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Screening of ten indian medicinal plant extracts for antioxidant activity

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ABSTACT

Currently there has been an increased interest globally to identify antioxidant compound that are pharmacologically potent and have low or no side effects. As plants are source of natural antioxidants, much attention has been gain to plants. The quest for natural antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenges over the last two decades. A variety of free radical scavenging antioxidants exist within the body in which many of them are derived from dietary sources like fruits, vegetables and teas. In this study methanolic crude extract of 10 traditionally used Indian medicinal plants were screened for their free radical scavenging and superoxide anion scavenging properties using Rutin and Curcumin as standard antioxidants. The overall antioxidant activity of ocimum sanctum was the strongest, followed in descending order by pterospermum acerifolium, Achyranthes aspera, Delonix regia, Mentha spicata, Datura stramonium, Coccinia indica, Hygrophilla auriculata, Cassia auriculata and Coriandrum sativum. Five plant, namely ocimum sanctum, pterospermum acerifolium, Achyranthes aspera, Delonix regia and Mentha spicata Showed strong super oxide anion scavenging activity. Both activities expressed as IC_{50} and strong correlation showed between total polyphenolic contents in plants and their antioxidant activity.

Keywords: Antioxidant; Free radical; Radical scavenging; Medicinal plant; Super oxide anions; DPPH.

INTRODUCTION

Reactive oxygen species (ROS) may caused great damage to cell membranes and DNA, including oxidation that causes membrane lipid peroxidation, decreased membrane fluidity and DNA mutations leading to cancer, degenerative diseases including atherosclerosis, ischemic

heart disease, ageing, diabetes mellitus and others [1-5]. Antioxidants are compounds that inhibit or delay the oxidation process by blocking the initiation or propagation of oxidizing chain reactions. So disease linked with free radicals can be therapy. Synthetic antioxidant like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in processed foods have side effects and are carcinogenic [6,7]. In recent years, the use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safely, nutritional and therapeutic value [8]. Antioxidant derived from fruits, vegetables, spices and cerials are very effective and have reduced interference with the body's ability to use free radicals constructively [9,10]. On continuation of our screening project for the search of antioxidant activity of popular Indian medicinal plants, we studied 10 plant extracts. The radical scavenging activity against 2, 2-diphenyl-1-picrylhdydrazyl (DPPH) and superoxide anions were studied in this report. Curcumin and Rutin were used as antioxidant reference compound [11-15].

MATERIALS AND METHODS

Plant material

The medicinal plant materials were purchased from the local herbal market of Jabalpur (M.P), India. Voucher specimens from all plant materials were deposited at the garden of Sanjay Nikunj Dahalwada, Vidisha (M.P). The plants were identified by Mr. R.P. Meena, Garden Superintendent, Sanjay Nikunj Dahalwada, Vidisha, M.P, India, where a voucher specimen no. G-22 were deposited for identification. The scientific name, family name, English name, part used and traditional uses are presented in Table 1.

Chemicals:

The chemical & reagents used were petroleum ether, chloroform, n-butanol, ethyl acetate, DPPH (Diphenyl picryl hydrazyl), NADH, phenazene methosulphate nicotinamide (PMS), Nitro blue terazolium (NBT), Ferric chloride, Potassium hydrogen phosphate, hydrogen peroxide, ascorbic acid (all chemicals purchased from Loba Chem. Lab). Curcumin were purchased from Lancaster Research Lab, Chennai, India. 2-2-Diphenyl-1-picryhydrazyl (DPPH) and rutin were purchased from Himedia Lab, Mumbai, India. All other chemicals and solvents were of analytical grade from Merck.

Extraction:

A quantity (100 g) of each powdered plant material was soaked in 300 ml of methanol after 1h stirring at room temperature overnight. The solvent was decanted and the residue was macerated two more days with the same solvent. The pooled solvents were combined and filtered. The filtrates were concentrated under reduced pressure and their extract yields were calculated [16-21]. The extraction yields (% dry weight basis) are presented in Table 1.

