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Research Article

Analysis of Free Radical Scavenging Activity of Plants Located Near Magnesite Mines, Salem, Tamil Nadu, India.

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ABSTRACT
Antioxidants defend against free radicals and free radical induced damage. The plants such as *Azadirachta indica*, *Santalum album*, *Casuarina equisetifolia*, *Annona squamosa*, *Wrightia tinctoria*, *Ailanthus excelsa*, *Tectona grandis*, *Millettia pinnata*, *Tamarindus indica*, *Mangifera indica*, *Syzygium cumini*, *Morinda pubescens*, *Albizia amara*, *Prosopis juliflora*, *Ficus religiosa* were selected for the present study. Higher phenolics, flavonoid, content was observed in most of the plants studied. *Tectona grandis* showed highest metal chelating activity. Whereas, total antioxidant activity, reducing power activity, nitric oxide scavenging activity, hydrogen peroxide scavenging activity was found to be moderate with all the plants studied from the experimental site.

Key words: Antioxidants, Free radicals, plants, Scavengers.

INTRODUCTION

Defence mechanisms to oxidative damages are exerted by the action of various antioxidants. Hence, it is essential to study the antioxidant status of plants located in the surroundings where we live, as natural antioxidants are multifunctional. Multifunctional nature of antioxidants requires various tests to be performed in order to have an insight in to it. Hence, the present study was designed and secondary metabolites, antioxidant activities are measured for the plants selected from the experimental site. The following plants were collected from the study area: *Azadirachta indica*, *Santalum album*, *Casuarina equisetifolia*, *Annona squamosa*, *Wrightia tinctoria*, *Ailanthus excelsa*, *Tectona grandis*, *Millettia pinnata*, *Tamarindus indica*, *Mangifera indica*, *Syzygium cumini*, *Morinda pubescens*, *Albizia amara*, *Prosopis juliflora*, *Ficus religiosa*.

MATERIALS AND METHODS

Leaf sample collection

For the present study, fresh leaves from each plant was collected from the experimental site near Magnesite mines, Salem, Tamil Nadu, India during the month of December 2014-January 2015. Common plants identified were selected from the study area. 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 reagent. After 40minutes incubation, the color developed was read at 725nm using UV- Spectrophotometer. Total phenolic contents calculated was expressed as mg of Gallic acid equivalents/g of sample using standard calibration curve constructed.[1]

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. All the tubes were allowed to remain at room temperature for 30minutes, 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.[2,3]

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 tubes were 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.[4]

Reducing power assay

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0.1ml of plant extract was mixed with 1ml of Phosphate buffer (0.2M, pH6.6) and 1% Potassium ferricyanide, shaken well and incubated at 50°C for 20minutes. After incubation, 1ml of 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 and 0.1ml ferric chloride (0.1%) was mixed and incubated for 10minutes, the absorbance was read at 700nm using UV Visible spectrophotometer. Standard calibration curve was plotted using ascorbic acid.[5]

Nitric oxide scavenging activity

To 0.1ml of extract, 2ml of 10mM Sodium nitroprusside, 0.5ml of 1M Phosphate buffered saline 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 and mixed, 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 which can be estimated by the use of Griess illsovery reaction at 540nm.[6,7] Quercetin was used as a standard.

Ferrous 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 10minutes 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.[8]

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 by the extracts and standard compounds was calculated as follows: % Scavenged $[H_2O_2]$ = [(Ao − A1)/Ao] × 100 where Ao was the absorbance of the control and A1 was the absorbance in the presence of the sample of extract.[2, 9]

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 = Sum of x values / n (Number of values), $s = \frac{\sqrt{\sum(X-M)^2}}{n-1}$.

RESULTS AND DISCUSSION

Table.1.shows the results of secondary metabolites and Table.2. shows the results of antioxidant activities.

Total phenolics

The total phenolics studied for the plants selected were shown as follows: *Azadirachta indica* 2.13±0.80, *Santalum album* 5.40±3.63, *Casuarina equisetifolia* 3.70±2.94, *Annona squamosa* 5.63±2.71, *Wrightia tinctoria* 5.76±2.48, *Ailanthus excelsa* 5.50±2.07, *Tectona grandis* 6.06±1.09, *Millettia pinnata* 4.76±1.96, *Tamarindus indica* 3.53±2.02 , *Mangifera indica* 5.13±0.28, *Syzygium cumini* 4.15±2.16, *Morinda pubescens* 4.06±1.96, *Albizia amara* 4.26±2.82, *Prosopis juliflora* 5.23±2.28, *Ficus religiosa* 5.10±0.86. The results were found to be similar with reports of Krishnaveni et.al for *Syzygium cumini*,[15] *Albizia spe.*[19]

