Research Article

Antioxidant Activities of Plants Studied in Yercaud Road Sides, Salem, Tamilnadu, India

Marimuthu Krishnaveni[1]*, Kalimuthu R[2], Ponraj K[2], Lavanya K[2], Magesh P[2], Jasbin shyni G[2]
*[1]Assistant Professor, Department of Biochemistry, Periyar University, Salem, Tamilnadu, India.
[2]M.Sc Students, Department of Biochemistry, Periyar University, Salem, Tamilnadu, India.
***Corresponding author's E-mail:** logasarvesh@gmail.com

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ABSTRACT

The medicinal value of plants is enhanced by its most important biologically active components like secondary metabolites, which induces antioxidant activities. Richer antioxidant property makes a plant medicinally important and finds its use in pharmacology. Hence, an attempt was taken to study the plants located at Yercaud road sides. The fresh plant leaves were collected from road side trees from bend 1 to bend 20. For convenience, the study locations were divided in to three points and samples were collected for the research at those points. 12 plants were selected for the study from point 1 and 11 plants was selected for the study from point 2 and 3. Standard protocols were adopted for secondary metabolites as well as for antioxidant activities. From the obtained results, it is concluded that plant leaves collected within 2 kilometers from bend 20 of Yercaud road side was showing more potent antioxidative activity when compared to other two study points, the observed result might be due to higher phenol and flavonoid content. The higher antioxidant property might also be due to the nature of soil nutrients present in the particular location which enhances plant growth and plant nutrients.

Keywords: Antioxidant activity, Bend, Road sides, Secondary metabolites, Yercaud.

INTRODUCTION

Phytochemicals are of plant sources and found to contain phenolics, flavonoids as major compounds that play a major role in scavenging free radicals. More research studies are being performed for its bioactive molecules. Hence, an initiative has been taken to study the antioxidant potential of the plants selected from Yercaud road at three different points such as roadsides of bend 1 (Point 1), between bend 10 and 11 (Point 2) and third location within two kilometer from twentieth bend (Point 3). The plants selected from point 1 includes *Pisivum Sativam, Cassia fistula, Morinda tinctoria, Bambusa bambos, Peltophorum acutifolium, Aayamaram, Cassia alata, Manilkara zapota, Pongamia pinnata, Azadirachitaindica, Ficus religiosa, Tamarindus indica.* The plants selected from point 2 includes *Callistemon, Cardia sebestena, Plumeria alba, Polyalthia longifolia, Psidium guajava, Mangifera indica, Annona squamosa, Ficus benghalensis, Tectona grandis, Mimusops elengi, Anacardium oxidetale.* The plants selected from point 3, *Morinda tinctoria, Nerium oleander, Tecoma stans, Cardia Sebestina, Eucalyptus, Citrus limon, Atrocarphous heterophyllus, Psidium guajava, Jatropa carcus, Causarina equistifolia, Tectona grandis.* The plant leaves collected from each points were studied for its secondary metabolites (Phenol and flavonoid) and various antioxidant activities such as reducing power assay, metal chelating activity, total antioxidant activity, nitric oxide scavenging activity was performed.

MATERIALS AND METHODS

Leaf sample collection

For the present study, fresh leaves from each plant were collected from the experimental sites -Yercaud road sides, Salem, Tamil Nadu, India. The plant leaves were collected during the month of January to March, 2014. For, our convenience, the total bends of Yercaud road sides were divided in to three points. And point 1 was considered as bend 1, point 2 was considered as between bend 10 and 11 and point 3 was considered within 2 kilometers from bend 20.

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 was determined by Folil-ciocalteau method.[1,2] The extract (0.1ml) was mixed with folin-ciocalteau reagent (5 ml, 1:10 diluted with distilled water) for 5 min and added aqueous $NaCo_3$ (4ml, 1M). The mixture was allowed to stand for 15min and the phenol content present was determined by colorimetric method at 765 nm. The standard curve was prepared. Total phenol values were expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

Estimation of flavonoids

The aluminium chloride method[3] was used for the determination of total flavonoid content. Extract solution were taken and added 0.1ml of $AlCl_3$ (10%) sequentially. The test solution was vigorously shaken. Absorbance at 415 nm 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 oxide scavenging assay, Reducing power, Total antioxidant assay, Metal chelating activities was performed.

Nitric oxide scavenging activity

This was estimated by the method of Ebrahimzadeh et.al 2009d**.**[4] This 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 ferric cyanide (2.5ml %). The mixture was incubated at 50^0c 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 phosphor molybdenum method assay is based on the reduction of Mo (V1) to Mo (V) by the sample analyte and subsequent formation of green phosphate/Mo (V) complex at acidic pH. The phosphor 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 (160µl), 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 (160µl) 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 by taking concentration in X axis and OD in Y axis.