Determination of total phenolics:

The concentration of phenolic content in all the fractions were determined with Folin-Ciocalteu's phenol reagent (FCR) according to the method of slinkard and singleton (1977) [22-28]. 1 ml of the solution (contains 1 mg) of the fraction in methanol was added to 46 ml of distilled water and 1 ml of FCR, and mixed thoroughly. After 3 min, 3 ml of sodium carbonate (2%) were added to the mixture and shaken intermittently for 2 hour at room temperature. The absorbance was

measured at 760 nm. The concentration of phenolic compound was calculated according to the following equation that was oriented from standard pyrocatechol graph:

$$\textbf{Absorbance} = .001 \times \textbf{pyrocatechol (µg)} + .0033$$

Table 1. Characteristics of the used medicinal plants.

Extract yield (%)	Traditional uses	Part Used	English Name	Family Name	Scientific Name
10.8	Laxative, stomachic, carminative and useful in treatment of vomiting, bronchitis, heart disease, piles [11,12]	Leaves	Chirchita	Amaranthaceae	*Achyranthes*
6.9	For rheumatism, conjunctivitis and diabetes [13]	Leaves	Avaram	Caesalpiniaceae	*Cassia auriculata*
11.2	Various part of the plant get relief from diabetes mellitus [14, 15]	Leaves	Little Gourd	Cucurbitaceae	*Coccinia auriculata*
15.2	Jaundice, Hepatic obstruction, rheumatism, inflammation, urinary infection and gout.	Seeds	Kulekhara	Acanthaceae	*Hygrophila auriculata*
9.3	Small Pox, Cancer, Migraine[17,18]	Leaves	Muchkund	Sterculiaceae	*Pterspermum acerifolium*
7.9	Anticarcinogenic, anthelmintic, antiseptic, antirheumatic, antistress and antibacterial properties [19,20,21]	Leaves	Tulsi	Labiatae	*Ocimum sanctum*
5.4	Antiparasitics and repellents [22]	Leaves	Datura	Solanaceae	*Datura stramonium*
8.9	Antidiabetic, anti-inflamatory and cholesterol lowering [23,24]	Seeds	Coriander	Umbeliferaeae	*Coriendrum sativum*
16.8	Carminative, stomach pain [25,26]	Leaves	Mint	Labiatae	*Mentha spicata*
6.2	Antibacterial [27]	Flowers	Gulmohar	Leguminosae	*Delonix regia*

DPPH radical scavenging activity:

The DPPH assay measured hydrogen atom (or one electron) donating activity and hence provided an evaluation of antioxidant activity due to free radical scavenging. DPPH, a purple-colored stable free radical, was reduced into the yellow-colored diphenylpicryl hydrazine which is measured spectrophotometrically at 517 nm [29] . Briefly, 0.1 mM solution of DPPH solution in methanol was prepared and 1 ml of this solution was mixed with 3ml of sample solution in water at different concentration. Finally, after 30 min, the absorbance was measured at 517 nm. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. DPPH radical-scavenging activity was calculated according to the following equation:

$$\textbf{\% Inhibition} = ((A_0 - A_1) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (without extract) and A_1 was the absorbance in the presence of the extract.

Superoxide anion scavenging activity assay:

The scavenging activity of the different fractions towards superoxide anion radicals was measured by method of Nishimiki (1972) with slight modification [30]. Phenazine methosulfate-nicotinamide adinine dinucleotide (PMS-NADH) system was used for the generation of

superoxide anion. It was assayed by the reduction of nitroblue tetrazolium (NBT). About 1 ml of nitroblue tetrazolium (156µM), 1 ml NADH (648µM) in 100 mM phosphate buffer of pH 7.8 and 0.1 ml of sample solution of different concentrations were mixed. The reaction started by adding 100 µl PMS (60 µM). The reaction mixture was incubated at 25^0C for 5 min and absorbance of the mixture was measured at 560 nm against blank samples.

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

Where A_0 was the absorbance of the control (blank, without extract) and A1 was the absorbance in the presence of the extract.

Statistical Analysis:
All data on all antioxidant activity tests are the average of triplicate analyses. The data were recorded as mean ±SD. The statistical significance of differences between groups was determined by analysis of variances (ANOVA), followed by Dunnett's test for multiple comparisons among groups. Differences of $p<0.05$ were considered statistically significant.

