

CAROTENOID CONTENT AND ANTIOXIDANT ACTIVITY OF SEVERAL MEDICINAL PLANTS IN SURIGAO DEL SUR

Gemma A. Gruyal

College of Teacher Education, Surigao del Sur State University, 8317, Philippines

Abstract: Plant constituents with antioxidant activity can exert protective effects against oxidative stress in biological system. The methanolic leaf extracts of the nine species of medicinal plants *Chromolaena odorata*, *Cyperus kyllingia*, *Ceiba pentandra*, *Eleusine polydactyla*, *Laportea interrupta*, *Trema amboinensis*, *Conyza cinerea*, *Phyllanthus niruri* and *Mimosa pudica* were evaluated for their total carotenoids and antioxidant activity using DPPH. Results indicated that *M. pudica* has the highest carotenoid content with an equivalent value of 1310.6 $\mu\text{mol/gram}$ dried material followed by *C. odorata* (1112.7 $\mu\text{mol/gram}$), *C. pentandra* (859.5 $\mu\text{mol/gram}$), *C. cinerea* (645.4 $\mu\text{mol/gram}$), *L. interrupta* (481.0 $\mu\text{mol/gram}$), *P. niruri* (417.8 $\mu\text{mol/gram}$), *C. kyllingia* (96 $\mu\text{mol/gram}$), *E. polydactyla* (86.76 $\mu\text{mol/gram}$) and *T. amboinensis* (75.2 $\mu\text{mol/gram}$). For their radical scavenging activity, various results were obtained. *P. niruri* showed the highest percentage (93.53%) of scavenging effect and the least is *C. odorata* Linn (2.84%). Findings showed that the elevated values of total carotenoids and radical scavenging activity has a strong correlation on medicinal bioactivities of plant extracts, which could lead to the development of medications for clinical usage.

Keywords: *Free radical scavenging, Plant extract, Carotenoids, secondary metabolites*

INTRODUCTION

Reactive oxygen species (ROS) or reactive nitrogen species (RNS) are free radicals produced in a living system due to the various metabolic processes that are taking place inside and generally occur in the mitochondrial respiratory chain. It may also be due to atmospheric pollutants, drugs and xenobiotics [1]. The uncontrolled generation and associated increase of ROS level in the body results in "oxidative stress". Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases [2].

Plant constituents with antioxidant activity can exert protective effects against oxidative stress in biological system [3]. Antioxidants are naturally abundant in fruits and vegetables and can neutralize free radicals donating an electron and converting them to harmless molecules [4]. Among the numerous naturally occurring antioxidants are ascorbic acid, carotenoids and phenolic compounds [5]. They are known to inhibit lipid peroxidation (by inactivating lipooxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions [6].

Carotenoids are known to be very efficient physical and chemical quenchers of singlet oxygen ($^1\text{O}_2$), as well as potent scavengers of other reactive oxygen species (ROS) [7]. They effectively scavenge ROS and other free radicals of different origins [8] [9] delivering protection against oxidative damage. Carotenoids and some of their metabolites are suggested to play a protective role in many ROS-mediated disorders, like cardiovascular diseases, several types of cancer or neurological, as well as photosensitive or eye-related disorders. Carotenoids are structurally and functionally a very diverse group of natural pigments of the polyene type [10] and are important precursors of retinol (vitamin A). Carotenoids are abundantly present in fresh fruits vegetables, yellow-orange-red fruits and green leafy vegetables. In the present study, it aims to determine the carotenoid contents and evaluate the antioxidant activity using DPPH from different extracts obtained from leaves of nine medicinal plant which are used as herbal medicine in Surigao del Sur.

2.0 Research Methodology

A. Plant Materials. The fresh leaves of the nine species of medicinal plants were *Chromolaena odorata*, *Cyperus kyllingia*, *Ceiba pentandra*, *Eleusine polydactyla*, *Laportea interrupta*, *Trema amboinensis*,

Corresponding Author: Gemma A. Gruyal, College of Teacher Education, Surigao del Sur State University, 8317, Philippines.
gemma_gruyal@yahoo.com

Conyza cinerea, *Phyllanthus niruri* and *Mimosa pudica*. It was collected from the localities of Surigao del Sur, Philippines. The scientific names of this plant species were identified from the library of botanical plants in the Philippines and authenticated from Co's Digital Flora of the Philippines.

B. Plant material extractions. Twenty (20) grams of the grounded plant materials was soaked with 300 ml of methanol for 12 hours with occasional shaking. Another 300 ml of methanol was added for the next one hour, then for the next one hour 300 ml methanol was added again. The extract was then filtered in a one-millimeter filter paper. The filtrate was then concentrated to 100 ml using rotary evaporator. It was transferred into the volumetric flask and the volumes were raised to 250 ml. The extracts were then placed into the storage bottle and placed in the refrigerator.