Statistical Tool

Mean, Standard deviation (S) was calculated by using following formula:

Mean= Sum of x values / n (Number of values).

$$s = \frac{\sqrt{\sum (X - M)^2}}{n - 1}$$

RESULTS AND DISCUSSION

Plants studied at Yercaud road sides of bend 1 – point 1

The results of secondary metabolites and antioxidant activities of plants collected from point 1 are shown in Table 1.

Total phenol

The phenolic content was high with *Manilkara zapota, Cassia alata*. Moderate amount was observed with *Cassia fistula, Tamarindus indica, Bambusa bambos, Peltophorum acutifolium, Morinda tinctoria.* The phenolic content was very low with *Pongamia pinnata, Ficus religiosa, Aayamaram, Azadirachita indica* and *Pisvum Sativam* showing only 1.16mg/g. Similar result was reported by Krishnaveni et.al for *Azadirachita indica,*[8] *Ficus religiosa.*[9]

Flavonoids

The flavonoid content was high with *Cassia fistula.* Whereas all the other plants showed moderate amount of flavonoids and very low flavonoid content was observed in *Pisivum Sativam*. Similar result was reported by Krishnaveni et.al for *Ficus religiosa.*[8]

Reducing power

The reducing power activity was high with *Manilkara zapota, Pisivum Sativam, Morinda tinctoria,* rest of the plants showed moderate amount of reducing power activity. Reducing power activity was very low with *Tamarindus indica.*

Table 1: Secondary metabolites and antioxidant activities

Name of the plants in point1	Total phenol (mg/g)	Flavonoids (mg/g)	Reducing power assay (mg/g)	Total antioxidant activity (mg/g)	Metal chelating activity (mg/g)	Nitric oxide scavenging assay (mg/g)
Pisivum Sativam	1.16±0.11	7.4±0.11	8.68±0.06	6.76±0.24	3.2±0.08	8.0±0.30
Cassia fistula	4.66±1.36	11.7±0.17	7.32±0.12	7.4±0.89	1.1±0.25	6.52±0.24
Morinda tinctoria	4.96±0.56	4.7±0.11	8.28±0.12	4.96±0.90	2.14±0.12	8.52±0.31
Bambusa bambos	5.93±0.25	4.2±0.50	7.6±0.12	3.28±0.78	3.28±0.12	5.72±0.24
Peltophorum acutifolium	5.26±0.35	1.7±0.35	7.14±0.73	6.16±0.18	3.56±0.12	3.16±0.06
Aayamaram	3.5±0.17	8.6±0.17	7.7±0.65	4.64±0.13	3.54±0.12	9.28±0.36
Cassia alata	10.7±0.17	4.4±0.23	7.28±0.06	11.84±0.18	4.06±0.13	8.24±0.54
Manilkara zapota	12.4±0.11	9.5±0.11	8.8±0.18	6.5±0.27	5.3±0.18	5.44±0.24
Pongamia pinnata	4.9±0.45	8.5±0.45	5.8±0.05	3.6±0.34	3.02±0.15	7.84±0.42
Azadirachta indica	3.3±1.12	8.3±0.20	5.1±0.05	3.0±0.25	5.3±0.18	6.8±0.24
Ficus religiosa	3.6±0.50	9.6±0.10	7.3±0.10	3.1±0.40	2.4 ±0.15	8.48±0.30
Tamarindus indica	6.1±0.40	6.6±0.30	3.1±0.05	2.5±0.10	2.6±0.27	4.6±0.38

Values are Mean ± SD for three experiments

Table 2: Secondary metabolites and antioxidant activities

Name of the plants in point 2	Total phenol (mg/g)	Flavonoids (mg/g)	Total antioxidant activity (mg/g)	Reducing power assay (mg/g)	Nitric oxide (mg/g)	Metal chelating (mg/g)
Callistemon	3.2±0.30	4.72±0.48	4.32±1.32	3.0±0.22	6.0±0.40	5.31±0.02
Cardia sebestena	2.9±0.05	2.88±0.24	1.76±0.30	6.8±0.36	11.9±0.30	4.3±0.23
Plumeria alba	2.8±0.05	7.08±0.12	5.64±0.60	8.78±0.19	7.7±0.40	4.3±0.13
Polyalthia longifolia	2.6±0.05	2.36±0.78	7.06±0.98	12.12±0.24	6.8±0.35	2.3±0.15
Psidium guajava	2.0±0.28	4.4±0.06	6.08±0.06	8.43±0.65	6.7±0.10	3.8±0.16
Mangifera indica	3.5±0.30	5.16±0.12	5.44±0.18	12.5±0.50	8.2±0.25	4.9±0.12
Annona squamosa	6.6±0.52	3.6±0.48	2.67±0.42	2.08±0.18	3.1±0.40	1.8±0.12
Ficus benghalensis	1.8±0.05	3.8±0.18	3.84±0.48	8.2±0.50	11.9±0.45	2.8±0.15
Tectona grandis	2.6±0.25	6.08±0.54	5.12±0.06	1.9±0.30	7.5±0.40	6.4±0.20
Mimusops elengi	2.2±0.20	8.76±0.18	3.36±1.03	6.1±0.10	9.7±0.05	1.7±0.18
Anacardium oxidetale	2.6±0.05	3.12±0.12	5.22±0.20	5.16±0.12	7.0±0.25	5.95±0.10

