Research Article

Free Radical Scavenging Activity of Plants at Perumal Malai Hill

Krishnaveni M*, Chandrasekar R, Amsavalli L, Durairaj S, Madhaiyan P
*Department of Biochemistry, Periyar University, Salem, Tamil Nadu, India.
***Corresponding author's E-mail:** logasarvesh@gmail.com

Accepted on: 14-04-2013; **Finalized on:** 30-06-2013.

ABSTRACT

The present work aims to study the free radical scavenging activity of plants located at Perumal malai hill, Salem, Tamil Nadu, India. Totally 20 plant species were selected for the study and its aqueous extract were subjected to secondary metabolite analysis like phenolics, flavonoid and several antioxidant assay such as Nitric oxide scavenging, Metal chelating activity, Reducing power, Total antioxidant assay. Among the secondary metabolites tested phenol was found to be high in *Psidium guajava*, whereas it was low in *Ficus religiosa* plant. Results obtained with antioxidant assay was compared with plants collected from the site, in that Phosphomolybenum assay was found to be more predominant with *Azadirachata indica* plants. Whereas all the other plants showed moderate to low antioxidant activity.

Keywords: Antioxidant assay, Free radicals, Perumalmalai hill, Plants, Secondary metabolites.

INTRODUCTION

Most of the diseases are linked to oxidative stress due to free radicals.[1] Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism.[2] Living organisms have antioxidant defence systems that protects against oxidative damage by removal or repair of damaged molecules.[3] The term 'antioxidant' refers to the activity of numerous vitamins, minerals and phytochemicals which provide protection against the damage caused by ROS.[4] Antioxidants interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donors.[5] Medicinal plants contain several active principles with specific therapeutic effects. They represent a source of chemical compounds such as tannins, flavonoids, saponins, resins and alkaloids curative properties, often not provided by synthetic chemical compounds.[6] The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins.[7] Plant based antioxidants are preferred to the synthetic ones because of their multiple mechanisms of actions and non-toxic nature. Hence, an attempt has been initiated to evaluate the *in vitro* antioxidant activities of some common medicinal plants available in the experimental site - Perumalmalai hill which is located in Salem, Tamil Nadu, India

MATERIALS AND METHODS

Plant materials

Fresh leaves were collected from the experimental site - perumalmalai hill dring March - April 2013. 100mg of fresh leaves was extracted with 2ml water. 0.1ml of extract was used for the analysis.

Secondary metabolites

The phenol[8,9] and flavonoid[10] content of aqueous leaf extract was analyzed.

Determination of total phenol content

Total phenolic content were determined by Folin – ciocalteau method. The extract samples 0.1ml were 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 was used for the determination of the total flavonoid content. Aliquotes of extract solutions were taken and made up the volume 3ml with methanol. 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 caliberation plot was generated at 415nm using known concentration of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent/g of sample.

Antioxidant assay

Nitric oxide scavenging assay,[11] Reducing power[12], Metal chelating activity[13], Total antioxidant assay[14] were performed.

Nitric oxide scavenging activity

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

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

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 of the solution was then measured spectrophotometrically at 562nm. The Ethylene Diamine Tetra Acetic Acid calibration curve was plotted as a function of metal chelating activity.

Total antioxidant capacity

Total antioxidant capacity by Phosphomolybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid. [14]

