Research Article

Assay of Secondary Metabolites, Free Radical Scavenging Capacity of Plants Collected near Dalmia, Salem, Tamil Nadu, India.

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ABSTRACT

Oxygen is very important part for the survival of all living things. So, an attempt has been taken to study the plants and also its beneficial aspects. The plants have been selected from the study area i.e *Azadirachta indica, Tectona grandis, Tamarindus indica, Casuarina equisetifolia*, *Ficus religiosa, Ficus benghalensis*, *Morinda pubescens*, *Annona squamosa*, *Wrightia tinctoria*, *Millettia pinnata*, *Mangifera indica*, *Syzygium cumini*, *Ailanthus excelsa*, *Melia composite*, *Polyalthia longifolia.* All the plants selected have been studied for their secondary metabolites, antioxidant activities. *Annona squamosa, Mangifera indica, Wrightia tinctoria, Ficus benghalensis* showed highest phenolics. Likewise, flavonoids were high with *Polyalthia longifolia, Mangifera indica, Melia composite.* The antioxidant activity was good in *Ficus religiosa, Polyalthia longifolia showed higher reducing power activity, metal chelating activity, Millettia pinnata* had significant nitric oxide scavenging activity, *Wrightia tinctoria* showed good hydrogen peroxide scavenging activity.

Keywords: Dalmia, Flavonoids, Phenolics, Plants, Scavenging activity.

INTRODUCTION

Plants are studied as they have huge pharmacological activities, economic importance, lower toxicity. Free radicals have unpaired electron-unstable in nature and able to capture electrons from other substances in order to neutralize themselves.

Even though, the initial stabilization occurs, at the same time it generates another in the process, which creates a chain reaction and thousands of free radical reactions can occur within a few seconds on the primary reaction. Plants are studied for their antioxidant, free radical scavenging properties as they are rich in medicinal properties. Antioxidants are chemicals that block the activity of other chemicals and are capable of stabilizing, or deactivating, free radicals before they attack cells, significant in preventing lipid peroxidation, chelating metal ions etc. The antioxidant capacity of phenolic compounds are due to its redox properties, helps to adsorb and neutralize free radicals, quench singlet, triplet oxygen.[1] Hence, the secondary metabolites and antioxidants were studied for the plants selected from the experimental site, they are as follows: *Azadirachta indica, Tectona grandis, Tamarindus indica, Casuarina equisetifolia*, Ficus religiosa, *Ficus benghalensis*, *Morinda pubescens*, *Annona squamosa*, *Wrightia tinctoria*, *Millettia pinnata*, *Mangifera indica*, *Syzygium cumini*, *Ailanthus excelsa*, *Melia composite*, *Polyalthia longifolia*.

MATERIALS AND METHODS

Leaf Sample Collection

For the present study, fresh leaves from each plants were collected from the experimental site near Dalmia magnesite, Salem, Tamil Nadu, India during the month of December 2014-January 2015. Common plants identified were selected from the study areas.

All the selected plants were identified by Dr. A. Balasubramanian and also by comparing with book named Dictionary of Medicinal Plants written by Dr. A. Balasubramanian, Executive Director, ABS Botanical garden, Salem, Tamil Nadu, India.

Extract Preparation

Fresh leaves were used according to the standard prescribed methods adopted. 100mg of fresh leaves was ground to a paste in a mortar and pestle using 1ml of distilled water. 0.1ml of clear extract was used for the each experiment assessed.

Quantitative Assays

Secondary metabolites

Total Phenolics

To 0.1ml of extract, added 2.8ml of 10% sodium carbonate, 0.1ml of 2N folin ciocalteu phenol reagent. After 40 minutes incubation, the color developed was read at 725nm using UV-Spectrophotometer. Total phenolic content calculated was expressed as mg of gallic acid equivalents/g of sample using standard calibration curve constructed.[2]

Total Flavonoids

0.1ml of plant extract was mixed with 1.5ml of methanol, 0.1ml of 10% aluminium chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. It remained at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415nm with UV/Visible spectrophotometer. Total flavonoid content was

calculated from a calibration curve obtained using quercetin as a standard.[3,4]

Assay of Antioxidants

Total antioxidant activity by phosphomolybdenum complex method

0.1ml of extract was mixed with 4ml of reagent solution containing 0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate. The contents in the tube was incubated in a water bath at 95°C for 90minutes.

