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Full Length Research Paper

Evaluation of antioxidant activity index (AAI) by the 2, 2-diphenyl-1-picryl hydrazyl method of 40 medicinal plants

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Currently there has been an increased interest globally to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine and the food industry. As plants produce significant amount of antioxidants to prevent the oxidative stress caused by free radicals and oxygen, they represent a potential source of new compounds with antioxidant activity. In India and across the world, the traditional herbal medicine has its own importance in human health care and prevention of many diseases. Ayurveda is the oldest medical system in the world that provides potential leads to find active and therapeutically useful compounds from plants. Considering the growing interest in possessing the antioxidant capacity of medicinal plants, we tried to recognize the effect of antioxidants in different plants by using DPPH (1, 1 – diphenyl- 2- picryl hydrazyl) radical assay. Some of the plants reviewed are part of multi-herbal preparations while others are used singly. Some of the medicinal plants like *Azadirachta indica, Bacopa monnieri, Capsicum annuum, Carica papaya, Citrullus colocynthis, Citrus aurantifolia, Coriandrum sativum, Emblica officinalis, Mangifera indica , Madhuca indica , Momordica charantia , Ocimum sanctum, Syzygium cuminii, Tamarindus indica* are used as food sources, which helps in the emerging area of antioxidant research of medicinal plants.

Key words: Medicinal plants, 1,1 – diphenyl-2- picryl hydrazyl (DPPH), antioxidant activity.

INTRODUCTION

Antioxidants help the organisms in dealing with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Reactive oxygen species (ROS) formed, such as superoxide anion, hydroxyl radical and hydrogen peroxide are highly reactive and potentially damaging transient chemical species. These are continuously produced in the human body, as they are essential for energy supply, detoxification.

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It is possible to reduce the risks of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants (Stanner et al., 2004). This is one of the reasons why discovery and synthesis of novel antioxidants is a major active area. In recent years, the use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value (Ajila et al., 2007). Antioxidants derived from fruits, vegetables, spices and cereals are very effective and have reduced interference with the body's ability to use free radicals constructively (Kahkonen et al., 1999; Wolfe et al., 2003). Natural antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols,

tocotrienols) ascorbic acid and carotenoids. The quest for natural antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenge over the last two decades. Efforts to gain extensive knowledge regarding the power of antioxidants from plants and to tap their potential are therefore on the increase. Many Indian plants have been investigated for their beneficial use as antioxidants or source of antioxidants using presently available experimental techniques. Apart from these 20 plants numerous other plants used in Indian traditional medicine are reported to show antioxidant activity. The present work deals with the antioxidant activity of some medicinal plants and effectiveness of the activity with the standard gallic acid and natural antioxidant ascorbic acid.

MATERIALS AND METHODS

Plant materials

The plants were collected from Tirumala Hills, Tirupati, Chittoor district of Andhra Pradesh in the month of July – October 2008 and identified by Dr. K. Madhava chetty, Assistant Professor, Department of Botany, S.V.University, Tirupati.

Preparation of extract

Fresh parts (leaf, stem, bark, roots and whole plant) from all the plants were shade dried and milled to fine powder using a mechanical grinder separately. The powdered plant material was macerated and shaken in methanol and water separately for 48 h using a bath shaker. The extract was then filtered with filter paper (Whatman No.1) and concentrated to dryness under vacuum and reduced pressure using Rota evaporator at 40°C. The concentrate was then layered on aluminum foil and freeze dried for further use.

1,1–diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity assay

DPPH assay was carried out as described by Amarowicz et al. (2004) with minor modifications. Recently the assay has been used to determine antioxidant activity in Tanacetum (Tepe and Sokmen, 2007), Moldavian balm (Dastmalchi et al., 2007) and Phyllanthus amarus (Lim and Murtijaya, 2007). A total of 0.5 mg of extract was adjusted to a volume of 4.0 ml, then 0.5 ml of 1mM methanolic solution of DPPH was added to the sample solution. The contents were stirred vigorously for 15 s and then left to stand at room temperature for 30 min. Decrease in colorization was measured spectrophotometrically at 517 nm spectrophotometer.

The radical scavenging activity (RSA) was calculated using the equation shown below:

% of inhibition = [(absorbance of control – absorbance of test sample)/absorbance of control] x 100.