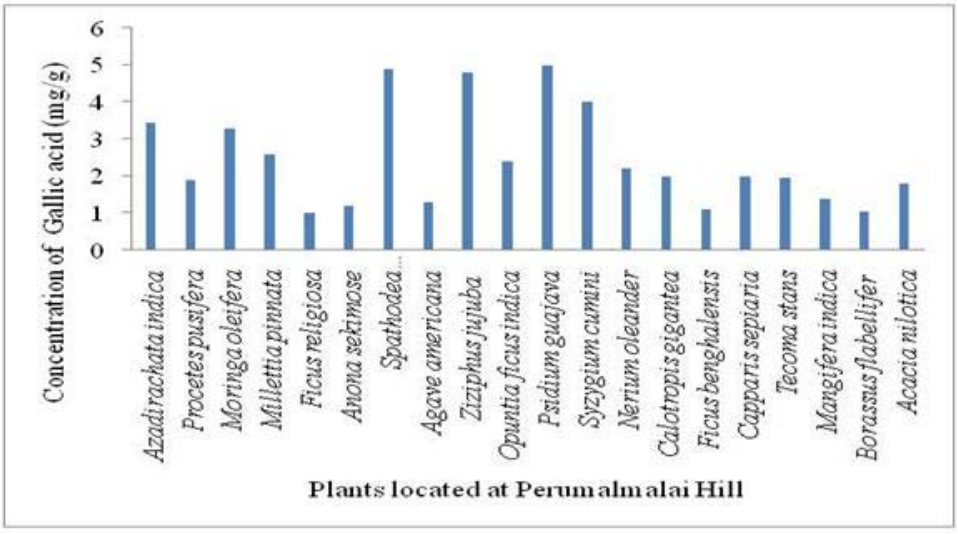

Figure 1: Total phenol content of plants

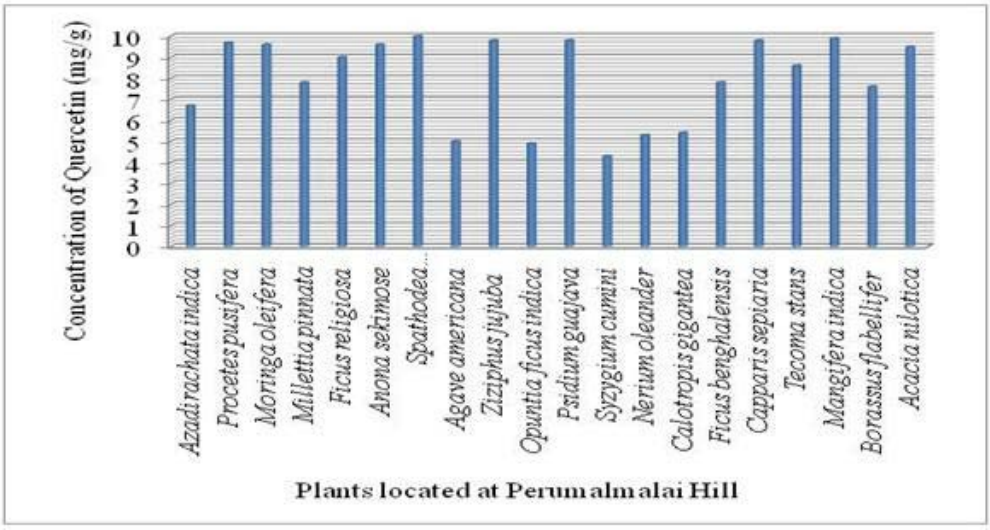

Figure 2: Total flavonoid content of plants

RESULTS AND DISCUSSION

Secondary metabolites

The results of phenol and flavonoid content of the plants were depicted in Figure 1 and Figure 2.

From the observed results, the phenol content was found to be high in *Psidium guajava, Spathodea campanulata, Ziziphus jujube* and low in *Borassus flabellifer, Ficus benghalensis, Ficus religiosa* whereas, moderate amount of phenol was observed in *Syzygium cumini, Nerium oleander, Azadirachata indica, Moringa oleifera, Millettia pinnata, Opuntia ficus indica* and also in other plants studied. The flavonoid content was found to be high in *Spathodea campanulata, Mangifera indica, Ziziphus jujube, Capparis sepiaria, Procetes pusifera, Moringa oleifera, Anona sekimose, Acacia nilotica* whereas, moderate amount in the range of 4.3 to 8.6 mg/g was observed with rest of the plants. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities.[15]

Antioxidant assay

The results of Nitric oxide scavenging assay, Reducing power, Metal chelating activity, Total antioxidant assay are illustrated in Figure 3, Figure 4, Figure 5, respectively.