Values are Mean ± SD for three experiments

Table 3: Secondary metabolites and antioxidant activities

Name of the plants in point 3	Total phenolics (mg/g)	Flavonoids (mg/g)	Reducing power (mg/g)	Total antioxidant activity (mg/g)	Nitric oxide (mg/g)	Metal chelating activity (mg/g)
Morinda tinctoria	4.48±0.06	4.1±0.25	3.36±0.36	7.76±0.66	12.28±0.18	3.5±0.49
Nerium oleander	4.92±0.12	3.8±0.15	3.04±0.66	9.04±0.42	13.48±0.86	3.7±0.77
Tecoma stans	6.48±1.2	5.2±0.35	5.68±0.18	2.80±0.18	13.68±0.95	3.4±0.20
Cardia Sebestina	6.72±1.93	4.6±0.75	7.60±0.60	5.04±0.36	10.12±0.54	3.2±0.26
Eucalyptus	6.48±0.48	5.2±0.35	1.52±0.24	5.2±2.26	11.88±0.72	4.7±0.69
Citrus limon	5.28±0.24	9.5±0.34	1.56±0.93	7.44±2.04	12.56±0.18	3.0±0.02
Atrocarphous heterophyllus	5.68±0.36	6.0±0.85	2.12±0.61	7.52±0.66	12.64±0.45	4.8±0.05
Psidium guajava	5.56±0.18	10.4±0.10	7.64±0.30	5.92±0.18	13.32±0.36	4.8±0.50
Jatropha carcus	4.32±0.36	6.5±0.75	3.90±0.20	7.88±0.18	14.24±0.42	3.7±0.29
Causarina equistifolia	5.32±0.90	2.5±0.45	1.32±0.12	5.72±0.18	9.88±1.78	3.9±0.87
Tectona grandis	5.90±0.40	7.3±0.90	4.75±0.43	10.44±0.12	14.48±0.30	3.1±0.35

Values are Mean ± SD for three experiments

Phosphomolybdenum activity

Total antioxidant activity was higher for *Cassia alata* showing 11.84mg/g, whereas the other entire plants showed moderate amount of total antioxidant activity which ranges from 2.5 to 6.76mg/g.

Metal chelating activity

Metal chelating activity was higher *Manilkara zapota, Azadirachta indica* while it was moderate with rest of the plants ranging from 1.1 to 4.06mg/g. Simlar result was reported by Krishnaveni et.al for *Ficus religiosa,*[10,11] *Tamarindus indica.*[11]

Nitric oxide assay

Nitric oxide scavenging activity was higher with *Aayamaram, Morinda tinctoria, Morinda tinctoria, Ficus religiosa, Cassia alata, Pisivum Sativam, Azadirachta indica, Cassia fistula, Bambusa bambos,* Whereas it was low with *Tamarindus indica, Peltophorum acutifolium.* Similar result was reported by Krishnaveni et.al for *Azadirachta indica.*[10]

Plants studied at yercaud road sides between bend 10 and 11- point 2

The results of plants studied near road sides of bend 10 and 11 are depicted in Table 2.

Secondary metabolites

The phenolic content was high with *Annona squamosa,* whereas it was moderate with rest of the plants. The flavonoid content was high with *Mimusops elengi*, *Plumeria alba, Tectona grandis, Mangifera indica*. But it was moderate with rest of the plants showing flavonoid content in the range of 2.36 to 4.72mg/g. Similar phenol, flavonoid content was reported for *Tectona grandis.*[11]

Antioxidant activity

Total antioxidant activity

Total antioxidant activity was higher for *Polyalthia longifolia, Psidium guajava.* Whereas the other entire plants showed moderate amount of total antioxidant activity which ranges from 1.76 to 5.64mg/g.

Reducing power

The reducing power activity was high with *Mangifera indica, Polyalthia longifolia,* while it was low with *Callistemon, Tectona grandis.* Whereas all the other plants moderate amount of reducing power activity. Similar result was reported by Krishnaveni et.al for *Tectona grandis.*[12]

Nitric oxide assay

Nitric oxide scavenging activity was higher with *Cardia sebestena, Ficus benghalensis*. Whereas it was moderate with rest of the plants studied.