RESULTS

Total polyphenolics contents of the extracts
The total phenolic compound amount was calculated as quite high in methanolic extract of Ocimum sanctum(79.2±7.5 mg mg^{-1} pyrocatechol equivalent) other than *Achyranyhes aspera* (53.2±7.5 mg mg^{-1} pyrocatechol equivalent) , *Cassia auriculata* (22.4±8.92 mg mg^{-1}), *Coccinia indica* (26.4±1.2 mg mg^{-1}) , *Mentha spicata* (38.2±3.39 mg mg^{-1}) , *Hygrophilla auriculata* (21.5±1.5 mg mg^{-1}) , *Datura stramonium* (22.6±0.2 mg^{-1}), *Delonix regia* (78.6±9.5 mg mg^{-1}) , *Coriandrum sativum* (168.2±0.5 µg mg^{-1}) and *Pterospermum acerifolium* (169.3±3.2 µg mg^{-1}). The high concentration of polyphenolics in methanol extract of *Ocimum sanctum* may be responsible for its high free radical scavenging activity. The FCR reducing capacity of different extracts are due to presence of hydroxyl groups present in the polyphenolics and flavonoids. The key role of phenolic compounds as scavengers of free radicals is emphasized in some report [31]. They were reported to eliminate radicals due to their hydroxyl groups, and they contribute directly to antioxidant effect of system and it also has an important role in stabilizing lipid oxidation. The results are depicted in Figure 1.

DPPH radical scavenging activity
The results of the free radical scavenging potentials of different fractions tested by DPPH method are depicted in Figure 2 and Figure 3, and values in table 2 and table 3. Antioxidant reacts with DPPH, which is a nitrogen-centered radical with a characteristic absorption at 517 nm and convert it to 1, 1,-diphenyl-2-picryl hydrazine, due to its hydrogen donating ability at a very rapid rate [32]. The degree of discoloration indicates the scavenging potentials of the antioxidant. The IC_{50} value of methanolic extract of *Ocimum sanctum* was found as 16.8 µg/ml, whereas the IC_{50} value of *Achyranyhes aspera*, *Cassia auriculata*, *Coccinia indica*, *Mentha spicata*, *Hygrophilla auriculata*, *Datura stramonium*, *Delonix regia*, *Coriandrum sativum* and *Pterospermum acerifolium* was found as 36.2 µg/ml, 68.4 µg/ml, 54.6 µg/ml, 47.3 µg/ml, 59.5 µg/ml, 48.3 µg/ml, 38.4 µg/ml, 89.3 µg/ml, and 26.3µg/ml respectively. The low IC_{50} value of

methanolic extract of *Ocimum sanctum* is due to presence of high polyphenolics and flavonoids in it.