Nitric oxide scavenging activity

The nitric oxide scavenging activity was good with *Opuntia ficus indica* (1.47mg/g), *Psidium guajava* showing 1.45mg/g. Whereas, it was low with *Procetes pusifera, Millettia pinnata, Spathodea campanulata* but changes observed was moderate with other plants which was in the range of 1.0 to 1.15mg/g.

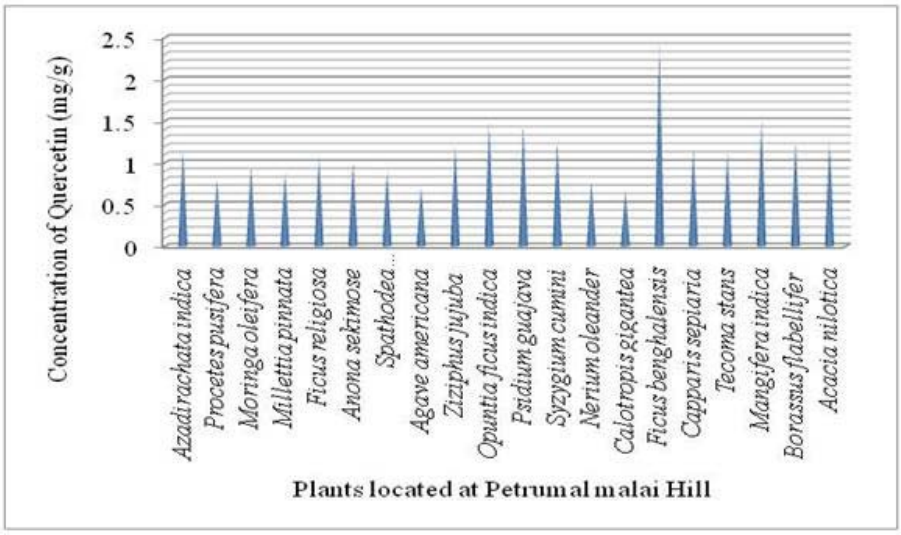

Figure 3: Showing Nitric oxide scavenging activity of plants

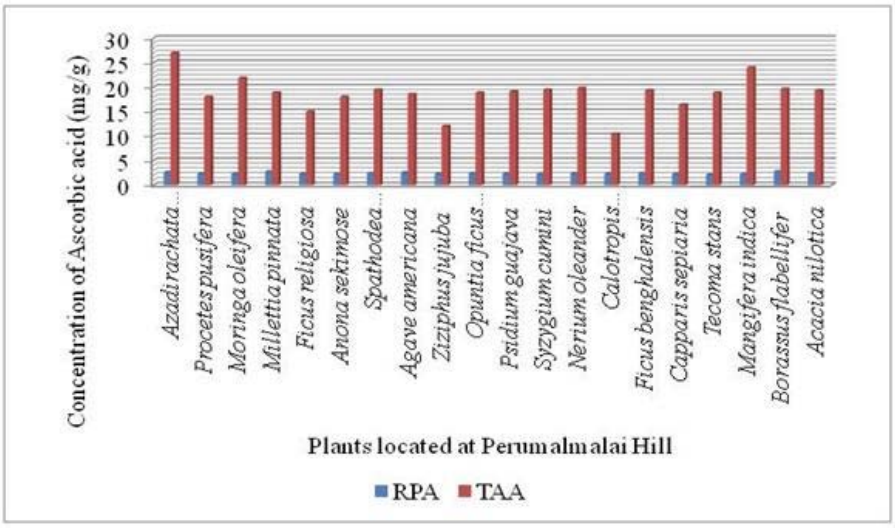

Figure 4: Depicting Reducing power and Total antioxidant assay

Reducing power assay (RPA), Total antioxidant capacity (TAA)

