

Research Article



A Comparative Study on Antioxidant Activities of Selected Plants From Road Sides, Salem, Tamil Nadu, India

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ABSTRACT

This study was undertaken to know the antioxidant potential of plants located at road sides. For this study, two sites were selected from Salem District, Tamil Nadu, India. The road sides of New bus stand (site 1) and Saradha college road (site 2), Salem. Commonly available plants were selected from both the study areas. The commonly available plants used for the present research are: *Polyalthiya longifolia*, *Pongamia pinnata*, *Ficus religiosa*, *Muntinga calabura*, *Azadirachta indica*, *Cocos nusifera*, *Tamarindus indica*, *Tectona grandis*, *Casurina equisetifolia*, *Psidium guajava*, *Delonix regia*, *Terminalia catappa*. Aqueous leaf extract was used for all the experiments. The fresh plant leaves collected were assessed for its secondary metabolites and antioxidant activities by different methods. The secondary metabolites were found to be high with *Muntinga calabura*, *Ficus religiosa* in site 1 and *Delonix regia*, *Terminalia catappa* in site 2. Antioxidant activity was high with *Polyalthiya longifolia*, *Casuarina equisetifolia*, *Azadirachta indica*, *Terminalia catappa* and *Cocos nusifera*. The changes observed might be due to the variation in nutrient content of the leaf as well as on the duration of exposure of pollution.

Keywords: Antioxidants, Flavonoids, New bus stand, Phenolics, Saradha college road.

INTRODUCTION

The idea of antioxidant capacity was first originated from chemistry and was later well-known to biology, medicine, epidemiology and nutrition. The free radicals are mainly derived from oxygen and nitrogen. Production of large number of free radicals leads to oxidative stress. All organisms are protected from free radical attack by two defense mechanisms: 1. Preventive antioxidant system that reduces the rate of free radical synthesis/formation, 2. A system to produce chain-breaking antioxidants that scavenge, stabilize free radicals. But, when the rate of free radical production exceeds the capacity of antioxidant defense mechanisms, a substantial tissue injury results. Plants are the major producers of a large number of antioxidants to manage the oxidative stress that make the plants good sources for novel compounds with antioxidant activity. Hence, a study was undertaken to analyze the secondary metabolites and antioxidant activities of plants selected from road sides of two study sites. Initial survey was done to know the plants present and a list was prepared. Among the plants available, common plants present in both the sites were selected for the present study to know the difference in each of their activities. The following is the list of thirteen common plants: *Polyalthiya longifolia*, *Pongamia pinnata*, *Ficus religiosa*, *Muntinga calabura*, *Albizia saman*, *Azadirachta indica*, *Cocos nusifera*, *Tamarindus indica*, *Tectona grandis*, *Casurina equisetifolia*, *Psidium guajava*, *Delonix regia*, *Terminalia catappa*. Thirteen common plants were used for the assessment of secondary metabolites, antioxidant assays.

MATERIALS AND METHODS

Leaf sample collection

For the present study, fresh leaves from each plants were collected from the experimental sites i.e road sides, Salem, Tamil Nadu, India. The plant leaves were collected during the month of January to Feb, 2014. The leaf samples were collected from two different sites that were selected for the present study. The two sites selected for the study includes plants located at road sides of new bus stand (site 1) and plants located at Saradha college road (site 2).

Extract preparation

Fresh leaves were used according to the standard prescribed methods adopted. Aqueous extract was used for the whole study.

Secondary Metabolites

The phenol and flavonoid content of aqueous leaf extract was analyzed.

Determination of total phenol content

Total phenolic content were determined by FolinCiocalteu method.^{1,2} The extract samples 0.1ml were mixed with folincioalteau reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous NaCo₃ (4ml, 1M) were added. The mixture was allowed to stand for 15min 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 of dry mass), which is a common reference compound.

Estimation of flavonoids

The aluminium chloride method³ was used for the determination of total flavonoid content. Extract solution were taken and then 0.1ml of $AlCl_3$ (10%) were added sequentially. The test solution was vigorously shaken. Absorbance at 415nm was recorded after 30min of incubation. A standard calibration plot was generated at 415nm using known concentration of quercetin. The concentration of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent/g of sample.

Antioxidant Assays

Nitric oxides scavenging assay, Reducing power, Total antioxidant assay, Metal chelating activities were performed.

