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Research Article

ANTIOXIDANT ACTIVITY OF SELECTED PLANTS

KRISHNAVENI.M *

Department of Biochemistry, Periyar University, Salem. Email: logasarvesh@gmail.com

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ABSTRACT

Objective: Plants are an important source of natural phytochemicals as it biosynthesise a vast number of antioxidant compounds which differ in concentration, chemical and physical properties. Secondary metabolites are compounds in specialized cells, not directly required for basic photosynthetic or respiratory metabolism but essential for plants survival in the environment. The present study aims to quantify the secondary metabolites in plants as well as to understand various antioxidant activities.

Methods: Standard methods were adopted for the analysis of secondary metabolites and antioxidant activities.

Results: Phosphomolybdenum and metal chelating activity was found to be high in *Delonix elata* (29.4mg/g, 7.60mg/g), whereas the nitric oxide scavenging activity was found to be high with *Opuntia ficus-indica* (9.7mg/g), likewise reducing power assay was moderate in *Alangium salvifolium* (3.75mg/g). Flavonoid content was high in *Tectona Grandis* (10.0mg/g).

Conclusion: From the obtained results, we can conclude, that presence of higher amount of flavonoids might play a major role in antioxidant activities of plants.

Keywords: Antioxidants; Aqueous extract; Flavonoids; Secondary metabolites.

INTRODUCTION

Life on earth requires oxygen for its existence. Oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent producing free radicals, leading to a chain reaction in a cell causing damage or death.

Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or poly-phenols. Plants are endowed with natural chemical constituents such as flavonoids, phenolic compounds which are responsible for free radical scavenging effect. Plants when exposed continuously to UV-B are altered in their metabolism generating free radicals. Flavonoids have indeed the capacity to absorb the most energetic solar wavelengths (*i.e.*, UV-B and UV-A), inhibit the generation of reactive oxygen species and then quench reactive oxygen species once they are formed [1]. In response to excess light stress, the biosynthesis of antioxidant flavonoid is almost exclusively enhanced [2-5].

Hence, the present study was initiated in order to study the antioxidant activity, secondary metabolites of plants such as *Syzygium cumini, Alangium salvifolium, Caesalpinia pulcherrima, Albizia lebbeck, Delonix elata, Holarrhena antidysenterica, Morinda pubescens, Tridax procumbens, Tectona Grandis, Opuntia ficus-indica, Strychnos nux-vomica, Albizia amara* located in and around salem city. Salem is a city located 11.669437º north 78.140865º east with an average elevation of 278 metres, at the tri-junction of Bangalore, Coimbatore and Cuddalore roads and surrounded by hills such as Sheveroys as well as Nagarmalai to the north, Jeeragamalai to the South, Kanjamalai to the west and Godumalai to the east. The river Thirumanimuthar runs through the city, it is a fifth largest city of Tamil Nadu after Chennai, Coimbatore, Madurai and Trichy.

MATERIALS AND METHODS

Plant materials

Fresh leaves from plants such as *Syzygium cumini, Alangium salvifolium, Caesalpinia pulcherrima, Albizia lebbeck, Delonix elata, Holarrhena antidysenterica, Morinda pubescens, Tridax procumbens, Tectona Grandis, Opuntia ficus-indica, Strychnos nux-vomica, Albizia amara* were collected for the antioxidant study.

Preparation of extract

Fresh leaves were collected during March, April 2013. 100 mg of fresh leaves extracted with 2 ml water. 0.1ml of aqueous extract was used for each analysis.

General experimental procedure

Analysis of secondary metabolites

Determination of total phenol content

Total phenolic content was determined by Folin - ciocalteau method [6,7]. The extract was mixed with folin ciocalteau reagent (5ml, 1:10 diluted with distilled water) for 5min and aqueous $NaCO_3$ (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 [8] was used to determine total flavonoid content. Aliquotes of extracts were treated with 0.1ml of 10% $AlCl_3$. The test solution was vigorously shaken. Absorbance at 415nm was recorded after 30min of incubation. A standard calibration plot was generated using known concentration of quercetin. The concentration of flavonoid was calculated from the calibration plot and expressed as mg quercetin equivalent/g of sample.

Assay of antioxidant activity

Nitric oxide scavenging assay [9], Reducing power [10], Metal chelating activity [11], Total antioxidant assay [12] was performed.

Determination of nitric oxide scavenging activity

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 chromophore formed was read at 546nm. Quercetin was used as a positive control.