The reducing power activity was high with *Millettia pinnata, Borassus flabellifer* showing 2.8mg/g ascorbic acid. Whereas, moderate amount was observed with rest of the plants which was in the range of 2.1 to 2.65mg/g. Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of

phenolic antioxidant action.[15] The total antioxidant activity was 27.0mg/g for *Azadirachata indica*, 24.0mg/g for *Mangifera indica*, 21.9 mg/g for *Moringa oleifera*, 19.8mg/g for *Nerium oleander*, 19.7 mg/g for *Borassus flabellifer*, 19.4mg/g ascorbic acid for *Ficus benghalensis*, 18.9mg/g for *Millettia pinnata*, *Opuntia ficus indica, Tecoma stans* and plants like *Acacia nilotica, Syzygium cumini, Psidium guajava, Spathodea campanulata, while, Ficus religiosa, Ziziphus jujuba, Calotropis gigantea* showed well below this range. Phytochemicals are plant chemicals or more appropriately defined as bioactive non-nutrient plant compounds in fruits, vegetables and other plant foods that have been linked to reduce the risks of major chronic diseases and cancers.[16] High consumption of vegetables containing phenolic antioxidants which inhibit the oxidation of LDL are said to slow down the process of atherosclerosis and may also reduce the risk of cancer and other diseases.[17-19]

Metal chelating activity

Metal chelating activity of plants is shown in Figure 5. 7.9mg/g was identified with *Acacia nilotica*. Plants such as *Psidium guajava, Agave Americana, Spathodea campanulata*, *Procetes pusifera,* exhibited 6.2, 6.0 mg/g. Wheras, all the other plants showed metal chelating ability in the range of 3.0 to 5.1 mg/g. Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton chemistry.[20]

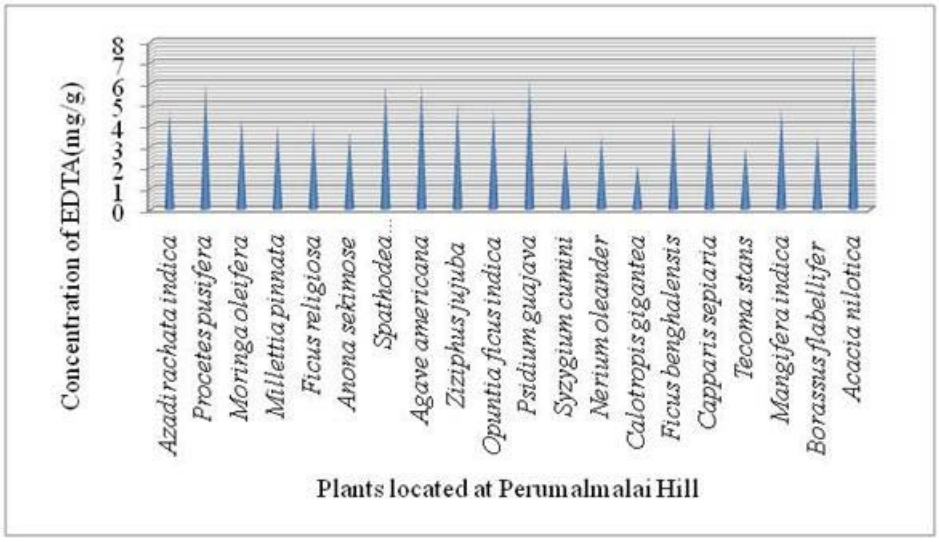

Figure 5: Metal chelating activity of plants

CONCLUSION

The present study revealed that leaves contain significant amount of phenol and flavonoid which impart differences in antioxidant activity. To highlight, phosphomolybdenum antioxidant activity was predominant with majority of plants located at this particular site compared to other antioxidant assays, which might be due to the variation in secondary metabolite content of plants as it depends on the place where it grows. Since, the study site is a hill there might be a nitrogen insufficiency which imparts significant differences.

