM ethanol solution of DPPH (1, 1, Diphenyl-2- Picrylhydrazl) was added to each of the test tubes and were shaken vigorously. They were then allowed to stand at 370 C for 20 minutes. The control was prepared without any extracts. Methanol was used for base line corrections in absorbance (OD) of sample measured at 517nm. A radical scavenging activity was expressed as 1% scavenging activity and was calculated by the following formula.

Figure 1

$$\% \text{ Radical scavenging activity} = \frac{\text{Control O.D-Sample O.D}}{\text{Control O.D}} \times 100$$

IC 50 Value was also calculated.

REDUCING POWER

Reducing activity was carried out by using the method of Oyaizu (1986).Different concentration (1000, 500,250,125 mg/ml) of A.longifolia and Delonix elata extracts (Methanol) were prepared with DMSO and taken in test tube as triplicates. To the test tubes 2.5 ml of sodium phosphate buffer and 2.5 ml of 1% Potassium ferric cyanide solution was added. These contents were mixed well and were incubated at 500 C for 20 minutes. After incubation 2.5ml of 10% TCA was added and were kept for centrifugation at 3000rpm for 10 minutes. After centrifugation 5ml of supernatant were taken and to this 5ml of distilled water was added. To this about 1ml of 1% ferric chloride was added and was incubated at 35 0 C for 20 minutes. The O.D (absorbance) was taken at 700nm and the blank was prepared by adding every other solution but without extract and ferric chloride (0.1%) and the control was prepared by adding all other solution but without extract. The reducing power of the extract is linearly proportional to the concentration of the sample

RESULTS AND DISCUSSION

Scavenging activity for free radicals of 1,1-diphenyl 1-2-picryl hydrazyl (DPPH) has been widely used to evaluate the antioxidant activity of natural products from plant and microbial sources. Plant extracts from 3 medicinal plants listed in table.1.were prepared for investigation of their antioxidant activities. Free radical scavenging activity of methanolic extract from the 3 medicinal plants was quantitatively determined using DPPH and reducing power assay. Table 1-3 shows the result of antioxidant assay.

Figure 2

Table 1a: Antioxidant activity of methanolic extracts of leaves.

Concentration (mg/ml)	Mean ± Standard deviation	IC_{50} Value (mg/ml)
1000	91.78±2.536	68.0±81.662
500	85.04±4.774	
250	77.04±1.910	
125	66.01±2.474	
62.5	50.80±8.289	
31.2	41.18±7.759	
Ascorbic acid		11.24±0.022

Figure 3

Table 1b: Antioxidant activity of methanolic extracts of

Concentration (mg/ml)	Mean ± Standard deviation	IC_{50} Value (mg/ml)
1000	56.16±13.488	375±176.77
500	34.68±2.304	
250	21.21±2.883	
125	13.03±2.487	
62.5	9.75±7.154	
31.2	4.21±11.227	
Ascorbic acid		11.24±0.022

Figure 4

Tables 1c: Antioxidant activity of methanolic extracts of

Concentration (mg/ml)	Mean ± Standard deviation	IC_{50} Value (mg/ml)
1000	58.17±10.58	
500	35.01±6.152	
250	25.53±6.257	
125	15.24±6.723	
62.5	10.81±6.431	
31.2	7.06±3.871	375± 87.834
Ascorbic acid		11.24±0.022

All the medicinal plant materials used contain varying degrees of antioxidants. It is well recognized that free radicals are critically involved in various pathological conditions such as cancer, cardiovascular disorders, arthritis, inflammation and liver diseases (Martin et al. 1993). Under normal physiological conditions low concentrations of lipid peroxidation products are found in tissues and cells. In the presence of oxidative stress more lipid peroxidation products are formed due to cell damage. Cellular antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase normally challenge oxidative stress (Agbor et al. 2002). In this study, a methanol solution of DPPH free radical was found to be stable for more than 30 mins by spectrophotometry at 517nm of an (0.1mM) solution (Blois, 1958). The radical scavenging effect of 3 medicinal plant extracts and fractions of them were then measured spectrophotometrically for DPPH free radical. The control intensity (absorbance of extracts, fractions and pure compounds) was taken as 100% and the percentage intensity was calculated. The concentration for 50% inhibition (IC_{50}) as well as the plant extracts examined in this study for scavenging effects on DPPH free radical is shown in Tables 1-3.

Figure 5

Table 2a: Reducing power of methanolic extracts of

Concentration (mg/ml)	O.D Value at 700nm
31.2	0.066
62.5	0.075
125	0.093
250	0.124
500	0.282
1000	0.593
Control	0.079

Figure 6

Table 2b: Reducing power of methanolic extracts of

Concentration (mg/ml)	O.D Value at 700nm (mg/ml)
31.2	0.073
62.5	0.086
125	0.101
250	0.120
500	0.159
1000	0.263
Control	0.079

Figure 7

Table 2c: Reducing power of methanolic extracts of

Concentration (mg/ml)	O.D Value at 700nm (mg/ml)
31.2	0.017
62.5	0.031
125	0.074
250	0.062
500	0.112
1000	0.150
Control	0.079

Values are expressed as mean ±S.D

Values are taken as a mean of three individual experiments

CONCLUSION

Against the backdrop of many known medicinal properties of these plants, results from the present work suggest that relatively low values of antioxidant and reducing power may not imply a low medicinal value. Emerging trends in antioxidant research point to the fact that low levels of phenolics (and other phytochemicals) and low value of antioxidant indices in plants do not translate to poor medicinal properties. The radical scavenging effects of Asteracantha longifolia were higher than all others in tested plants. Asteracantha longifolia found that major criteria for the evolution of anti-inflammatory activity in which it is caused a statistically significant suppression of ROS (Reactive Oxygen Species).

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