After the samples had been cooled to RT, the absorbance of mixture was measured at 695nm using UV Visible spectrophotometer. Standard calibration plot was prepared using ascorbic acid.[5]

Reducing Power Assay

0.1ml of plant extract was mixed with 1ml of phosphate buffer (0.2M, pH6.6) and 1% Potassium ferricyanide, mixed well and incubated at 50°C for 20minutes. After incubation, 1ml TCA (10%) was added to stop the reaction. It was centrifuged at 3000rpm for 10minutes. To 1.5ml of supernatant, 1.5ml of distilled water, 0.1ml ferric chloride (0.1%) was mixed and incubated for 10 minutes, the absorbance was read at 700nm using UV Visible spectrophotometer. Standard calibration curve was plotted using ascorbic acid.[6]

Nitric Oxide Scavenging Activity

To 0.1ml of extract, 2ml of 10mM sodium nitroprusside, 0.5ml of phosphate buffered saline 1M was added and then incubated at 25°C for 150minutes. After incubation, 1ml of sulphanilic acid reagent (0.33%), 1ml of naphthylene diamine dihydrochloride (1%) was added, mixed and allowed to stand for 30minutes. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions can be estimated by the use of Griess illsovery reaction at 540nm.[7,8] Quercetin was used as standard.

Metal Ion Chelating Activity

To 0.1ml of extract add 2.16ml of distilled water, 80µl of 2mM ferric chloride. The reaction was initiated by the addition of 160µl of ferrozine. The contents in the tube was mixed well and allowed to stand for 10 minutes at room temperature. After incubation the absorbance was read at 562nm using UV Visible spectrophotometer. The calibration plot was drawn using ascorbic acid as standard.[9]

Hydrogen Peroxide Scavenging Activity

To 0.1ml of extract add 0.6ml hydrogen peroxide solution (0.6ml, 40mM). The absorbance of hydrogen peroxide at 230nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. A solution of hydrogen peroxide (40 mM) was prepared in phosphate solution. The percentage of hydrogen peroxide scavenging activity exhibited by the extracts and standard compounds was calculated as follows:

$$\%\ Scavenged\ [H_2O_2] = \left[\frac{A_o - A_1}{A_o}\right] \times 100$$

where A_o was the absorbance of the control and A_1 was the absorbance in the presence of the sample of extract.[3,10]

Statistical Analysis

Each experiment was carried out in triplicate and the results are given as the Mean ± Standard deviation. The Mean and Standard deviation (S) was calculated by using the following formula:

$$Mean = \frac{Sum\ of\ x\ values}{n(Number\ of\ values)}$$

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

RESULTS AND DISCUSSION

The results of secondary metabolite analysis are shown in Table 1 and the results of antioxidant are shown in Table 2.

Table 1: Secondary Metabolites

S. No	Name of the plants	Total phenolics (mg/g)	Total Flavonoids (mg/g)
1.	*Azadirachta indica*	2.00 ± 0.17	4.26 ± 1.78
2.	*Tectona grandis*	4.66 ± 2.65	5.53 ± 2.88
3.	*Tamarindus indica*	7.23 ± 2.36	2.20 ± 0.00
4.	*Casuarina equisetifolia*	6.30 ± 2.59	4.80 ± 0.69
5.	*Ficus religiosa*	5.40 ± 2.59	3.86 ± 1.44
6.	*Ficus benghalensis*	7.26 ± 0.63	3.66 ± 1.27
7.	*Morinda pubescens*	6.90 ± 0.34	3.20 ± 0.00
8.	*Annona squamosa*	7.66 ± 0.05	4.70 ± 0.69
9.	*Wrightia tinctoria*	7.31 ± 1.06	2.60 ± 0.34
10.	*Millettia pinnata*	4.10 ± 1.55	4.13 ± 2.02
11.	*Mangifera indica*	7.50 ± 0.51	6.20 ± 0.34
12.	*Syzygium cumini*	8.20 ± 0.17	5.03 ± 2.19
13.	*Ailanthus excelsa*	6.06 ± 1.96	3.76 ± 0.75
14	*Melia composita*	4.46 ± 2.71	6.03 ± 3.23
15	*Polyalthia longifolia*	6.45 ± 1.81	6.86 ± 0.40

Values are Mean ± SD for three experiments

Total Phenolics

The phenolic content was high in *Syzygium cumini* 8.20±0.17, *Annona squamosa* 7.66±0.05, *Mangifera indica* 7.50±0.51, *Wrightia tinctoria* 7.31±1.06, *Ficus benghalensis* 7.26±0.63, *Tamarindus indica* 7.23±2.36, *Morinda pubescens* 6.90±0.34, *Polyalthia longifolia* 6.45±1.81, *Casuarina equisetifolia* 6.30±2.59, *Ailanthus excels* 6.06±1.96. Moderate amount was observed with *Ficus religiosa* 5.40±2.59, *Tectona grandis* 4.66±2.65, *Melia composite* 4.46±2.71, *Millettia pinnata* 4.10±1.55.