Table1: Secondary metabolites of plants

S.No	Name of the plants	Total phenolics (mg/g)	Total flavonoids (mg/g)
1.	*Azadirachta indica*	2.13±0.80	5.33±2.54
2.	*Santalum album*	5.40±3.63	5.03±0.28
3.	*Casuarina equisetifolia*	3.70±2.94	4.53±0.46
4.	*Annona squamosa*	5.63±2.71	4.43±1.84
5.	*Wrightia tinctoria*	5.76±2.48	5.56±1.78
6.	*Ailanthus excelsa*	5.50±2.07	5.73±2.19
7.	*Tectona grandis*	6.06±1.09	4.36±1.78
8.	*Millettia pinnata*	4.76±1.96	4.90±1.38
9.	*Tamarindus indica*	3.53±2.02	5.93±2.36
10.	*Mangifera indica*	5.13±0.28	6.60±0.51
11.	*Syzygium cumini*	4.15±2.16	3.23±0.98
12.	*Morinda pubescens*	4.06±1.96	4.70±2.07
13.	*Albizia amara*	4.26±2.82	6.36±2.48
14	*Prosopis juliflora*	5.23±2.28	4.43±1.15
15	*Ficus religiosa*	5.10±0.86	4.83±0.11

Values are Mean ± SD for three experiments

Total flavonoids

The calculated values for total flavonoids are given below: *Azadirachta indica* 5.33±2.54, *Santalum album* 5.03±0.28, *Casuarina equisetifolia* 4.53±0.46, *Annona squamosa* 4.43±1.84, *Wrightia tinctoria* 5.56±1.78, *Ailanthus excelsa* 5.73±2.19, *Tectona grandis* 4.36±1.78, *Millettia pinnata* 4.90±1.38, *Tamarindus indica* 5.93±2.36, *Mangifera indica* 6.60±0.51, *Syzygium cumini* 3.23±0.98, *Morinda pubescens* 4.70±2.07, *Albizia amara* 6.36±2.48, *Prosopis juliflora* 4.43±1.15, *Ficus religiosa* 4.83±0.11. Similar result was reported by Krishnaveni et.al for *Azadirachta indica*,[12] *Albizia spe.*,[12] *Tectona grandis*,[15] *Azadirachta indica.*[15,16]

Total antioxidant assay

The total antioxidant activity was measured in terms of phosphomolybdenum assay. All the plants showed moderate amount of antioxidant activity. *Azadirachta indica* 1.40±0.00, *Santalum album* 2.93±1.24, *Casuarina equisetifolia* 1.83±0.20, *Annona squamosa* 3.50±0.34, *Wrightia tinctoria* 3.23±1.32, *Ailanthus excelsa* 2.18±0.28, *Tectona grandis* 2.28±0.37, *Millettia pinnata* 3.38±0.37, *Tamarindus indica* 3.40±1.12, *Mangifera indica* 2.50±0.34, *Syzygium cumini* 3.85±0.86, *Morinda pubescens* 3.23±1.32, *Albizia amara* 1.71±0.14, *Prosopis juliflora* 3.28±1.32, *Ficus religiosa* 3.96±0.83. Similar result was reported by Krishnaveni et.al for *Albizia spe.*[13]

Reducing power assay

Reducing power activity obtained for the selected plants

Table2: Antioxidant activities of selected plants

S.No	Name of the plants	Total antioxidant activity (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*	1.40±0.00	1.81±0.49	3.83±1.58	3.86±0.40	2.39±0.00
2.	*Santalum album*	2.93±1.24	2.15±0.17	3.90±0.95	4.66±0.23	1.61±0.00
3.	*Casuarina equisetifolia*	1.83±0.20	2.65±0.60	4.55±0.34	3.40±1.03	2.26±0.21
4.	*Annona squamosa*	3.50±0.34	2.81±0.57	3.06±0.31	3.83±0.80	2.38±0.67
5.	*Wrightia tinctoria*	3.23±1.32	2.68±0.89	3.93±0.80	4.46±0.05	2.13±0.45
6.	*Ailanthus excelsa*	2.18±0.28	2.83±0.11	3.80±1.29	6.66±1.09	2.01±0.00
7.	*Tectona grandis*	2.28±0.37	3.45±0.51	2.73±0.46	7.43±1.67	2.39±0.00
8.	*Millettia pinnata*	3.38±0.37	3.23±0.20	2.00±0.00	2.80±1.55	2.26±0.21
9.	*Tamarindus indica*	3.40±1.12	2.81±0.49	4.83±2.45	3.33±0.63	2.13±0.45
10.	*Mangifera indica*	2.50±0.34	2.93±0.54	4.66±2.13	0.85±0.60	2.26±0.21
11.	*Syzygium cumini*	3.85±0.86	3.38±0.46	3.61±0.23	3.06±0.92	2.26±0.21
12.	*Morinda pubescens*	3.23±1.32	3.18±0.28	3.50±0.43	2.13±0.46	2.39±0.00
13.	*Albizia amara*	1.71±0.14	3.13±0.02	5.18±0.28	2.80±1.55	1.88±0.21
14	*Prosopis juliflora*	3.28±1.32	3.76±0.49	3.06±0.92	4.00±0.34	2.39±0.00
15	*Ficus religiosa*	3.96±0.83	1.95±0.60	3.31±0.83	4.26±0.57	2.51±0.43

