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Antioxidant properties of certain South Indian medicinal plants

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Abstract
Polyphenols and flavonoids are the most abundant compounds in nature. They are strong antioxidants. Antioxidant plays an important role in inhibiting and scavenging free radicals, thus providing protection to human against infection and degenerative diseases. Now the modern research is directed towards "Natural antioxidants" from the herbal plants due to safe therapeutic. (Abdul Quiyum Ansari et al., 2013). In the present paper antioxidant study of ethanolic extracts of six medicinal plants, namely *Gynodropsis pentaphyllum* (Capparidaceae family), *Solanum nigrum* (Solanaceae family), *Merremia gangitica* (Convolvulaceae family), *Cicca acida* (Euphorbiaceae family), *Erythrina variegata* (fabaceae family) and *Asparagus fysoxii* (Asparagaceae family) has been made for its free radical scavenging ability by adopting various *in vitro* methods. The extracts from these plants were investigated for the antioxidants property using 2,2- diphenyl-1-picryl hydrazyl (DPPH) radical scavenging method and the Ferric Reducing Ability of Plasma (FRAP) activity method.

Keywords: Medicinal plants, Antioxidant, DPPH, Anti radical, Gp, Sn, Mg, Ca, Ev and Af.

1. Introduction
In recent years growing evidence has been accumulated indicating the involvement of reactive oxygen species (ROS) in the pathogenesis of a number of diseases. Among the cellular and extracellular components, lipids, proteins, enzymes, DNA and RNA form the major targets for these reactive species, and the resulting damages are associated with degenerative ("Oxidative") diseases. Most living organisms possess efficient enzymatic and non-enzymatic defense systems against excess production of ROS. However, different external factors (smoke, diet, alcohol, some drugs) and aging decreases the capacity of protecting systems, resulting in disturbance of the redox equilibrium that is establishing in healthy conditions. Therefore, antioxidants that scavenge ROS may be great value in preventing the onset and or the progression of oxidative diseases.

Over the past few years, a number of medicinal plants have been investigated for their quenching activity of specific ROS. Interestingly, these medicinal plants contain large amounts of antioxidants other than vitamin C, vitamin E and carotenoids. The antioxidative effect is mainly due to phenolic components, such as flavonoids, phenolic acids and phenolic diterpenes. They are also a good source of dietary fiber, proteins and minerals and thus, serve as a rich source of bioactive constituents. Polyphenols, especially the flavonoids, exhibit a spectacular array a biological effects as a consequence of their antioxidant properties, with mechanisms involving both free radical scavenging and metal chelation and have been demonstrated to be responsible for several pharmacological activities claimed by folkloric traditions. Therefore, it is felt that the investigation of the antioxidant properties of the phenolic constituents of medicinal plants like *Gynodropsis pentaphyllum, Solanum nigrum, Merremia gangetica, Cicca acida, Erythrina variegate and Asparagus fysoxii* has become a vital task.

2. Materials and Methods
2.1 Collection of plant materials
Fresh aerial parts of six medicinal plants viz. *Gynodropsis pentaphyllum, Solanum nigrum, Merremia gangetica, Cicca acida, Erythrina variegate* and *Asparagus fysoxii* we collected in and around of Puducherry during the month of April 2013 and authenticated by the department of Botany K.M Centre for PG studies, Puducherry, where a voucher specimens of each plants were deposited.

2.2 Preparation of Plant extracts

The air dried aerial parts of the six medicinal plants each of 500 g collected from in and around Puducherry were extracted thrice with hot 95% ethanol under reflux (3 × 5 litre). These extracts were concentrated in vacuo. 10 ml of the concentrated ethanolic extracts of each plant was used for the antioxidant studies. The different concentrations of the ethanolic extract were used for both DPPH and FRAP assay method.

3. Determination of antioxidant Capacity

3.1 DPPH Method

Free radical scavenging ability by the use of stable DPPH radical 2, 2- diphenyl-1-picryl hydrazyl The DPPH radical scavenging activities of ethanolic extracts of six medicinal plants viz., *Ca, Af, Ev, Sn, Gp* and *Mg* were determined using the method proposed by Von Credow, Jaibert and Hansmann (1997) [2]. In this method 0.1 ml of ethanolic extract of each plant is added to 2.9 ml of 0.1M DPPH solution. The negative control prepared by mixing 0.1 ml of methanol with 2.9 ml of 0.1 M DPPH solution. The samples were kept in the dark for 30 minutes and after that the absorbance measured at 517 nm.

The percentage inhibition of DPPH radical by the samples was calculated according to the formula of Yen and Dush (1994)

$$\%\ of\ Inhibition\ of\ DPPH\ assay = \frac{AB - AA}{AB} \times 100$$

$$AB - Absorbance\ of\ control$$

$$AA - Absorbance\ of\ Test$$

3.2 Determination of Ferric Reducing/antioxidant power

The total antioxidant potential sample was determined using a ferric reducing ability of plasma (FRAP assay) of Benzie and Strain (1996) as a measure of antioxidant power FRAP assay measures the change in absorbance at 700 nm owing to the formation of a blue coloured $Fe^{II} - tripyridyltriazine\ compound$ from the colourless oxidized Fe^{III} form by the action of electron denoting antioxidant.