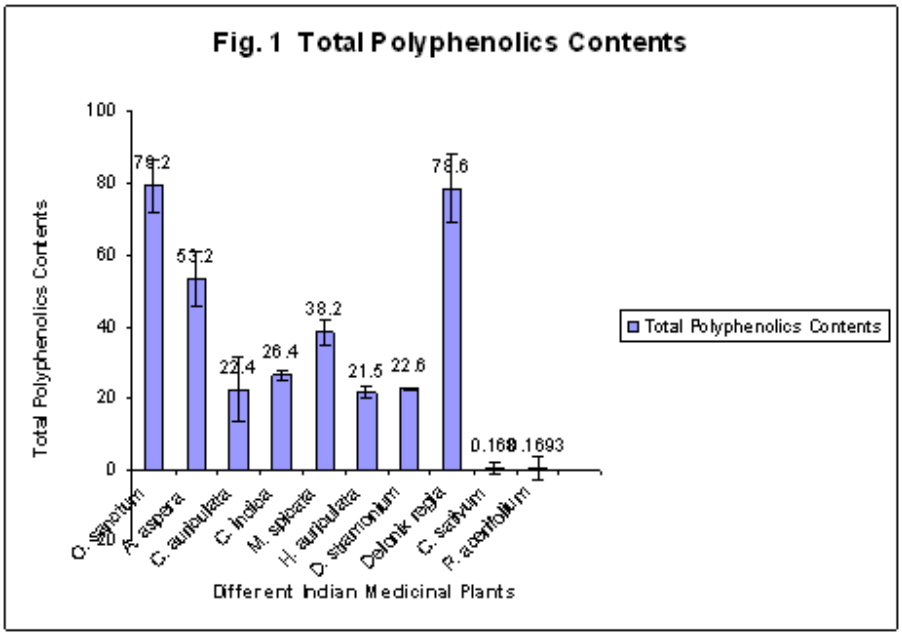


TABLE 2. IC_{50} value of different Plants in different free radical scavenging assays

Process	IC_{50} value (µg/ml)					
	A.A	C.A	C.I	H.A	D.S	STD
DPPH radical	16.8 ± 1.52	26.3±2.33	89.3±5.21	38.4±3.32	34.2±2.32	14.2±1.12(Rutin)
Super oxide radical	30.4±2.03	53.8±3.13	91.6±6.21	21.8±1.96	38.9±2.52	7.9 ±0.82 (Curcumin)

O.S- Ocimum sanctum *P.A- Pterospermum acerifolium* *C.S- Coriandrum sativum*
D.R- Delonix regia *M.S- Mentha spicata* *STD- Standard drug*
SD- Standard Deviation

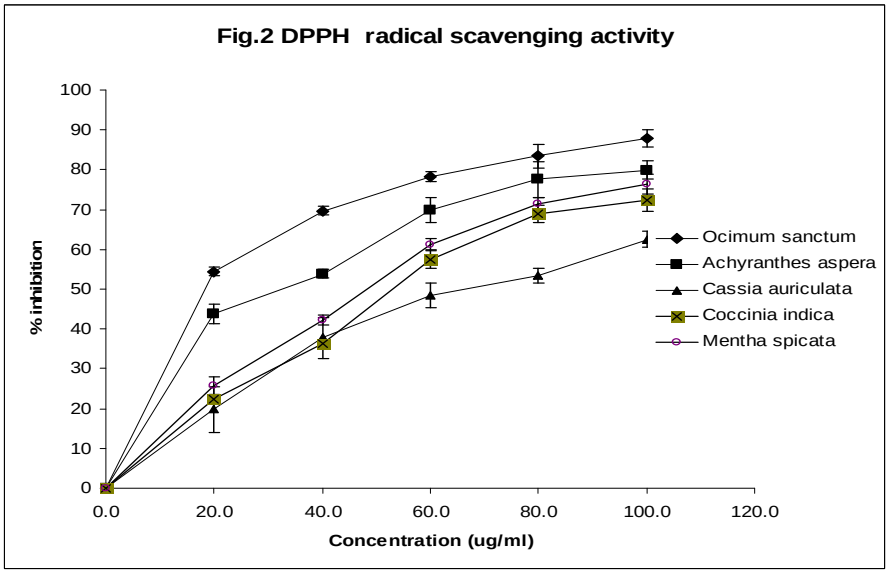
TABLE 3. IC_{50} value of different Plants in different free radical scavenging assays