The structure of DPPH and its reduction by an antioxidant are shown above. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm. The principle of this assay is based on the measurement of scavenging ability of the antioxidants towards the stable radical. The free radical DPPH is reduced to the corresponding hydrazine, when it reacts with hydrogen donors, this stability is evaluated by decolorizing assay which evaluates the decrease in absorbance at 517 nm produced by the addition of antioxidant to DPPH solution in ethanol or methanol.

The scavenging reaction between (DPPH.) and an antioxidant (H-A) can be written as:

$$\underset{\text{(Purple)}}{(DPPH)} + (H\text{-}A) \longrightarrow \underset{\text{(Yellow)}}{DPPH\text{-}H} + (A)$$

Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPH H and as consequence the absorbance's decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability (Oktay et al., 2003).

RESULTS AND DISCUSSION

The yield of the methanol extract of the various plant species and its total antioxidant capacity is given in Table 1. Total antioxidant capacity of *Emblica officinalis* is expressed as the number of equivalents of ascorbic acid. DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants (Oyaizu, 1986). DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). The reduction capability of DPPH radical is determined by the decrease in absorbance at 517 nm induced by antioxidants. Gallic acid and ascorbic acid are the reagents used as standards. The extracts are able to reduce the stable radical DPPH to the yellow-coloured diphenylpicrylhydrazine. The scavenging effect of methanol extracts and standards with the DPPH radical is in the following order: *Emblica officinalis* (95.24%) *Tamarindus indica* (95.14%), *Punica granatum* (94.41%), *Albizzia lebbeck* (94.11%), Ficus *glomerata* (93.36), *Terminalia arjuna* (90.78%), *Syzygium cuminii* (89.82%), *Mangifera indica* (85.86%), *Syzygium aromaticum* (75.48%) at the dose of 50 mg/ml. The scavenging effect of water extracts and standards with the DPPH radical is in the following order: *Punica granatum* (92.34%), *Albizzia lebbeck* (85.07%), *Ficus glomerata* (76.00), *Syzygium aromaticum* (75.47%), *Syzygium cuminii* (74.97%), *Emblica officinalis* (74.97%), *Terminalia arjuna* (64.36%), *Tamarindus indica* (63.76%), *Mangifera indica* (62.41%). The experimental data of these species reveal that all these extracts are likely to have the effect of scavenging free radical. We observed that a dose–response relationship is found in the DPPH radical scavenging activity; the activity increased as the concentration increased for each sample. The involvement of free radicals, especially their increased production, appears to be a feature of most, if not all human diseases, including cardiovascular disease and cancer (Deighton et al., 2000). It has been found that cysteine, glutathione, ascorbic acid, tocopherol, flavonoids, tannins,

Table 1. Antioxidant activity of different medicinal plants using DPPH scavenging method.

