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IN VITRO FREE RADICAL SCAVENGING ACTIVITY OF AQUEOUS LEAF EXTRACT OF PLANTS NEAR THERMAL POWER PLANT, METTUR, SALEM

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ABSTRACT: The present study was undertaken to know about the free radical scavenging activity of plants located near Thermal power plant, Mettur, Salem. In this study, *Polyalthia longifolia, Derris indica, Holopetela integrifolia, Tamarindus indica, Terminalia tomentosa, Psidium guajava, Nerium indicum, Azadirachta indica, Calotropis gigantea, Ricinus communis, Carica papaya, Ficus benghalensis, Embilica officinalis, Tectona grandis, Ficus religosa, Mangifera indica, Moringa oleifera, Acacia Arabica, Opuntia ficus indica* are the plants that were collected during March to April 2013. Among the plants selected, *Holopetela integrifolia, Mangifera indica, Tamarindus indica, Acacia arabica, Azadirachta indica* were found to have high flavonoid content. Total antioxidant activity was measured in terms of phospho-molybdenum activity was high in *Carica papaya* when compared to the other entire antioxidant assay, *Acacia arabica* showed maximum ability to chelate metal ions.

INTRODUCTION: Plants contain many bioactive molecules which are responsible for various benefits offered by plants. A major antioxidant compound present in the plants are phenols, flavonoid. Literature survey has revealed a direct relationship between antioxidant activity and total phenolic content. It has been reported by Halliwell [1] that for a polyphenol to be defined as an antioxidant it must satisfy two basic conditions: first, when present in low concentrations relative to the substrate to be oxidized it can delay, retard or prevent the autoxidation of free radical mediated oxidation; second, the resulting radicals formed after scavenging must be stable through intramolecular hydrogen bonding on further oxidation [2].

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Since, plant extracts are studied for its antioxidant properties, the present study was planned to determine the contents of phenolics and flavonoids as well as its ability to perform antioxidant function in aqueous extracts of plants collected from the experimental site – Thermal power plant, Mettur, Salem, Tamil Nadu, India.

MATERIALS AND METHODS:

Plant Materials: Fresh leaves were collected from the experimental site near Thermal power plant, Mettur, Salem during March - April 2013. 100 mg of fresh leaves was extracted with 1ml water. 0.1 ml of extract was used for the analysis.

Secondary Metabolites: The phenol [3, 4] and flavonoid [5] content of aqueous leaf extract was analysed.

Determination of Total Phenol Content: Total phenolic content was determined by Folin-Ciocalteu method.

To 0.1 ml extract add Folin-Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na_2Co_3 (4 ml, 1M) were added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetric method at 765 nm. The standard curve was prepared. Total phenol values are expressed in terms of gallic acid equivalent (mg/g), which is a common reference compound.

Estimation of Flavonoids: The aluminium chloride method was used for the determination of the total flavonoid content. Extract solutions were taken and then 0.1 ml of $AlCl_3$ (10%) were added sequentially. The test solution was vigorously shaken. Absorbance at 415 nm was recorded after 30 min of incubation. A standard calibration plot was generated using known concentration of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent / g of sample.

Antioxidant assay: Nitric oxide scavenging assay [6], Reducing power [7], Metal chelating activity [8], and Total antioxidant assay [9] were performed.

Nitric oxide scavenging activity: The procedure is based on the principle that, sodium nitroprusside in aqueous solution, at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. For this experiment, sodium nitroprusside (10 mM), in phosphate buffered saline was mixed with extract and incubated at room temperature for 150 min. After the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546 nm. Quercetin was used as a positive control.

Reducing power assay: Aqueous extract was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml 1%). The mixture was incubated at 50˚C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as positive control.

Metal chelating activity: The chelating ability of ferrous ion was estimated by adding extract to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM Ferrozine (0.2 ml), the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The ethylene diamine tetra acetic acid calibration curve was plotted as a function of metal chelating activity.

Total Antioxidant capacity: Total antioxidant capacity by phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate / Mo (V) complex at acidic pH. The phosphomolybdenum method is quantitative since, the total antioxidant activity is expressed as the number of equivalents of ascorbic acid [14].

RESULTS AND DISCUSSION:

Phenol and flavonoid content: The results of phenol and flavonoid content are depicted in **Table 1**. The phenol content was found to be in the range of 4.0 to 4.4 mg/g with *Carica papaya, Embilica officinalis, Ficus religosa, Polyalthia longifolia* (Table 1). Whereas the phenol content in all the other plants were well below this range (Table 1). Likewise, the flavonoid content of plants studied was found to be more when compared to phenol content (Table 1).

Antioxidant activities: The antioxidant activity was assessed by means of different antioxidant tests which are shown in **Table 2**. The nitric oxide radical scavenging activity was low and found to range from 0.60 to 1.52 mg/g (Table 2) while, the reducing power assay was higher than nitric oxide activity thus confirming Fe^{3+} to Fe^{2+} confirmation by plant extract. Highest metal chelating activity was observed with *Acacia arabica***,** *Embilica officinalis***,** *Holopetela integrifolia* and moderate amount was observed with *Azadirachta indica***,** *Polyalthia longifolia, Moringa oleifera, Mangifera indica, Derris indica* (Table 2). But the same was lower with rest of the plants (Table 2).

