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Original Article

FREE RADICAL SCAVENGING ACTIVITY (*IN VITRO*) OF PLANTS COLLECTED NEAR CEMENT INDUSTRY

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ABSTRACT

Plants such as *Azadirachta indica*, *Millettia pinnata*, *Wrightia tinctoria*, *Eucalyptus tereticornis*, *Delonix elata*, *Albizia amara*, *Mangifera indica*, *Citrus aurantiaca*, *Psidium guajava*, *Holoptelea integrifolia*, *Delonix regia*, *Bambusa tulda*, *Morinda pubescens*, *Manilkara zapota*, *Tetelia* were selected for the experimental study of secondary metabolites, various antioxidant assays. The study shows that all plants contain phenolics and flavonoid in significant amount. The hydrogen peroxide activity was found to be high compared to other activities tested. All the other parameters were found to be moderate with most of the plants studied. The experiments performed show that high phenolics, flavonoid content of plants might be a significant factor in stimulating antioxidant activities, which suits its application in various pharmacological drugs.

Key words: Antioxidants, Free radicals, Plants, Scavenging activity.

INTRODUCTION

Phenolic compounds of plants have a very good antioxidant activity in order to protect cells from damage, hence antioxidants are otherwise called as metal chelators, hydrogen donars, reducing agents, singlet oxygen quenchers, free radical scavengers. Antioxidant rich plant extracts are neutraceuticals, helps to reduce the oxidative stress. Recently, there has been a growing interest towards the research on antioxidants. Hence, the present study was initiated, and the below listed plants were studied for their secondary metabolites, antioxidant activities studied through different assays. *Azadirachta indica*, *Millettia pinnata*, *Wrightia tinctoria*, *Eucalyptus tereticornis*, *Delonix elata*, *Albizia amara*, *Mangifera indica*, *Citrus aurantiaca*, *Psidium guajava*, *Holoptelea integrifolia*, *Delonix regia*, *Bambusa tulda*, *Morinda pubescens*, *Manilkara zapota*, *Tetelia*.

MATERIALS AND METHODS

Leaf sample collection

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For the present study, fresh leaves from each plants were collected from the experimental site near Cement factory, 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 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. It remained 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 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.[4]

Reducing power assay

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 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 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, 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.

Table.1. Secondary metabolites

S.No	Name of the plants	Total phenolics (mg/g)	Total Flavonoids (mg/g)
1.	*Azadirachta indica*	5.46±4.38	5.46±0.75
2.	*Millettia pinnata*	5.13±3.75	5.80±1.21
3.	*Wrightia tinctoria*	2.41±0.31	4.30±1.55
4.	*Eucalyptus tereticornis*	5.53±3.05	3.43±0.80
5.	*Delonix elata*	6.40±2.94	3.73±1.32
6.	*Albizia amara*	5.86±2.48	3.43±0.28
7.	*Mangifera indica*	4.20±2.85	4.70±1.38
8.	*Citrus aurantiaca*	5.96±2.13	4.56±0.40
9.	*Psidium guajava*	6.53±3.05	3.93±1.15
10.	*Holepetela integrifolia*	6.35±1.81	4.20±1.55
11.	*Delonix regia*	3.46±1.35	2.90±0.34
12.	*Bambusa tulda*	4.03±0.54	5.70±1.21
13.	*Morinda pubescens*	7.16±2.74	5.10±1.03
14	*Manilkara sapota*	7.36±2.56	4.53±1.50
15	*Tetelia*	6.80±4.24	4.36±1.78

Values are Mean ± SD for three experiments

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 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 a 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]