The aliquot of various concentrations of the test sample extracts $(100\mu g/ml)$ were mixed with 2.5 ml of $(PH\ 6.61)$ phosphate buffer and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at $50℃$ in water both for 20 minutes. After cooling, aliquots of 2.5 ml of (10%) trichloroacetic acid were added to the mixture, which was then centrifuged at 300 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with 2.5 ml distilled water and a freshly prepared 0.5 ml of (0.1%) ferric chloride solution.

The absorbance was measured at 700 nm in a UV visible spectrometer. A blank was prepared without adding the extract. Increased absorbance of the reacting mixture indicates increase of reducing capacity.

4. Results and Discussion

The antioxidant activity of the extract of the leaves was assessed by spectrophotometry in the presence of the DPPH radical, which is often used to compare the activities of ethanolic extracts of plants. DPPH radical is a stable free radical which dissolves in methanol and shows characteristic absorption at 517 nm, when an antioxidant scavenges free radicals by hydrogen donation, the DPPH solution become lighter in colour (Molynux, 2004: Villiano *et al.,* 2007). Samples with a raw material concentration of $25\mu g/ml$ were analyzed.

Table 1: Absorbance of the ethanolic extract of plants at 517 nm

EXTRACT	ABSORBANCE	SCAVENGING % (DPPH)
Ca	0.183	42.81
Af	0.295	7.81
Ev	0.304	5.00
Sn	0.261	18.44
Gp	0.313	2.19
Mg	0.127	60.31

Tabular column shows the antioxidant activities of the extracts tested as the percentage of deactivation of the DPPH free radicals. The quality of antioxidants in the ethanolic extracts was determined by the absorbance and scavenging percentage. The DDPH method revealed that the scavenging of the free radicals was found to be highest for the ethanolic extract of *Merremia gangetica* (60%) followed by *Cicca acida* (42.8%) and *Solanum nigrum* (18.4%) respectively.

Antioxidant activities of the ethanolic extracts of the plants determined by FRAP are depicted in Tabular column II. All polyphenols reduce Fe^{3+} ions to Fe^{2+} which are complex TPTZ at a concentration of 10 to $100\mu g/ml.$ The antioxidant activities of ethanolic extracts of six medicinal plants have greater capacity of antioxidants than the antioxidant activities of standard Ascorbic acid. It was proved by a FRAP assay method that the absorbance of *Merremia gangetica* was found to be maximum (0.234) followed by *Asparagus fysoxii* absorbance (0.148), *Cicca acida* absorbance (0.141) and *Erythrina variegata* absorption (0.134) respectively at the concentration of $\frac{100\mu g}{ml}$ Similarly $80\ mcg/ml$, $60\ mcg/ml, 40\ mcg/ml$ And $20\ mcg/ml$ were also found to be greater values of absorbance than the standard drug Ascorbic acid. Out of six medicinal plants the antioxidant capacity of the plant *Merremia gangetica* was found to be greater than the standard Ascorbic acid at the concentration of $100\ mcg/ml$.

Table 2: Absorbance of plant extracts and standard ascorbic acid at 700 nm

Concentration in mcg/ml	Standard Ascorbic acid	Absorbance at 700 nm					
		Ca	Mg	Gp	Sn	Ev	Af
20	0.083	0.106	0.098	0.128	0.093	0.098	0.096
40	0.088	0.099	0.124	0.106	0.106	0.092	0.104
60	0.090	0.119	0.164	0.091	0.153	0.107	0.117
80	0.108	0.114	0.196	0.076	0.153	0.105	0.131
100	0.145	0.141	0.234	0.098	0.134	0.098	0.148

The reducing capacity of the ethanolic extracts of *Gynodropsis pentaphyllum* and *Cicca acida* were found to be onpar with that of the standard antioxidant Ascorbic acid at $100\ mcg/ml$ concentration.

5. Conclusion

In the present study, the antioxidant capacities of the plant extracts were analyzed using free radicals scavenging activity (DPPH) and Ferric reducing ability of plasma (FRAP) methods as a measure of antioxidant power.

The DPPH test is the oldest indirect method of determining the antioxidants activity based on the ability of free radicals $2,2-diphenyl-1-picrylhydrazyl$ to react with hydrogen donors with phenol. The ethanolic extracts of all the six medicinal plants have the ability to scavenge the free radicals. Out of the six medicinal plants *Merremia gangetica* is found to be with *a 60*% scavenging activity followed *Cicca acida* 42.8% and *Solanum nigrum* 18.4% respectively.

The antioxidant capacity of the plant extracts *Merremia gangetica* and *Asparagus fysoxii* under study is found to be greater than standard Ascorbic acid at the concentration of $20\ mcg/ml$,$40\ mcg/ml$, $60\ mcg/ml$, $80\ mcg/ml$ and $100\ mcg/ml$ than the other medicinal plants that have desirable values of antioxidant capacity at different concentration. Ethanolic extracts of leaves of six medicinal plant species are found to be good antioxidants as proved by the FRAP assay method. Ethanol is the best solvent for preparing plant extracts for yielding strongest antioxidant activity in the extract.

From the above methods it is found that the antioxidant capacity of the plant extracts have the maximum ability to scavenge the free radicals is useful to cure diabetes to some extent with a reducing level of the oxidative stress. It has been found that the intake of antioxidant rich diet is effective in reducing the deleterious effects of aging and behaviour.

Several South Indian medicinal plants tested are rich sources of polyphenolic compounds which are free radical scavengers. Some medicinal plants, thus can be considered as promising sources of natural antioxidants for medicinal and commercial use.

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