Research Article

Antioxidant Activity of Plants Studied at Thoppur Hill Road Sides, Dharmapuri, Tamil nadu, India

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ABSTRACT

Plants are the major scavenging tool for oxidation process. Hence, an initiative was taken to study the antioxidant activities of plants located near road sides of Thoppur hill. Plants were collected from three different locations. Totally 12 plants from location 1, 2, and 11 plants from location 3 were collected. From all locations, few commonly available plants as well as plants not commonly found were also selected from their respective sites. Fresh leaves were collected at early morning and used for the study. The free radical scavenging activity of auqeous leaf extract was studied using different methods. On the whole, plants such as Ficus *religiosa, Azadirachta indica, Syzygium cumini, Albizia amara, Tectona grandis, Tamarindus indica, Ficus benghalensis, Mangifera indica, Manilkara zapota, Pongamia pinnata* showed very good antioxidant activity.

Keywords: Antioxidants, Dharmapuri, Metabolites, Plants, Thoppur hill.

INTRODUCTION

Phenolic compounds are known to inhibit oxidizing enzymes, a potential mechanisms make diverse group of phenolic compounds an interesting objective in the search for valuable phytochemicals. Phenolics, a recognized group of plant secondary metabolites, are well-known free radical scavengers and also responsible for exhibiting multiple therapeutic and physiological functions in animals and plants. The value of polyphenols can be very well recognized at the time of stress. It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion.[1] 11km is the total length of the road sides of Thoppur hill. The total length was divided in to three locations. The locations are given here while going up via Thoppur Hill from Salem.

The plants selected from location 1 were taken from road sides i.e between 1st and 2nd kilometer: *Madhuca longifolia, Cardia sebestena, Psidium guajava, Tectona grandis, Mangifera indica, Ficus religiosa, Peltophorumacutifolium, Annona squamosa, Manilkara zapota, Causarina equistifolia, Tamarindus indica, Azadirachta indica.*

The plants selected from location 2 were taken from road sides i.e near 6th kilometer: *Polyalthialongifolia, Syzygium cumini, Tamarindus indica, Ficus religiosa, Ficus benghalensis, Albizia saman, Pongamia pinnata, Albizia amara, Azadirachta indica, Tectona grandis, Carica papaya, Morinda tinctoria*

The plants collected from location 3 were taken from road side's i.e near 10th kilometer: *Peltophorum acutifolium, Tectona grandis, Tamarindus indica, Azadirachta indica, Annona squamosa, Madhuca longifolia, Psidium guajava, Cardia sebestena, Mangifera indica, Pongamia pinnata, Polyalthialongifolia*

MATERIALS AND METHODS

Leaf sample collection

For the present study, fresh leaves from 12 commonly available plants were collected early in the morning from Thoppur hill road sides. This study was carried out during the month of January to March, 2014.

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 Folil-ciocalteau method.[2] The extract samples 0.1ml were mixed with folinciocalteau reagent (5ml, 1:10 diluted with distilled water) for 5 min and aqueous Sodium carbonate (4ml, 1M) were added. The mixture was allowed to stand for 15min and the phenols were determined by colorimetric method at 765nm. 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 method[3] was used for the determination of the 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 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] 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 ferriccyanide (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 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 (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. All antioxidant studies were carried out in triplets. 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

The results of secondary metabolites and antioxidant activities of plants studied at three different locations in the roadsides of Thoppur hill, Dharmapuri, Tamil Nadu, India are presented here in Table 1 to Table 6.

Total Phenolics

Total Phenolics was found to be high with *Ficus religiosa, Manilkara zapota, Annona squamosa* and it was moderate with *Psidium guajava, Peltophorum acutifolium, Mangifera indica, Tamarindus indica, Cardiasebestena, Tectona grandis, Madhuca longifolia, Azadirachta indica* (Table 1).