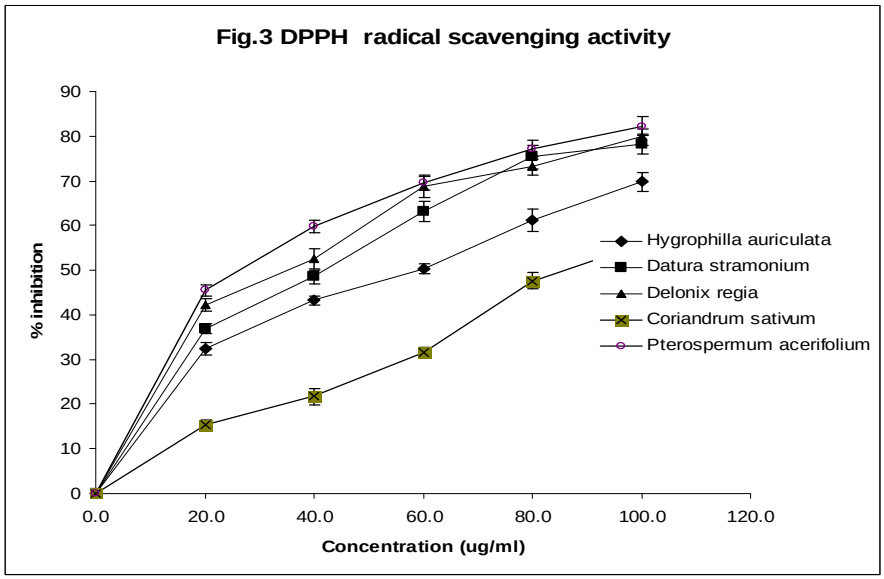
Process	IC_{50} value (µg/ml)					
	A.A	C.A	C.I	H.A	D.S	STD
DPPH radical	36.2 ±2.55	68.2±4.56	59.5±3.26	48.3±2.83	34.2±2.32	7.9±0.82(Rutin)
Super oxide radical	28.3±2.03	89.7±6.33	59.2±3.21	92.4±6.23	33.43±2.30	54.6±3.21(Curcumin)

A.A- Achyranthes aspera *C.A- Cassia auriculata* *C.I- Coccinia indica*
H.A- Hygrophilla auriculata *D.S- Datura stramonium* *STD- Standard drug*
SD- Standard Deviation

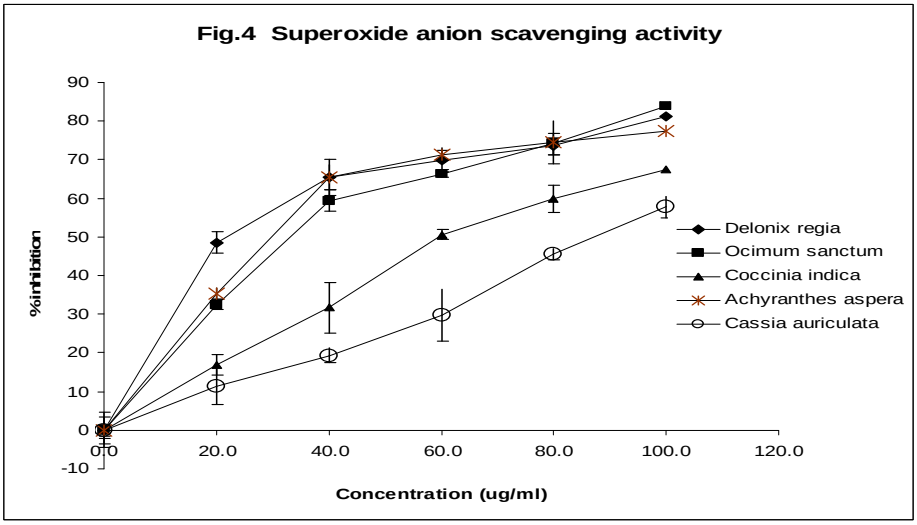
Superoxide anion scavenging assay

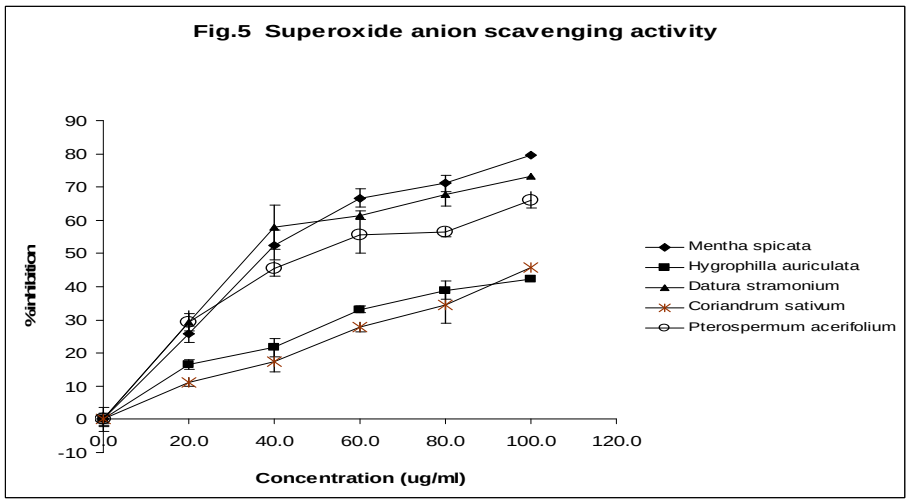
In the PMS/NADH-NBT system, superoxide anion is generated using a non-enzymatic reaction of phenazine methosulphate in the presence of NADH and molecular oxygen [33]. Super oxide anion reduces NBT into formazan at pH 7.8 at room temperature and formazan generation can be determined spectrophotometry at 560 nm. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion by the active fractions of the extracts.