Metal chelating activity

Metal chelating activity was higher with *Tectona grandis, Anacardium oxidetale, Callistemon, Mangifera indica,* while it was moderate with rest of the plants.

Plants studied at Yercaud road sides within 2 kilometer from bend 20-point 3

The results of plants studied near yercaud road sides within 2 kilometer from bend 20 are exhibited in Table.3.

Total phenol

The phenolic content was found to be high with all the plants studied in point 3 and it falls in the range of 4.32 to 6.72mg/g. Similar result was reported by Krishnaveni et.al for *Psidium guajava.*[9]

Flavonoids

The flavonoid content was high with *Psidium guajava, Citrus limon*. Moderate amount of flavonoid was observed with most of the plants studied and falls in the range of 2.5 to 6.5mg/g. Similar result was reported by Krishnaveni et.al for *Psidium guajava*.[9]

Reducing power

The reducing power activity was high with *Psidium guajava, Cardia Sebestina.* while it was moderate with other plants and found to have values in the range of 1.32 to 4.75mg/g.

Total antioxidant activity

Total antioxidant activity was higher for *Tectona grandis, Nerium oleander,* whereas it was moderate with all the other plants studied.

Nitric oxide assay

Nitric oxide scavenging activity was higher with *Tectona grandis, Jatropha carcus, Tecoma stans, Nerium oleander, Psidium guajava, Atrocarphous heterophyllus, Citrus limon, Morinda tinctoria, Eucalyptus Cardia Sebestina*. Whereas it was moderate with *Causarina equistifolia*.

Metal chelating activity

Metal chelating activity was higher with *Atrocarphous heterophyllus, Psidium guajava, Eucalyptus,* while it was moderate with rest of the plants showing values in the range of 3.0 to 3.9mg/g. Similar result was reported by Krishnaveni et.al for *Nerium* sp.[10] and *Causarina equistifolia.*[10]

CONCLUSION

Total phenolics was higher in plants collected at third point and at first point, whereas it was lower for plants collected at point 2. The flavonoid content was found to be rich for all the plants studied near yercaud road. Reducing power activity was found to be high with plants collected at point 1, while it was moderate with point 2 and point 3. Total antioxidant, nitric oxide scavenging activity as well as metal chelating ability was higher with

point 3 while it was moderate with point 2, point 1. The higher secondary metabolites and antioxidant activities of plants studied at point 3 might be due to the soil structure that holds most of the nutrients and later on used for the development of plant growth.

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REFERENCES

1. Ebrahimzadeh MA, Hosseinimehr SJ, Hamidinia A, Jafari M, Antioxidant and free radical scavenging activity of *Feijoa sallowiana* fruits peel and leaves, Pharmacology online, 1, 2008, 7-14.
2. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A, Bekhradni AR, Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica,*Pharmacology online, 2, 2008, 560-567.
3. 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, 3, 2009, 3609-3616.
4. Ebrahimzadeh MA, Nabavi SF, Nabavi SM, Antioxidant activities of methanol extract of *Sambus ebulus L.*flower, Pak. J. Biol. Sci, 12, 2009, 447-450.
5. Yen GC, Chen HY, Antioxidant activity of various tea extracts in relation to their Antimutagenicity, J. Agri. Food Chem, 43, 1995, 27-32.
6. Priet 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, 269, 1999, 337-341.
7. Ebrahimzadeh MA, Pourmorad F and Bekhradnia AR, Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran, Afr. J. Biotechnol, 2008a, 32, 43-49.
8. Krishnaveni M, Chandrasekar R, Amsavalli L, Durairaj S, Madhiyan P, Free radical scavenging activity of plants at Perumal malai Hill, Int. J. Pharm. Sci. Rew. Res, 21, 2013, 155-159.
9. Krishnaveni M, Amsavalli L, Chandrasekar R, Madhiyan P, Durairaj S, Antioxidant activities of plants at Govt. college of Engineering Campus, Salem, Tamil Nadu, India, Int. J. Pharm. Sci. Rew. Res, 21, 2013, 160-163.
10. Krishnaveni M, Madhaiyan P, Durairaj S, Amsavalli L, Chandrasekar R, Antioxidant activity of plants at Chinnathirupathi, Salem, Tamil Nadu, India, International Journal of Pharmaceutical Sciences Research, 4, 2013, 3917-3919.
11. Krishnaveni M, Durairaj S, Madhiyan P, Amsavalli L, Chandrasekar R, In vitro free radical scavenging activity of Aqueous leaf extract of plants near thermal power plant, Mettur, Salem, International Journal of Pharmaceutical Sciences Research, 4, 2013, 3659-3662.
12. Krishnaveni M, Antioxidant activity of selected plants, International Journal of Pharmacy and Pharmaceutical Sciences, 6, 2014, 126-128.

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