Flavonoids

The flavonoid content was high with *Mangifera indica, Azadirachta indica, Tamarindus indica, Peltophorum acutifolium,* whereas *Annona squamosa, Causarina equistifolia, Manilkara zapota, Madhuca longifolia, Tectona grandis, Ficus religiosa, Psidium guajava* showed moderate amount of flavonoid and very low amount was observed with *Cardia sebestena* (Table 1). Similar result was reported by Krishnaveni et.al for *Tamarindus indica.*[8]

Reducing power assay

The reducing power activity was found to be high with *Mangifera indica, Psidium guajava, Tamarindus indica, Cardia sebestena.* Moderate amount was observed with *Manilkara zapota, Causarina equistifolia, Madhuca longifolia, Ficus religiosa, Annona squamosa, Tectona grandis, Peltophorum acutifolium* (Table 1).

Nitric oxide assay

The nitric oxide activity was found to be high with *Psidium guajava, Tamarindus indica, Cardiasebestena, Tectona grandis, Mangifera indica.* Nitric oxide scavenging activity was moderate with *Ficus religiosa, Causarinaequistifolia, Manilkara zapota, Annona squamosa, Peltophorum acutifolium, Azadirachta indica, Madhuca longifolia* (Table 1).

Table 1: Showing results of Secondary metabolites and Antioxidant activities

Name of plants	Total phenolics (mg/g)	Flavonoids (mg/g)	Reducing power assay (mg/g)	Nitric oxide assay (mg/g)	Phosphomolybdenum assay (mg/g)	Metal chelating assay (mg/g)
Madhuca longifolia	2.6	2.9	5.9	7.84	9.56	4.96
Cardia sebestena	2.1	1.3	7.1	8.68	10.28	4.24
Psidium guajava	3.4	2.1	7.4	9.52	7.48	2.72
Tectona grandis	4.8	7.0	4.0	8.56	4.24	7.22
Mangifera indica	4.6	12.0	7.9	8.53	9.32	3.86
Ficus religiosa	9.0	7.3	4.4	3.17	4.0	2.66
Peltophorum acutifolium	6.0	8.0	7.1	7.28	3.0	4.04
Annona squamosa	8.3	6.3	6.6	5.56	5.68	4.24
Manilkara zapota	8.8	6.6	6.5	5.92	3.2	3.86
Causarina equistifolia	5.7	4.1	4.8	6.76	5.2	3.86
Tamarindus indica	6.7	8.0	7.3	8.68	5.6	5.12
Azadirachta indica	3.76	8.8	4.8	6.24	10.76	3.32

(Location 1 between 1st and 2nd kilometer)

Table 2: Showing results of Secondary metabolites and Antioxidant activities

Name of plants	Total phenolics (mg/g)	Flavonoids (mg/g)	Reducing power assay (mg/g)	Nitric oxide Assay (mg/g)	Phosphomolybdenum assay (mg/g)	Metal chelating activity (mg/g)
Polyalthialongifolia	3.8	4.04	8.28	9.9	4.64	2.0
Syzygium cumini	4.5	10.16	2.72	7.73	9.72	3.3
Tamarindus indica	4.5	3.76	2.32	6.9	6.76	2.7
Ficus religiosa	2.4	7.20	9.6	11.46	12.24	3.7
Ficus benghalensis	3.3	4.40	5.88	13.2	9.08	5.91
Albizia saman	6.6	5.28	5.2	9.2	5.32	3.05
Pongamia pinnata	3.4	05.68	3.76	7.86	8.72	5.10
Albizia amara	1.8	7.52	5.28	0.36	15.16	2.4
Azadirachta indica	4.5	6.00	7.2	10.3	16.56	3.3
Tectona grandis	2.0	4.56	3.08	13.86	6.4	4.2
Carica papaya	2.3	7.12	6.68	13.3	4.16	2.8
Morinda tinctoria	5.5	4.96	2.96	6.0	10.96	1.4

(Location 2 near 6th kilometer)

Total antioxidant assay

Total antioxidant activity was high with *Azadirachta indica, Cardia sebestena, Madhuca longifolia*. Moderate amount of antioxidant activity was observed with *Manilkara zapota, Psidium guajava, Causarina equistifolia, Mangifera indica, Annona squamosa, Peltophorum acutifolium, Ficus religiosa, Peltophorum acutifolium, Tamarindus indica* (Table 1).