Nitric oxide scavenging activity

This was estimated by the method of Ebrahimzadeh et.al 2009d.⁴ 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 the experiment, sodium nitroprusside (10mM), in phosphate buffered saline, was mixed with extract and incubated at room temperature for 150min. After the incubation period, 0.5ml of griess reagent was added. The absorbance of the chromophore formed was read at 546nm. Quercetin was used as positive control.

Reducing power assay

Reducing power assay was performed according to the method of Yen et.al 1995.⁵ Aqueous extract was mixed with phosphate buffer (2.5ml, 0.2M, P^H 6.6) and potassium ferricyanide (2.5ml %). The mixture was incubated at 50^oc for 20min. 1.0 ml of trichloro acetic acid (10%) was added to stop the reaction, which was then centrifuged at 3000rpm for 10min. The upper layer of solution (1.5ml) was mixed with distilled water (1.5ml) and $FeCl_3$ (0.1ml, 0.1%) after mixing, the contents were incubated for 10 min and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as positive control.

Total antioxidant capacity

Total antioxidant capacity by phospho-molybdenum method assay is based on the reduction of Mo (V1) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. The phospho-molybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid. Assay was carried out according to the method of Prieto P et.al 1999.⁶

Metal chelating activity

The chelating ability of ferrous ion was estimated by the method of Oyaizu M 1986.⁷ Add extract to a solution of 2mM $FeCl_2$ (0.05ml). The reaction was initiated by the addition of 5mM Ferrozine (160 μ l), the mixture was shaken vigorously and left standing at room temperature for 10min. Absorbance of the solution was then measured spectrophotometrically at 562nm. Standard curve was plotted using ascorbic acid. Distilled water (1.6ml) instead of sample solution was used as a control. Distilled water (160 μ l) instead of ferrozine was used as a blank, which is used for error correction because of unequal color of sample solution.

For all estimations readings were taken using UV- Visible spectrophotometer- Shimadzu, Japan make. Model UV 1800. Standard graph were plotted for all experiments using their respective standards and samples were plotted against standard by taking concentration in X axis and OD in Y axis.

RESULTS AND DISCUSSION

Secondary Metabolites

Total phenolics

The results of total phenolics are shown in Figure 1.

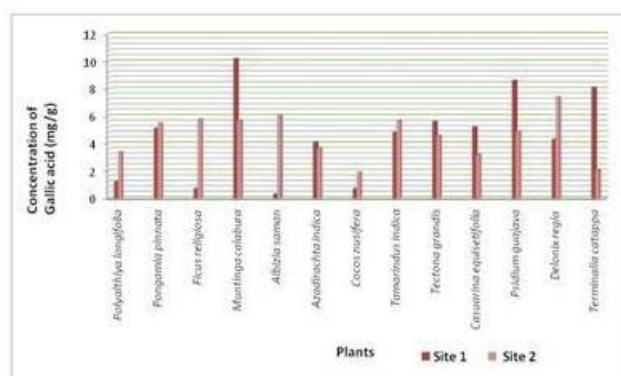


Figure 1: Showing results of Total Phenolics

The phenolic content was higher with *Muntinga calabura*, *Psidium guajava*, *Terminalia catappa* in site 1 and lower level was observed with *Polyalthiya longifolia*, *Ficus religiosa*, *Cocos nucifera*, *Albizia saman*. The phenolics observed was moderate in all the other plants studied. Whereas, in site 2 it was higher with *Delonix regia* and it was low with *Cocos nucifera*, *Terminalia catappa* but moderate level was observed with rest of the plants studied. Similar result was reported for *Azadirachta indica*, *Polyalthiya longifolia* in site 2 by Krishnaveni et.al.^{8,9}

Flavonoid

The results of flavonoid content are shown in Figure 2.