Low level was observed with *Azadirachta indica* 2.00±0.17.

Similar result was reported by Krishnaveni for *Annona squamosa,*[15] *Azadirachta indica,*[14] with respect to phenolics. (Table 1)

Total Flavonoids

The flavonoid content was high in *Polyalthia longifolia* 6.86±0.40, *Mangifera indica* 6.20±0.34, *Melia composite* 6.03±3.23, *Tectona grandis* 5.53±2.88, *Syzygium cumini* 5.03±2.19, *Casuarina equisetifolia* 4.80±0.69, *Annona squamosa* 4.70±0.69, *Azadirachta indica* 4.26±1.78, *Millettia pinnata* 4.13±2.02. Moderate amount was observed in *Ficus religiosa* 3.86±1.44, *Ailanthus excelsa* 3.76±0.75, *Ficus benghalensis* 3.66±1.27, *Morinda pubescens* 3.20±0.00, *Tamarindus indica* 2.20±0.00.

Similar result was reported by Krishnaveni for *Polyalthia longifolia,*[11] *Tectona grandis,*[15] *Ficus religiosa,*[15,19] *Ficus benghalensis,*[16] *Casuarina equisetifolia,*[17] *Syzygium cumini,*[19] with respect to flavonoids. (Table 1)

Table 2: Antioxidant activities of selected plants

S. No	Name of the plants	Phosphomolybdenum assay (mg/g)	Reducing power assay (mg/g)	Nitric oxide scavenging assay (mg/g)	Metal chelating activity (mg/g)	Hydrogen peroxide scavenging activity (%)
1.	*Azadirachta indica*	0.88 ± 0.11	2.01 ± 0.49	2.43 ± 0.20	3.26 ± 0.40	2.87 ± 0.00
2.	*Tectona grandis*	1.58 ± 0.54	2.80 ± 0.34	4.08 ± 0.11	3.53 ± 0.63	3.80 ± 0.45
3.	*Tamarindus indica*	0.98 ± 0.20	2.95 ± 0.08	3.41 ± 0.57	2.90 ± 0.34	3.00 ± 0.48
4.	*Casuarina equisetifolia*	2.91 ± 1.35	3.06 ± 1.01	2.75 ± 0.25	2.10 ± 0.69	3.00 ± 0.48
5.	*Ficus religiosa*	4.73 ± 2.62	2.08 ± 0.37	4.01 ± 1.09	2.10 ± 0.69	3.00 ± 0.48
6.	*Ficus benghalensis*	2.91 ± 0.57	3.28 ± 1.32	5.41 ± 1.70	4.33 ± 1.50	3.11 ± 0.97
7.	*Morinda pubescens*	3.28 ± 1.24	3.01 ± 0.14	2.30 ± 0.00	3.63 ± 0.98	2.44 ± 0.00
8.	*Annona squamosa*	2.98 ± 1.50	2.45 ± 0.17	2.86 ± 0.66	3.13 ± 0.28	3.52 ± 0.93
9.	*Wrightia tinctoria*	3.16 ± 0.49	2.50 ± 0.08	3.71 ± 0.83	3.76 ± 0.92	3.93 ± 0.21
10.	*Millettia pinnata*	2.76 ± 0.31	2.71 ± 1.09	5.08 ± 0.80	4.40 ± 1.55	2.54 ± 1.31
11.	*Mangifera indica*	3.63 ± 0.72	3.48 ± 0.89	2.48 ± 0.11	4.43 ± 1.15	3.41 ± 0.46
12.	*Syzygium cumini*	3.78 ± 0.80	3.41 ± 0.05	2.36 ± 0.14	3.86 ± 0.75	2.87 ± 0.00
13.	*Ailanthus excelsa*	4.46 ± 1.01	1.81 ± 0.31	2.63 ± 0.28	3.40 ± 0.69	2.72 ± 0.24
14	*Melia composita*	2.13 ± 0.37	3.01 ± 1.01	2.61 ± 0.05	3.70 ± 1.03	2.57 ± 0.50
15	*Polyalthia longifolia*	4.28 ± 0.63	3.65 ± 1.03	3.35 ± 0.17	4.56 ± 1.27	3.41 ± 0.46

Values are Mean ± SD for three experiments

Total Antioxidant Assay

The total antioxidant activity was measured in terms of phosphomolybdenum assay. The antioxidant activity was high in *Ficus religiosa* 4.73±2.62, *Ailanthus excelsa* 4.46±1.01, *Polyalthia longifolia* 4.28±0.63.