Metal chelating activity

Higher amount of metal chelating activity was observed with *Tectona grandis* alone. But the activity was moderate with Psidium *guajava, Madhuca longifolia, Azadirachta indica, Manilkara zapota, Peltophorum acutifolium, Cardiasebestena, Causarina equistifolia, Mangifera indica, Tamarindus indica, Annona squamosa* (Table.1). Similar result was reported by Krishnaveni et.al for *Ficus religiosa.*[11]

Total phenolics

The phenolics content was high for *Albizia saman.* Moderate amount of total phenolics was observed in all the other plants studied at site 2 (Table 2) Similar result was reported by Krishnaveni et.al for *Tamarindus indica.*[8]

Flavonoids

The observed flavonoid content was high for *Syzygium cumini.* While it was moderate for all the other plants studied (Table 2). Similar result was reported by Krishnaveni et.al for *Azadirachta indica*[9]

Reducing power assay

The reducing power activity was high with *Polyalthia longifolia, Ficus religiosa.* Only moderate amount was observed for *Pongamia pinnata, Cardia sebestena, Albizia saman, Tectona grandis, Syzygium cumini, Albizia amara, Tamarindus indica, Carica papaya, Morinda tinctoria* (Table 2). Similar result was reported by Krishnaveni et.al for *Syzygium cumini,*[10] *Tamarindus indica.*[11,12]

Nitric oxide activity

The nitric oxide scavenging activity was high with *Ficus religiosa, Ficus benghalensis, Tectona grandis, Carica papaya, Azadirachta indica.* Moderate amount of scavenging activity was observed with *Albizia saman, Tamarindus indica, Syzygium cumini, Polyalthialongifolia, Annona squamosa, Pongamia pinnata* and it was very less with *Albizia amara* (Table 2).

Phosphomolybdenum assay

Phosphomolybdenum activity was higher for *Morinda tinctoria, Azadirachta indica, Albizia amara,Pongamia pinnata,Ficus benghalensis, Ficus religiosa, Syzygium cumini.* Plants such as *Tamarindus indica, Tectona grandis, Albizia saman, Polyalthialongifolia, Annona squamosa* showed moderate amount of phosphomolybdenum activity (Table 2).

Metal chelating activity

The metal chelating activity was found to be high for *Ficus benghalensis, Pongamia pinnata* and found to be moderate with Tectona *grandis, Ficus religiosa, Annona squamosa, Albizia amara, Tamarindus indica, Cardia sebestena, Polyalthia longifolia, Syzygium cumini.* Fe2+ causes the production of oxyradicals and lipid peroxidation, minimizing its concentration give protection against oxidative damage (Table 2). Similar result was reported by Krishnaveni et.al for *Ficus religiosa,*[9] *Albizia amara.*[10]

Table 3: Showing results of Secondary metabolites and Antioxidant activities

Name of plants	Total Phenolics (mg/g)	Flavonoids (mg/g)	Reducing power assay (mg/g)	Nitric oxide Assay (mg/g)	Phosphomolybd Enum assay (mg/g)	Metal chelating activity (mg/g)
Peltophorum acutifolium	7.16	5.60	7.06	12.48	8.77	6.18
Tectona grandis	5.45	5.61	6.56	9.76	10.2	6.18
Tamarindus indica	11.30	3.76	8.60	13.08	6.08	7.22
Azadirachta indica	8.56	5.70	8.12	12.24	8.56	3.64
Annona squamosa	7.32	3.12	8.08	4.04	8.24	1.8
Madhuca longifolia	3.80	5.08	2.12	4.12	6.12	1.94
Psidium guajava	8.84	6.08	4.49	7.60	5.93	7.92
Cardia sebestena	6.01	5.16	3.32	7.96	6.60	3.74
Mangifera indica	8.97	3.88	8.06	6.68	10.72	4.46
Pongamia pinnata	6.96	4.96	6.52	10.92	3.36	7.56
Polyalthia longifolia	7.52	5.44	4.56	10.04	9.32	2.70

(Location 3 near 10th kilometer)

Total phenolics

Total phenolics was high with *Tamarindus indica, Psidium guajava, Azadirachta indica*, *Mangifera indica* but it was moderate with *Cardia sebestena, Polyalthia longifolia, Tectona grandis, Pongamia pinnata, Peltophorum acutifolium, Annona squamosa, Madhuca longifolia* (Table 3)

Flavonoids

The flavonoid content was moderate with all the plants studied (Table 3). Similar result was reported by Krishnaveni et.al for *Tamarindus indica.*[12]

Reducing power activity

The reducing power activity was high with *Tamarindus indica, Mangifera indica, Azadirachta indica, Annona squamosa.* Moderate amount was observed with rest of the plants studied (Table 3).