The flavonoid content was found to be high with *Ficus religiosa*, *Muntinga calabura*, *Terminalia catappa* while it was moderate with rest of the plants studied with respect to site 1. The flavonoid content was higher with

Terminalia catappa, *Albizia saman*, *Delonix regia* but *Azadirachta indica* showed lower flavonoid content when compared to other plants. Similar result was reported by Krishnaveni et.al for *Albizia speies*, *Ficus religiosa*, *Tamarindus indica* in site 1.^{10,8,11}

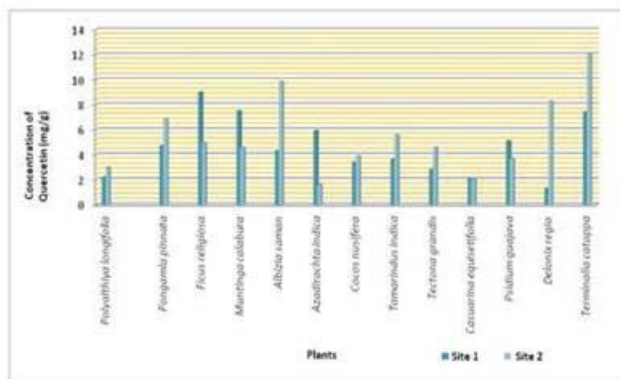


Figure 2: Showing results of Flavonoid

Reducing power assay

The results of reducing power assays are shown in Figure 3.

The reducing power activity was higher with *Polyalthiya longifolia* whereas it was moderate for the rest of the plants studied in site 1. In site 2, the reducing power assay was higher for all the plants studied which falls in the range of 7.0- 9.2mg/g ascorbic acid.

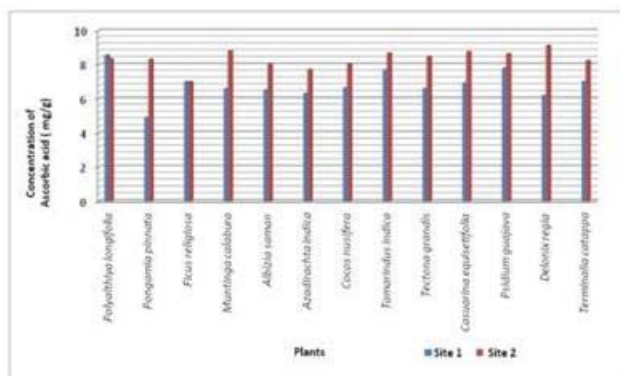


Figure 3: Showing results of Reducing Power Assay

Total antioxidant activity

The results of total antioxidant activities estimated through phosphomolybdenum assay are given in Figure 4.

The antioxidant activity of several plants studied. Among the plants studied in site 1, the activity was higher with *Casuarina equisetifolia*, *Pongamia pinnata*, *Terminalia catappa*, *Tamarindus indica*. Moderate level of phosphomolybdenum activity was observed with rest of the plants. In study site 2, it was higher for *Delonix regia*, *Cocos nucifera*, *Muntingia calabura*, *Psidium guajava*. While, moderate level was observed with rest of the plants which falls in the range of 3.2 to 8.24mg/g ascorbic acid.

Nitric oxide scavenging assay

The results of nitric oxide scavenging activity are shown in Figure 5.

The nitric oxide scavenging activity was higher for *Azadirachta indica*, *Delonix regia*, *Tectona grandis*, *Psidium guajava* studied from site 1. All the other plants from site 1 showed moderate levels of nitric oxide scavenging activity. When plants collected from site 2 were analysed for its nitric oxide scavenging activity, the obtained results exhibited higher activity for *Delonix regia*, *Psidium guajava*, *Tamarindus indica*, *Terminalia catappa* and it was slightly lower for the rest of the plants studied. Similar result was reported for *Azadirachta indica* for site 2 by Krishnaveni et. al.¹¹