In this assay, ethyl acetate fraction shows maximum superoxide anion scavenging activity and the results are presented in Figure 4 and Figure 5, and values in table 2 and table 3. The IC_{50} value of methanolic extract of *Delonix regia* (21.8 µg/ml) was low other than *Ocimum sanctum* (IC_{50}=30.4 µg/ml) , *Coccinia indica* (IC_{50} 59.2 µg/ml), *Achyranthes aspera (* IC_{50}=28.3 µg/ml), *Cassia auriculata*(IC_{50} 89.7 µg/ml), *Mentha spicata*(IC_{50} 38.9 µg/ml), *Hygrophilla auriculata*(IC_{50} > 100 µg/ml), *Datura stramonium*(IC_{50} 33.9 µg/ml), *Coriandrum sativum*(IC_{50} >100 µg/ml), and *Pterospermum acerifolium*(IC_{50} 53.8 µg/ml).





DISCUSSION

There is evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices herbs, and medicinal plants [34,35]. Our attention has been focused, in particular, on the parts of 10 commonly used Indian medicinal plants. The plant extracts tested showed low absorbance values, which indicated a high level of antioxidant activity. None of the plant extracts showed absorbance values greater than the negative controls (without plant extracts) indicating the presence of antioxidant activity. The extracts of *Ocimum sanctum*, *Pterospermum acerifolium*, *Achyranthes aspera*, *Delonix regia* and *Mentha spicata* and some other plants exerted good antioxidant activity by both methods. In the present experiment, methanolic extracts of 10 plants were evaluated for their free radical scavenging activity using the DPPH radical assay and Super oxide anion scavenging assay.

Different plant extracts reduced DPPH radicals significantly. Values of percent decolorization of DPPH radicals are shown in Figure 2 and figure 3. Of the 10 plants tested 8 plants, namely *Pterospermum acerifolium, Achyranthes aspera, Delonix regia, Mentha spicata, Datura stramonium, Coccinia indica,* and *Hygrophilla auriculata, showed* more than 70% decolorization. The activity of *Ocimum sanctum* was at par when compared with the standard Rutin. Some variations in the extent of extract antioxidant activity were observed for each type of assay used in this study. The extracts of *Hygrophilla auriculata*, had good DPPH radical scavenging activity, but low Superoxide anion scavenging activity, while the extracts of *Pterospermum acerifolium* having low value of Total polyphenolics contents but showed good DPPH and Superoxide radical scavenging activity. These differences may be due to their different antioxidant mechanisms. A fair correlation between total phenolic content and antioxidant activity was observed in 8 of the 10 plants, whereas no direct relationship could be detected in *Pterospermum acerifolium*, and *Hygrophilla auriculata* .These observations clearly indicated a close linkage between phenolics and antioxidant activity but, due to the phytochemical diversity in the antioxidant phytocompounds, the above variation is expected.

CONCLUSION

In conclusion, the results further support the view that some traditionally used Indian medicinal plants are important sources of potential antioxidants and may be efficient as preventive agents in some diseases. Further study will be aimed at isolating and identifying the substances responsible for the antioxidant activity of plant extracts, and essential to characterize them as biological antioxidants. It should also be kept in mind that antioxidant activity measured by in vitro methods may not reflect in vivo effects of antioxidants [36]. Many other factors such as absorption/metabolism are also important.

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