The metal chelating action of plant depends on the flavonoid composition in plants. Phosphomolybdenum antioxidant assay was performed as a measure of antioxidant activity. It was higher for *Carica papaya*, *Tamarindus indica*, *Terminalia tomentosa*, *Mangifera indica*, *Acacia arabica*, *Derris indica*, *Tectona grandis*, *Nerium indicum* (Table 2), but all the other plants showed moderate activity (Table 2).

TABLE 1: SHOWING PHENOL, FLAVONOID CONTENT OF PLANTS

S. No.	Medicinal Plants	Phenol (mg/g)	Flavonoid (mg/g)
1	*Polyalthia longifolia*	4.4	7.0
2	*Derris indica*	1.2	8.0
3	*Holopetela integrifolia*	1.4	9.9
4	*Tamarindus indica*	1.1	9.7
5	*Terminalia tomentosa*	1.3	6.1
6	*Psidium guajava*	1.7	7.8
7	*Nerium indicum*	3.0	8.4
8	*Azadirachta indica*	1.7	9.4
9	*Calotropis gigantea*	2.3	6.4
10	*Ricinus communis*	2.4	8.0
11	*Carica papaya*	4.0	6.0
12	*Ficus benghalensis*	2.9	4.8
13	*Embilica officinalis*	4.1	6.3
14	*Tectona grandis*	2.0	7.8
15	*Ficus religosa*	4.2	8.8
16	*Mangifera indica*	2.6	9.9
17	*Moringa oleifera*	1.6	9.0
18	*Acacia arabica*	2.3	9.4
19	*Opuntia ficus indica*	2.0	5.3

TABLE 2: SHOWING DIFFERENT ANTIOXIDANT ACTIVITIES OF PLANTS

S. No.	Medicinal Plants	Nitric oxide scavenging activity (mg/g)	Reducing Power (mg/g)	Metal chelating activity (mg/g)	Total antioxidant activity (mg/g)
1	*Polyalthia longifolia*	1.52	2.6	4.6	24.0
2	*Derris indica*	1.50	2.85	4.1	27.9
3	*Holopetela integrifolia*	0.75	2.45	5.0	12.6
4	*Tamarindus indica*	1.22	2.25	3.5	29.4
5	*Terminalia tomentosa*	1.47	2.7	2.2	29.1
6	*Psidium guajava*	1.32	2.5	4.2	19.2
7	*Nerium indicum*	1.52	2.15	2.9	27.6
8	*Azadirachta indica*	0.60	3.25	4.7	23.7
9	*Calotropis gigantea*	1.15	2.4	3.0	13.2
10	*Ricinus communis*	1.12	2.9	3.9	24.6
11	*Carica papaya*	1.27	3.5	4.9	29.7
12	*Ficus benghalensis*	1.62	3.1	3.6	15.6
13	*Embilica officinalis*	1.72	3.6	5.4	18.6
14	*Tectona grandis*	1.37	2.6	4.7	27.1
15	*Ficus religosa*	0.97	3.45	2.7	23.1
16	*Mangifera indica*	0.97	2.45	4.5	29.1
17	*Moringa oleifera*	0.65	3.25	4.6	26.7
18	*Acacia arabica*	1.10	2.4	6.4	28.5
19	*Opuntia ficus indica*	1.42	2.5	2.0	27.1

CONCLUSION: The free radical scavenging effect measured, predicts the phenol and flavonoid content of selected plants. In this study, the aqueous extract was used which could extract flavonoid fully from the plants as it is a water soluble compound. The observed results showed significant changes with metal chelating activity. Increased total antioxidant activity might be due to the addition of hydrogen atom to form a stable product, which terminates free radical chain

reaction. So, with our results we can say that plants can be used as natural antioxidant rather than using chemical to be free from side effects caused by synthetic antioxidants.

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REFERENCES:

1. Halliwell B: How to characterize a biological antioxidant. Free. Rad. Res. Comm. 1990; 9: 1 - 32.
2. Shahidi F, Wanasundara PKJPD, Hong C: Phenolic compounds in foods and their effects on health. ACS Symposium Series 506. Am. Chem. Soc. 1992; 214 - 222.
3. Ebrahimzadeh MA, Hosseinimehr SJ, Hamidinia A, Jafari M: Antioxidant and free radical scavenging activity of *Feijoa sallowiana* fruits peel and leaves. Pharmacology online, 2008a; 7- 14.
4. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A, Bekhradnia AR. Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica* Mey. Pharmacology online 2008; 2: 560-567.
5. Mervat MM, Far E, Hanan A, Taie A: "Antioxidant activities, total anthrocynins, phenolics & flavonoids contents of some sweet potato genotypes under stress of different concentration of sucrose and sorbitol. Australian J Basic Applied Sci. 2009; 3: 3609-3616.
6. Ebrahimzadeh MA, Nabavi SF, Nabavi SM: Antioxidant activities of methanol extract of *Sambus ebulus L.* flower. Pak. J. Biol. Sci. 2009d; 12: 447- 450.
7. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimuta-genicity. J. Agri. Food Chem. 1995; 43: 27-32.
8. Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR: Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. Afr. J. Biotechnol. 2008a; 32: 43-49.
9. Prieto P, Pineda M, Aguilar M: Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of Vitamin E. *Anal. Biochem*. 1999; 269: 337-341.

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