Values are Mean ± SD for three experiments

are shown as follows: *Azadirachta indica* 1.81±0.49, *Santalum album* 2.15±0.17, *Casuarina equisetifolia* 2.65±0.60, *Annona squamosa* 2.81±0.57,*Wrightia tinctoria* 2.68±0.89, *Ailanthus excelsa* 2.83±0.11, *Tectona grandis* 3.45±0.51,*Millettia pinnata* 3.23±0.20, *Tamarindus indica* 2.81±0.49, *Mangifera indica* 2.93±0.54, *Syzygium cumini* 3.38±0.46, *Morinda pubescens* 3.18±0.28, *Albizia amara* 3.13±0.02, *Prosopis juliflora* 3.76±0.49, *Ficus religiosa* 1.95±0.60. The reducing power activity was found to be moderate with all the plants studied. Similar study was reported by Krishnaveni et.al for *Casuarina equisetifolia*,[10]*Prosopis juliflora*,[11] *Tectona grandis*,[12] *Annona squamosa*,[14] *Mangifera indica*.[18]

Nitric oxide scavenging assay

Nitric oxide scavenging activity of studied plants are as follows: *Azadirachta indica* 3.83±1.58, *Santalum album* 3.90±0.95, *Casuarina equisetifolia* 4.55±0.34, *Annona squamosa* 3.06±0.31, *Wrightia tinctoria* 3.93±0.80, *Ailanthus excelsa* 3.80±1.29, *Tectona grandis* 2.73±0.46, *Millettia pinnata* 2.00±0.00, *Tamarindus indica* 4.83±2.45, *Mangifera indica* 4.66±2.13, *Syzygium cumini* 3.61±0.23, *Morinda pubescens* 3.50±0.43, *Albizia amara* 5.18±0.28, *Prosopis juliflora* 3.06±0.92, *Ficus religiosa* 3.31±0.83. Among the plants studied, *Tamarindus indica* showed higher nitric oxide scavenging activity, while, all the other plants showed moderate level of nitric oxide scavenging activity. Similar result was reported by Krishnaveni et.al for *Annona squamosa*.[14]

Metal chelating activity

The metal chelating activity of the experimented plants studied are depicted below: *Azadirachta indica* 3.86±0.40, *Santalum album* 4.66±0.23, *Casuarina equisetifolia* 3.40±1.03, *Annona squamosa* 3.83±0.80, *Wrightia tinctoria* 4.46±0.05, *Ailanthus excelsa* 6.66±1.09, *Tectona grandis* 7.43±1.67, *Millettia pinnata* 2.80±1.55, *Tamarindus indica* 3.33±0.63, *Mangifera indica* 0.85±0.60, *Syzygium cumini* 3.06±0.92, *Morinda pubescens* 2.13±0.46, *Albizia amara* 2.80±1.55, *Prosopis juliflora* 4.00±0.34, *Ficus religiosa* 4.26±0.57. The metal chelating activity was higher for *Tectona grandis, Ailanthus excelsa.* Rest of the plants showed moderate amount of metal chelating activity. Similar result was reported by Krishnaveni et.al for *Tectona grandis*,[13] *Casuarina equisetifolia*,[14] *Tectona grandis*,[15] *Azadirachta indica*,[15] *Ficus religiosa*.[17]

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity calculated are as follows: *Azadirachta indica* 2.39±0.00, *Santalum album* 1.61±0.00, *Casuarina equisetifolia* 2.26±0.21, *Annona squamosa* 2.38±0.67, *Wrightia tinctoria* 2.13±0.45, *Ailanthus excelsa* 2.01±0.00, *Tectona grandis* 2.39±0.00, *Millettia pinnata* 2.26±0.21, *Tamarindus indica* 2.13±0.45, *Mangifera indica* 2.26±0.21, *Syzygium cumini* 2.26±0.21, *Morinda pubescens* 2.39±0.00, *Albizia amara* 1.88±0.21, *Prosopis juliflora* 2.39±0.00, *Ficus religiosa* 2.51±0.43. The hydrogen peroxide scavenging activity was moderate for all the plants studied.

CONCLUSION

Findings of the present study revealed that, all plants possess sufficient secondary metabolites such as phenolics, flavonoids, which finds a major role in antioxidant activities and thus serve as a very good antioxidant useful in various pharmaceutical and other industries.

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