Determination of reducing power assay

Aqueous extract was mixed with phosphate buffer (2.5ml, 0.2M, pH6.6), potassium ferricyanide (2.5ml1%) and incubated at 50˚C for 20min. A portion (2.5ml) of trichloroacetic acid (10%) was added to stop the reaction, then centrifuged at 3000rpm for 10min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml), $FeCl_3$ (0.5ml, 0.1%). The absorbance was measured at 700nm. Vitamin C was used as a positive control.

Determination of metal chelating activity

The chelating ability of ferrous ion was estimated by adding extract to a solution of 2mM $FeCl_2$ (0.05ml). The reaction was initiated by the addition of 5mM ferrozine (0.2ml), the mixture was shaken vigorously and left standing at room temperature for 10min. Absorbance was then measured spectrophotometrically at 562nm. The ethylene diamine tetra acetic acid calibration curve was plotted as a function of metal chelating activity.

Determination of total antioxidant capacity

Total antioxidant capacity by phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of green phosphate/Mo (V) complex at acidic pH. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid.

RESULTS AND DISCUSSION

The results of various antioxidant tests and secondary metabolites such as phenol and flavonoid are depicted in Table.1. The flavonoid content was found to be high in *Tectona Grandis, Delonix elata, Albizia lebbeck, Syzygium cumini,* whereas it was less with *Alangium salvifolium, Caesalpinia pulcherrima, Tridax procumbens* while moderate amount (6.15 to 8.1 mg/g) was observed with rest of the selected plants. Flavonoids act through scavenging or chelating process [13, 14]. Moderate amount of phenol content was present in *Albizia lebbeck*, *Delonix elata, Alangium salvifolium, Holarrhena antidysenterica,* while it was low with *Caesalpinia pulcherrima* whereas all the other plants showed very lesser phenol content. Phosphomolybdenum activity was high in *Delonix elata, Morinda pubescens, Strychnos nux-vomica*, *Opuntia ficus-indica*, *Albizia lebbeck*, while moderate amount was observed with remaining plants. Metal chelating activity was high with *Delonix elata*, *Strychnos nux-vomica*, *Albizia lebbeck*, whereas it was found to be moderate with rest of the plants. Reducing power assay showed similar results in all plants except *Alangium salvifolium.* Likewise nitric oxide assay was high in *Opuntia ficus-indica, Albizia amara, Morinda pubescens,* whereas it was in the range of 1.1 to 2.0 mg/g in all the other plants studied.

Table 1: Showing antioxidant activity and secondary metabolites of plants

Plant name	Family name	Nitricoxide assay-mg/g	Reducing Powerassay-mg/g	Metal chelating assay – mg/g	Phospho molybdenum assay-mg/g	Phenol mg/g	Flavonoid mg/g
Syzygium cumini (L.) Skeels	Myrtaceae	1.6	2.50	4.0	13.5	0.26	9.0
Alangium salvifolium Lam.	Alangiaceae	1.8	3.75	3.9	19.0	3.50	5.0
Caesalpinia pulcherrima (L.) Sw.	Caesalpiniaceae	1.82	2.2	2.1	19.8	1.90	4.2
Albizia lebbeck (L.) Willd.	Mimosaceae	1.50	2.15	4.90	21.6	4.85	9.7
Delonix elata (L.) Gamble	Caesalpiniaceae	2.0	2.75	7.60	29.4	3.50	9.9
Holarrhena antidysenterica (Roxb.) Wall.	Apocynaceae	0.10	2.0	2.60	18.90	3.15	7.3
Morinda pubescens J.E. Smith	Rubiaceae	8.2	2.3	3.2	25.5	0.06	6.3
Tridax procumbens L.	Asteraceae	1.4	2.10	3.1	15.0	0.15	4.15
Tectona Grandis	Lamiaceae	1.1	2.1	2.1	19.5	0.21	10.0
Opuntia ficus-indica	Cactacea	9.7	2.05	2.1	22.8	0.33	6.15
Strychnos nux-vomica L.	Loganiaceae	1.1	2.5	4.8	23.5	0.48	8.1
Albizia amara (Roxb.) Boivin	Mimosaceae	9.0	2.5	2.1	19.5	0.26	6.2

CONCLUSION

Injury to plant occurs when the capacity of antioxidant and detoxification processes are slower than the amount of reactive oxygen production. The antioxidant activities of obtained results show that *Opuntia ficus-indica* have high nitric oxide scavenging activity and *Delonix elata* had good phosphomolybdenum activity, reducing power activity was high with *Alangium salvifolium.* The observed antioxidant activity might be due to the presence of secondary metabolites especially flavonoid in aqueous extract. The difference in antioxidant activity is neither restricted to a particular part nor to a specific families of plant.

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