Table.2. Antioxidant activities

S.No	Name of the plants	Total antioxidant Activity (mg/g)	Reducing power assay (mg/g)	Nitricoxide scavenging assay (mg/g)	Metal chelating activity (mg/g)	Hydrogen peroxide scavenging activity (%)
1.	*Azadirachta indica*	2.63±0.11	3.48±1.06	4.40±2.07	3.40±0.17	2.24±0.52
2.	*Millettia pinnata*	5.30±0.00	2.68±1.06	2.11±0.14	4.96±0.23	3.39±0.35
3.	*Wrightia tinctoria*	3.66±0.40	2.23±0.54	2.36±0.28	4.63±0.80	8.79±5.03
4.	*Eucalyptus tereticornis*	2.06±0.40	3.15±0.43	2.93±0.63	3.50±0.17	3.10±0.65
5.	*Delonix elata*	3.13±0.46	5.46±0.14	2.73±0.46	3.63±0.11	8.65±5.27
6.	*Albizia amara*	3.06±0.05	3.65±0.43	3.46±0.05	5.23±0.63	2.61±0.85
7.	*Mangifera indica*	2.56±0.23	3.38±0.37	3.11±1.09	6.73±2.97	3.56±0.50
8.	*Citrus aurantiaca*	2.63±0.46	2.58±0.28	2.73±0.98	4.30±0.69	7.24±3.69
9.	*Psidium guajava*	4.76±0.92	2.60±1.38	4.70±1.81	6.10±2.42	9.10±6.92
10.	*Holepetela integrifolia*	3.50±0.51	2.71±0.31	4.01±2.74	3.86±0.05	11.68±4.27
11.	*Delonix regia*	5.43±0.89	2.90±1.38	2.88±0.28	3.96±0.40	3.78±0.79
12.	*Bambusa tulda*	4.53±2.02	3.61±1.52	2.43±0.02	4.96±1.09	7.21±3.55
13.	*Morinda pubescens*	3.76±1.44	4.11±1.61	3.50±0.17	4.30±0.34	8.23±4.43
14	*Manilkara sapota*	4.40±0.86	3.00±1.47	4.71±0.75	4.06±0.57	3.17±0.53
15	*Tetelia*	4.16±1.78	3.60±1.38	4.08±1.24	4.73±0.80	4.41±0.25

Values are Mean ± SD for three experiments

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

The results of secondary metabolites are exhibited in Table.1 and the results of antioxidant activities are shown in Table.2.

Total phenolics

The total phenolics was high with *Manilkara sapota* 7.36±2.56, *Morinda pubescens* 7.16±2.74, *Tetelia* 6.80±4.24, *Psidium guajava* 6.53±3.05, *Delonix elata* 6.40±2.94, *Holepetela integrifolia* 6.35±1.81. Moderate amount of phenolics was observed with *Citrus aurantiaca* 5.96±2.13, *Albizia amara* 5.86±2.48, *Eucalyptus tereticornis* 5.53±3.05, *Azadirachta indica* 5.46±4.38, *Millettia pinnata* 5.13±3.75, *Mangifera indica* 4.20±2.85, *Bambusa tulda* 4.03±0.54, *Wrightia tinctoria* 2.41±0.31. Similar result was reported by Krishnaveni et.al for *Mangifera indica,*[12,17] *Mangifera indica-phe,*[15] *Albizia sp.*[17]

Total flavonoids

All plants studied for flavonoids show moderate amount of flavonoids, each plants flavonoid content are shown as below: *Millettia pinnata* 5.80±1.21, *Bambusa tulda* 5.70±1.21, *Azadirachta indica* 5.46±0.75, *Morinda pubescens* 5.10±1.03, *Mangifera indica* 4.70±1.38, *Citrus aurantiaca* 4.56±0.40, *Manilkara sapota* 4.53±1.50, *Tetelia* 4.36±1.78, *Wrightia tinctoria* 4.30±1.55, *Holepetela integrifolia* 4.20±1.55, *Psidium guajava* 3.93±1.15, *Delonix elata* 3.73±1.32, *Albizia amara* 3.43±0.28, *Eucalyptus tereticornis* 3.43±0.80, *Delonix regia* 2.90±0.34. Similar result was reported by Krishnaveni et.al for *Azadirachta indica,*[12] *Mangifera indica,*[12] *Psidium guajava,*[14] *Azadirachta indica,*[15,16] *Morinda sp.*[15]

Total antioxidant activity

Total antioxidant activity was measured in terms of Phospomolybdenum assay. It was found to be moderate with all the plants studied. The antioxidant capacity of the plants assessed were as follows: *Delonix regia* 5.43±0.89, *Millettia pinnata* 5.30±0.00, *Psidium guajava* 4.76±0.92, *Bambusa tulda* 4.53±2.02, *Manilkara sapota* 4.40±0.86, *Tetelia* 4.16±1.78, *Morinda pubescens* 3.76±1.44, *Wrightia tinctoria* 3.66±0.40, *Holepetela integrifolia* 3.50±0.51, *Delonix elata* 3.13±0.46, *Albizia amara* 3.06±0.05, *Azadirachta indica* 2.63±0.11, *Citrus aurantiaca* 2.63±0.46, *Mangifera indica* 2.56±0.23, *Eucalyptus tereticornis* 2.06±0.40. Similar result was reported by Krishnaveni et.al for *Azadirachta indica.*[13]