Sl. No.	Tested material	Code	% DPPH Scavenging activity at 50 µg/ml	IC50 (µg/ml)
1	*Aegle marmelos*	Methanolic extract	23.63	-
		Water extract	24.94	-
2	*Albizzia lebbeck*	Methanolic extract	94.11	10.18 (8.46-12.18)
		Water extract	85.07	33.65 (28.44-40.62)
3	*Allium cepa*	Methanolic extract	14.10	-
		Water extract	2.69	-
4	*Azadirachta indica*	Methanolic extract	22.02	-
		Water extract	23.76	-
5	*Bacopa monnieri*	Methanolic extract	12.59	-
		Water extract	10.62	-
6	*Capsicum annuum*	Methanolic extract	12.03	-
		Water extract	4.51	-
7	*Carica papaya*	Methanolic extract	3.18	-
		Water extract	8.74	-
8	*Cassia fistula*	Methanolic extract	4.60	-
		Water extract	16.63	-
9	*Citrullus colocynthis*	Methanolic extract	0.96	-
		Water extract	7.10	-
10	*Citrus aurantifolia*	Methanolic extract	3.46	-
		Water extract	10.43	-
11	*Coriandrum sativum*	Methanolic extract	2.32	-
		Water extract	12.91	-
12	*Crataeva nurvula*	Methanolic extract	NA	-
		Water extract	16.77	-
13	*Cuminum cyminum*	Methanolic extract	4.16	-
		Water extract	18.87	-
14	*Dalbergia sissoo*	Methanolic extract	2.89	-
		Water extract	37.08	-
15	*Daucus carota*	Methanolic extract	0.63	-
		Water extract	1.00	-
16	*Emblica officinalis*	Methanolic extract	95.24	6.47 (5.36-7.82)
		Water extract	74.97	16.04 (13.25-19.12)
17	*Ficus glomerata*	Methanolic extract	93.36	8.03 (6.4-9.61)
		Water extract	76.00	21.35 (17.57-25.87)
18	*Foeniculum vulgare*	Methanolic extract	NA	-
		Water extract	13.17	-
19	*Gardenia gummiphora*	Methanolic extract	32.03	-
		Water extract	36.07	-
20	*Madhuca indica*	Methanolic extract	NA	-
		Water extract	25.67	-
21	*Mangifera indica*	Methanolic extract	75.86	15.92 (13.02-19.68)
		Water extract	62.41	28.46 (23.19-35.79)
22	*Melia azadirachta*	Methanolic extract	11.23	-
		Water extract	16.71	-
23	*Momordica charantia*	Methanolic extract	5.13	
		Water extract	11.06	
24	*Moringa oleifera*	Methanolic extract	7.44	-
		Water extract	17.68	-
25	*Morus alba*	Methanolic extract	13.88	-
		Water extract	38.85	-

Table 1. Continued.

26	*Nelumbo nucifera*	Methanolic extract	7.37	-
		Water extract	31.57	-
27	*Ocimum sanctum*	Methanolic extract	13.44	
		Water extract	20.31	-
28	*Picrorrihiza kurrao*	Methanolic extract	8.80	-
		Water extract	7.42	
29	*Punica granatum*	Methanolic extract	94.41	10.57 (8.73-12.54)
		Water extract	92.34	11.98 (9.82-14.3)
30	*Rubia cordifolia*	Methanolic extract	2.89	-
		Water extract	8.10	-
31	*Santalum album*	Methanolic extract	3.02	-
		Water extract	NA	-
32	*Sauropus androgynus*	Methanolic extract	8.74	-
		Water extract	5.88	-
33	*Sesamum indicum*	Methanolic extract	9.75	-
		Water extract	NA	-
34	*Sesbania grandiflora*	Methanolic extract	13.51	
		Water extract	6.65	
35	*Syzygium aromaticum*	Methanolic extract	75.48	26.01 (21.19-32.53)
		Water extract	75.47	27.70 (22.67-34.5)
36	*Syzygium cuminii*	Methanolic extract	81.82	14.62 (11.92-19.59)
		Water extract	84.97	15.90 (12.76-19.59)
37	*Tamarindus indica*	Methanolic extract	93.14	6.85 (5.63-8.34)
		Water extract	63.76	33.32 (25.64-46.03)
38	*Terminalia arjuna*	Methanolic extract	86.78	16.52 (13.22-21.54)
		Water extract	64.36	38.90 (28.18-62.8)
39	*Vetiveria zizinoids*	Methanolic extract	8.45	-
		Water extract	10.71	-
40	*Withania somnifera*	Methanolic extract	4.38	-
		Water extract	NA	-
	Gallic acid			1.93 (1.69-2.19)

and aromatic amines (p-phenylene diamine, p-aminophenol, etc.), reduce and decolourise DPPH by their hydrogen donating ability (Blois, 1958; Yokozawa et al., 1998). In the present study we conclude that all the maximum number of plants we have selected for the experiment showed moderate antioxidant activity in methanolic extracts. The results from various free radical scavenging systems reveal that the ten selected plants have shown significant antioxidant activity. The extracts were found to have different levels of antioxidant activity in all the systems tested. The antioxidant activity of the ten plants is moderately appreciable in both methanolic and water extracts. They are *Emblica officinalis, Tamarindus indica, Punica granatum, Albizzia lebbeck, Ficus glomerata, Terminalia arjuna, Syzygium cuminii, Mangifera indica, Syzygium aromaticum.* Further studies are warranted for the isolation and identification of individual phenolic compounds and also *in vivo* studies are needed for better understanding their mechanism of action as an antioxidant.

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