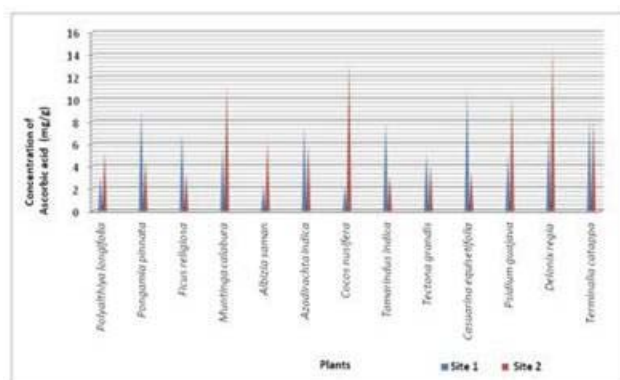


Figure 4: Showing results of Total antioxidant activity

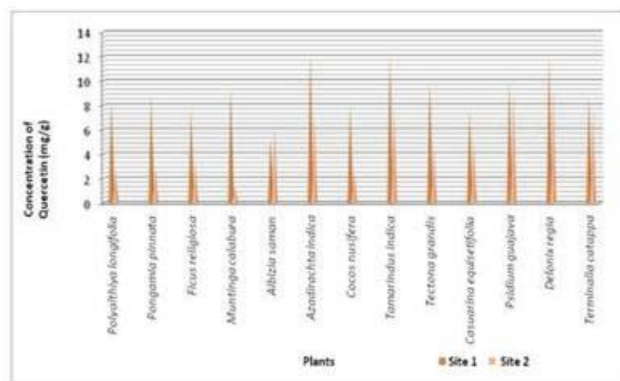


Figure 5: Showing results of Nitric oxide scavenging activity

Metal chelating activity

The observations of metal chelating activities are shown in Figure 6 and percent inhibition in Figure 7.

The ability to chelate metal ions were found to be high with *Terminalia catappa*, *Psidium guajava* for the plants studied at site 1. It was lesser for the rest of the plants studied. Among the plants studied at site 2, the activity was higher for *Delonix regia*, whereas it was moderate for the remaining plants assessed. Similar result was reported by Krishnaveni et al., for *Tectona grandis* in site 2, *Psidium guajava* in site 1 and *Casuarina equisetifolia* in both the sites studied.^{8, 11, 12}

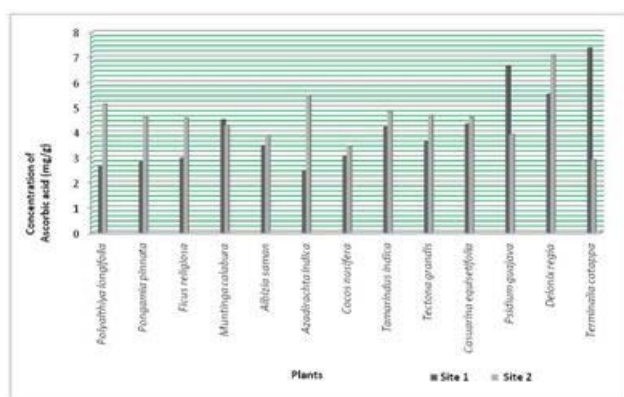


Figure 6: Showing results of Metal chelating activity

Percent inhibition of metal chelating activity

The results of percent inhibition of metal chelating activity are depicted in Figure 7. The plants such as *Azadirachta indica*, *Tamarindus indica*, *Muntingia calabura*, *Delonix regia* showed higher inhibition among the thirteen plants studied. Inhibition was moderate with remaining plants studied in site 1 and all the plants studied at site 2 showed higher level.

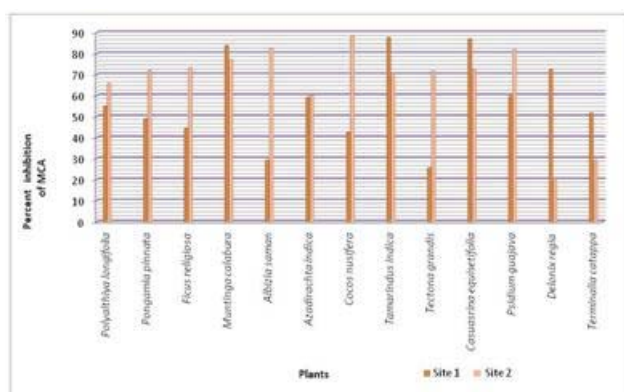


Figure 7: Showing percent inhibition (Metal Chelating Activity)

CONCLUSION

Site 1

The phenolic content was higher for *Muntingia calabura* and *Ficus religiosa* showed higher flavonoid content. The reducing power activity was more for *Polyalthiya longifolia*. Likewise, total antioxidant activity was high for *Casuarina equisetifolia*. The nitric oxide scavenging activity was high with *Azadirachta indica* and metal chelating activity was more for *Terminalia catappa*.

Site 2

The phenolic content was found to be high for *Delonix regia*. While flavonoid content was more with *Terminalia catappa*. Reducing power activity was high with *Casuarina equisetifolia*. *Phosphomolybdenum* and nitric oxide activity was high with *Delonix regia*. Metal chelating ability was high with *Azadirachta indica* whereas the percent inhibition observed was more with *Cocos nucifera*. The changes observed among the plants, might

depend upon the topography and upon the nature, amount of exposure to pollution.

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