Nitric oxide scavenging assay

Plants such as *Tamarindus indica, Peltophorum acutifolium, Azadirachta indica, Pongamia pinnata, Polyalthia longifolia* showed higher scavenging activity. Moderate activity was observed with rest of the plants studied (Table 3).

Total antioxidant activity

Mangifera indica, Tectona grandis, Peltophorum acutifolium, Polyalthia longifolia showed higher level of total antioxidant activity. Remaining plants showed moderate amount of antioxidant activity. Phosphoolybdenum assay detects antioxidants such as ascorbic acid, some phenolics, tocopherols and carotenoids (Table 3).

Metal chelating activity

Plants like *Annona squamosa, Madhuca longifolia* showed very low metal chelating activity while it was high for *Psidium guajava, Pongamia pinnata, Tamarindus indica.* The remaining plants showed moderate amount. Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydro peroxide decomposition reactions via Fenton chemistry.[13] The transition metal, iron is capable of generating free radicals from peroxides. Presence of reducers causes the conversion of the fe3+/ferricyanide complex to the ferrous form which serves as a significant indicator of its antioxidant capacity (Table 3).

Percent inhibition of metal chelating activity

The percent inhibition of metal chelating activity is depicted in Table 4, 5 and Table 6.

Table 4 depicts the results of percent inhibition of metal chelating activity of location 1. The activity was high with *Cardia sebestena, Annona squamosa, Madhuca longifolia, Tamarindus indica.* All the other plants showed moderate amount of inhibition.

Table 5 explains the results of percent inhibition of metal chelating activity of location 2. The activity was high with *Syzygium cumini, Azadirachta indica, Ficus religiosa, Morinda tinctoria.* Moderate amount was observed with remaining plants.

Table 6 explains the results of percent inhibition of metal chelating activity of location 3. The activity was high with *Annona squamosa.* All the other plants showed moderate amount of inhibition.

Table 4: Showing results of % Inhibition of MCA

Name of plants	% Inhibition of MCA
Madhuca longifolia	78.69
Cardia sebestena	87.31
Psidium guajava	51.67
Tectona grandis	53.08
Mangifera indica	16.35
Ficus religiosa	54.0
Peltophorum acutifolium	11.26
Annona squamosa	87.18
Manilkara zapota	16.60
Causarina equistifolia	16.27
Tamarindus indica	77.37
Azadirachta indica	33.47

(Location 1 between 1st and 2nd kilometer), MCA- Metal Chelating Activity

Table 5: Showing results of % Inhibition of MCA

Name of plants	% Inhibition of MCA
Polyalthia longifolia	57.84
Syzygium cumini	89.79
Tamarindus indica	34.47
Ficus religiosa	84.11
Ficus benghalensis	54.43
Albizia saman	22.45
Pongamia pinnata	67.22
Albizia amara	44.57
Azadirachta indica	89.69
Tectona grandis	77.16
Carica papaya	32.97
Morinda tinctoria	81.55

(Location 2 near 6th kilometer), MCA- Metal Chelating Activity.

Table 6: Showing results of % Inhibition of MCA

Name Of Plants	% Inhibition of MCA
Peltophorum acutifolium	64.65
Tectona grandis	63.37
Tamarindus indica	52.52
Azadirachta indica	23.94
Annona squamosa	79.38
Madhuca longifolia	53.40
Psidium guajava	44.92
Cardia sebestena	21.86
Mangifera indica	58.98
Pongamia pinnata	48.29
Polyalthia longifolia	51.58

(Location 3 near 10th kilometer), MCA- Metal Chelating Activity

CONCLUSION

The plants studied showed good antioxidant activity. There are variations within each antioxidants assessed, this might be due to the soil nutrients in that area and also on the amount of stress that each tree exposes, the duration of exposure.

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