The antioxidant activity was moderate in *Syzygium cumini* 3.78±0.80, *Mangifera indica* 3.63±0.72, *Morinda pubescens* 3.28±1.24, *Wrightia tinctoria* 3.16±0.49, *Annona squamosa* 2.98±1.50, *Ficus benghalensis* 2.91±0.57, *Casuarina equisetifolia* 2.91±1.35, *Millettia pinnata* 2.76±0.31, *Melia composite* 2.13±0.37, *Tectona grandis* 1.58±0.54. Very low level of antioxidant activity was observed with *Tamarindus indica* 0.98±0.20, *Azadirachta indica* 0.88±0.11. Similar result was reported by Krishnaveni for *Tectona grandis,*[15] *Polyalthia longifolia,*[18] *Mangifera indica.*[19] (Table.2)

Reducing Power Assay

The reducing power activity was moderate in all the plants studied. The reducing power was given as follows: *Polyalthia longifolia* 3.65±1.03, *Mangifera indica* 3.48±0.89, *Syzygium cumini* 3.41±0.05, *Ficus benghalensis* 3.28±1.32, *Casuarina equisetifolia* 3.06±1.01, *Morinda pubescens* 3.01±0.14, *Melia composite* 3.01±1.01, *Tamarindus indica* 2.95±0.08, *Tectona grandis* 2.80±0.34, *Millettia pinnata* 2.71±1.09, *Wrightia tinctoria* 2.50±0.08, *Annona squamosa* 2.45±0.17, *Ficus religiosa* 2.08±0.37, *Azadirachta indica* 2.01±0.49, *Ailanthus excelsa* 1.81±0.31. Similar result was reported by Krishnaveni for *Azadirachta indica,*[11-13] *Ficus religiosa,*[12,13] *Tectona grandis,*[13] *Tamarindus indica,*[14,16] *Annona squamosa.*[16](Table 2)

Nitric Oxide Scavenging Activity

The nitric oxide scavenging activity was high with *Ficus benghalensis* 5.41±1.70, *Millettia pinnata* 5.08±0.80, *Tectona grandis* 4.08±0.11, *Ficus religiosa* 4.01±1.09. Moderate amount of nitric oxide scavenging activity was observed with *Wrightia tinctoria* 3.71±0.83, *Tamarindus indica* 3.41±0.57, *Polyalthia longifolia* 3.35±0.17, *Annona squamosa* 2.86±0.66, *Casuarina equisetifolia* 2.75±0.25, *Ailanthus excelsa* 2.63±0.28, *Melia composite* 2.61±0.05, *Mangifera indica* 2.48±0.11, *Azadirachta indica* 2.43±0.20, *Syzygium cumini* 2.36±0.14, *Morinda pubescens* 2.30±0.00. (Table 2)

Metal Chelating Activity

The metal ion chelating activity was moderate in all the plants studied. The ion chelating activity of the plants

studied are as follows: *Polyalthia longifolia* 4.56±1.27, *Mangifera indica* 4.43±1.15, *Millettia pinnata* 4.40±1.55, *Ficus benghalensis* 4.33±1.50, *Syzygium cumini* 3.86±0.75, *Wrightia tinctoria* 3.76±0.92, *Melia composite* 3.70±1.03, *Morinda pubescens* 3.63±0.98, *Tectona grandis* 3.53±0.63, *Ailanthus excelsa* 3.40±0.69, *Azadirachta indica* 3.26±0.40, *Annona squamosa* 3.13±0.28, *Tamarindus indica* 2.90±0.34, *Casuarina equisetifolia* 2.10±0.69, *Ficus religiosa* 2.10±0.69. Similar result was reported by Krishnaveni for *Mangifera indica,*[12] *Ficus benghalensis,*[12] *Tamarindus indica,*[13,16] *Tectona grandis,*[16] *Azadirachta indica,*[17] *Ficus religiosa,*[17] *Morinda pubescens,*[20] *Syzygium cumini.*[20] (Table 2)

Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity was found to be moderate for all the plants studied. The results are given in percentage inhibition. *Wrightia tinctoria* 3.93±0.21, *Tectona grandis* 3.80±0.45, *Annona squamosa* 3.52±0.93, *Mangifera indica* 3.41±0.46, *Polyalthia longifolia* 3.41±0.46, *Ficus benghalensis* 3.11±0.97, *Tamarindus indica* 3.00±0.48, *Casuarina equisetifolia* 3.00±0.48, *Ficus religiosa* 3.00±0.48, *Syzygium cumini* 2.87±0.00, *Azadirachta indica* 2.87±0.00, *Ailanthus excelsa* 2.72±0.24, *Melia composite* 2.57±0.50, *Millettia pinnata* 2.54±1.31, *Morinda pubescens* 2.44±0.00. (Table 2)

CONCLUSION

From the present study, it is concluded, that plants are endowed with secondary metabolites and antioxidants which might play a major role in scavenging free radicals induced by stress.

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