Acknowledgement: Authors are thankful to Honourable Vice Chancellor Dr. K. Muthuchelian Avl, Registrar Dr. K. Angamuthu Avl, for their support and Dr. V. Raj, Head, Department of chemistry in rendering timely help. Dr. P. Nazni Head, Department of Food Science, Dr. T. Poongodi Vijayakumar, Department of Food Science for providing UV Spectrophotometer for the analysis.

REFERENCES

1. Gutteridgde J, Free radicals in disease process: a complication of cause and consequence, Free. Radic. Res. Comm., 19, 1995, 141–58.
2. Tiwari A, Imbalance in antioxidant defence and human diseases: multiple approach of natural antioxidants therapy, Curr. Sci., 81, 2001, 1179–87.
3. Sun J, Chen Y, Li M, Ge Z, Role of antioxidant enzymes on ionizing radiation resistance, Free Radical Biology and Medicine, 42, 1998, 586-592.
4. Khilfi S, Hachimi E, Khalil A, Es-Safi A, Belahyam A, Tellal A, El Abbouyi A, *In-vitro* antioxidant properties of *Salvia verbenaca*. L. hydromethanolic extract, Indian Journal of pharmacology, 38, 2006, 276-280.
5. Gulcin I, Alici HA, Cesur M, Determination of *in- vitro* antioxidant and radical scavenging activities of propofol, Pharmacology Bulletin, 53, 2005, 281-285.
6. Fabricant DS, Farnsworth NR, The value of plants used in traditional medicine for drug discovery, Environ Health Persp., 109, 2001, 69-75.
7. Aqil F, Ahmed I, Mehmood Z, Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants, Turk J Biol., 30, 2006, 177–18.
8. Ebrahimzadeh MA, Hosseinimehr SJ, Hamidinia A and Jafari M, Antioxidant and free radical scavenging activity of *Feijoa sallowiana* fruits peel and leaves, Pharmacologyonline, 1, 2008a, 7-14.

9. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A and Bekhradnia AR, Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica Mey.,* Pharmacologyonline, 2, 2008, 560-567.

10. Mervat MM, Far E, Hanan A, Taie A, "Antioxidant activities, total anthrocynins, phenolics & flavonoids contents of some sweet potato genotypes under stress of different concentration of sucrose and sorbitol, Australian J Basic Applied Sci., 3, 2009, 3609-3616.

11. Ebrahimzadeh MA, Nabavi SF, and Nabavi SM, Antioxidant activities of methanol extract of *Sambus ebulus L.flower,* Pak. J. Biol. Sci., 12(5), 2009d, 447-450.

12. Yen GC and Chen HY, Antioxidant activity of various tea extracts in relation to their antimutagenicity, J. Agri. Food Chem., 43(1), 1995, 27-32.

13. Ebrahimzadeh MA, Pourmorad F and Bekhradnia AR, Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran, Afr. J. Biotechnol., 32, 2008a, 43-49.

14. Prieto P, Pineda M & Aguilar M, Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of Vitamin E, Anal. Biochem., 269, 1999, 337-341.

15. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M, Eslami B, *In vitro* antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran, Pharmacognosy Magazine, 4(18), 2009a, 123-127.

16. Sun J, ChuYF,Wu X, Liu RH, Antioxidant and antiproliferative activities of fruits, J. Agric. Food. Chem., 50, 2002, 7449-7454.

17. Beteridge DJ, Diabetes, lipoprotein metabolism and atherosclerosis, Br. Med. Bull., 45, 1989, 285 - 311.

18. Baynes JW, Perspectives in diabetes: role of oxidative stress in development of complications in diabetes, Diabetes, 37, 1991, 550 - 557.

19. Wolff SP, Diabetes mellitus and free radicals, Br. Med. Bull., 49, 1993, 642 - 652.

20. Halliwell B, Antioxidants: the basics - what they are and how to evaluate them, Adv. Pharmacol., 38, 1997, 3-20.

Source of Support: Nil, **Conflict of Interest:** None.