Reducing power assay

The reducing power activity of the plants was found to be moderate for the plants analysed from the experimental site. The plants reducing power activities are exhibited below : *Delonix elata* 5.46±0.14, *Morinda pubescens* 4.11±1.61, *Albizia amara* 3.65±0.43, *Bambusa tulda* 3.61±1.52, *Tetelia* 3.60±1.38, *Azadirachta indica* 3.48±1.06, *Mangifera indica* 3.38±0.37, *Eucalyptus tereticornis* 3.15±0.43, *Manilkara sapota* 3.00±1.47, *Delonix regia* 2.90±1.38, *Holepetela integrifolia* 2.71±0.31, *Millettia pinnata* 2.68±1.06, *Psidium guajava* 2.60±1.38, *Citrus aurantiaca* 2.58±0.28, *Wrightia tinctoria* 2.23±0.54. Similar result was reported by Krishnaveni et.al for *pinnata* sp.[10] *Holepetela integrifolia,*[18] *Azadirachta indica,*[18] *Psidium guajava.*[18]

Nitric oxide scavenging assay

Nitric oxide scavenging activity observed was also moderate for the plants assessed from experimental site. The nitric oxide scavenging activity calculated was as follows: *Manilkara sapota* 4.71±0.75, *Psidium guajava* 4.70±1.81, *Azadirachta indica* 4.40±2.07, *Tetelia* 4.08±1.24, *Holepetela integrifolia* 4.01±2.74, *Morinda pubescens* 3.50±0.17, *Albizia amara* 3.46±0.05, *Mangifera indica* 3.11±1.09, *Eucalyptus tereticornis* 2.93±0.63, *Delonix regia* 2.88±0.28, *Delonix elata* 2.73±0.46, *Citrus aurantiaca* 2.73±0.98, *Bambusa tulda* 2.43±0.02, *Wrightia tinctoria* 2.36±0.28, *Millettia pinnata* 2.11±0.14. Similar result was reported by Krishnaveni et.al for *Psidium guajava.*[13]

Metal chelating activity

Metal chelating activity was expressed in mg/g. The obtained values are given as follows: *Azadirachta indica* 3.40±0.17, *Millettia pinnata* 4.96±0.23, *Wrightia tinctoria* 4.63±0.80, *Eucalyptus tereticornis* 3.50±0.17, *Delonix elata* 3.63±0.11, *Albizia amara* 5.23±0.63, *Mangifera indica* 6.73±2.97, *Citrus aurantiaca* 4.30±0.69, *Psidium guajava* 6.10±2.42, *Holoptelea integrifolia* 3.86±0.05, *Delonix regia* 3.96±0.40, *Bambusa tulda* 4.96±1.09, *Morinda pubescens* 4.30±0.34, *Manilkara zapota* 4.06±0.57, *Tetelia* 4.73±0.80. Similar result was reported by Krishnaveni et.al for *pinnata* sp.,[10] *Psidium guajava,*[11,12] *Azadirachta indica,*[15,17] *Albizia sp.*[19]

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity was expressed in %. The calculated percent activity was shown below: *Holepetela integrifolia* 11.68±4.27, *Psidium guajava* 9.10±6.92, *Wrightia tinctoria* 8.79±5.03, *Delonix elata* 8.65±5.27, *Morinda pubescens* 8.23±4.43, *Citrus aurantiaca* 7.24±3.69, *Bambusa tulda* 7.21±3.55, showed high level of hydrogen peroxide scavenging activity. The activity was moderate with *Tetelia* 4.41±0.25, *Delonix regia* 3.78±0.79, *Mangifera indica* 3.56±0.50, *Millettia pinnata* 3.39±0.35, *Manilkara sapota* 3.17±0.53, *Eucalyptus tereticornis* 3.10±0.65, *Azadirachta indica* 2.24±0.52.

CONCLUSION

This study shows that all plants were found to contain phenolics, flavonoids, which influences various antioxidant activities to scavenge hydrogen peroxide, chelate metal ions etc. The antioxidant property of plants contribute its use in any therapeutic preparations.

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