C. UV-Vis spectrophotometric analysis for total carotenoid content. The total carotenoid content of samples was determined according to the method of [11] with slight modification. Briefly, about 0.200 g of plant dried material was placed into the vial. The extract was then added with 5 ml ammoniacal acetone and shaken for 5 minutes. The solution was then subjected to centrifuge for 3-5 minutes and the supernatant liquid was transferred into another vial. The extraction was done again with another 5 ml of ammoniacal acetone. The first supernatants were combined with the new one. The empirical supernatants were subjected to centrifuge to ensure clean layer of solvent solution for the use in absorbance measurement. The absorbance was read at 480,645,663 and 710 nm against solvent blank. The absorbance @710 nm is isobestic point or theoretical base and is deducted from all other absorbance readings from the same solution. The corrected absorbance can be computed using this formula:

$$\text{Corrected Absorbance} = \text{Abs}@480,645,663 - \text{Abs}@710\text{nm}$$

Carotenoid content can be computed using the formula:

$$\text{Carotenoid as } \mu\text{mol/unit area or wt. sample} = \frac{(A_{480} + 0.114 * A_{663} - 0.638 * A_{645}) * V * 1000}{112.5 * \text{unit area or wt. sample}}$$

Where:

- A480 – corrected Absorbance @480 nm
- A645 – corrected Absorbance @645 nm
- A663 – corrected Absorbance @663 nm
- V – Total volume of the plant extract
- V= 10ml

D. DPPH radical scavenging activity.

The DPPH solution (0.006% w/v) was prepared in 95% methanol. The methanol extract of the leaves from the nine medicinal plants were mixed with 95% methanol to prepare the stock solution (1 mg/mL). Freshly prepared DPPH solution was taken in the test tubes and extracts was added followed by serial dilutions (100-1000 ug) to every test tube so that the final volume is 2 mL and discoloration was measured at 517 nm after incubation for 30 minutes in the dark (Themo UV1 spectrophotometer). A measurement was performed at least in three trials. The control sample was prepared and contained the same volume without any extract and 95% methanol. It was used as the blank. The percentage scavenging of the DPPH free radical was measured using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

where, A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the sample (methanolic leaf extract of the medicinal plants).

E. Statistical Analysis

The data were expressed as the mean \pm standard deviation of triplicate separate observations. Significant correlation between total carotenoids and antioxidant activity were statistically analyzed using Statistical package for Social Sciences (SPSS) version 17.

3.0 Results and Discussion

A. Total Carotenoid Content

Carotenoids are lipid-soluble antioxidant. According to [12] [13], carotenoids have been credited with health-promoting effects including immune-enhancement and reduction of the risk of developing degenerative diseases such as cancer, cardiovascular disease (CVD), cataract and macular degeneration. In the present study the total carotenoid content of each medicinal plant extract is presented in table 1.

The results showed that carotenoid content varies from each other. The values as revealed indicated that *M. pudica* has the highest carotenoid content with an equivalent value of 1310.6 $\mu\text{mol/gram}$ dried material followed by *C. odorata* (1112.7 $\mu\text{mol/gram}$), *C. pentandra* (859.5 $\mu\text{mol/gram}$), *C. cinerea* (645.4 $\mu\text{mol/gram}$), *L. interrupta* (481.0 $\mu\text{mol/gram}$), *P. niruri* (417.8 $\mu\text{mol/gram}$), *C. kyllingia* (96 $\mu\text{mol/gram}$),

Table 1. Quantitative determination of total carotenoid contents among the nine medicinal plants.

Name of the Medicinal Plants	Total Carotenoid	
	Mean μmol/g dried material	SD
<i>Chromolaena odorata</i>	1112.70 ±	56.00
<i>Cyperus kyllingia</i>	96.00 ±	33.90
<i>Ceiba pentandra</i>	859.50 ±	44.90
<i>Eleusine polydactyla</i>	86.76 ±	4.80
<i>Laportea interrupta</i>	481.00 ±	32.70
<i>Trema amboinensis</i>	75.20 ±	33.30
<i>Conyza cinerea</i>	645.40 ±	18.10
<i>Phyllanthus niruri</i>	417.80 ±	59.90
<i>Mimosa pudica</i>	1310.60 ±	144.50

Results are the mean of triplicate determinations ± SD

polydactyla (86.76 μmol/gram) and *T amboinensis* (75.2 μmol/gram). According to [14] in his study, some carotenoids even serve as precursors for the synthesis of vitamin A while some provide protection against damaging reactions in the body, acting as physiological antioxidants and thereby enhancing immune responses. In the study of Joseph [15], it was mentioned that *M. pudica* has a capacity of arresting bleeding and it fastens the process of healing of wounds, cure skin diseases, bronchitis, general weakness impotence and treat neurological problems. Result of the present study conferred with [15] study since it has higher carotenoid contents.

B. DPPH radical scavenging activity.

The free radical scavenging capacity of the extracts was determined using DPPH. The DPPH test provides information on the activity of the test compounds with a stable free radical. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron that is responsible for the absorbance of 517 nm and for the visible deep purple colour. When the DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. The decrease in absorption is taken as a measure of the extent of radical scavenging.

The percent DPPH scavenging activity of the nine plant extracts is shown in Figure 1. Results revealed that the percentage scavenging activity varied

from each other. *P. niruri* showed the highest percentage (93.53%) of scavenging effect and the least is *Chromolaena odorata* Linn with 2.84% scavenging effect. In the present study, *P. niruri* appeared that there is less correlation with antioxidant activity and the carotenoid content, which suggest that, besides carotenoids other chemical constituents may contribute to the antioxidant activity. According to [16] free radical scavenging activity may be due to its high total phenolic content. In addition, [17] stated that carotenoids are part of the antioxidant defence system. It interacts synergistically with other antioxidants. Mixtures of carotenoids are more effective than single compounds. Moreover, in the study of [18] aqueous leaf extract of *P. niruri* was found to display in vitro free radical scavenging activity.

It reduces endogenous LPO product formation, and increase activity levels of endogenous antioxidant enzymes in the kidney, which makes this herb a potential treatment for diabetic nephropathy.

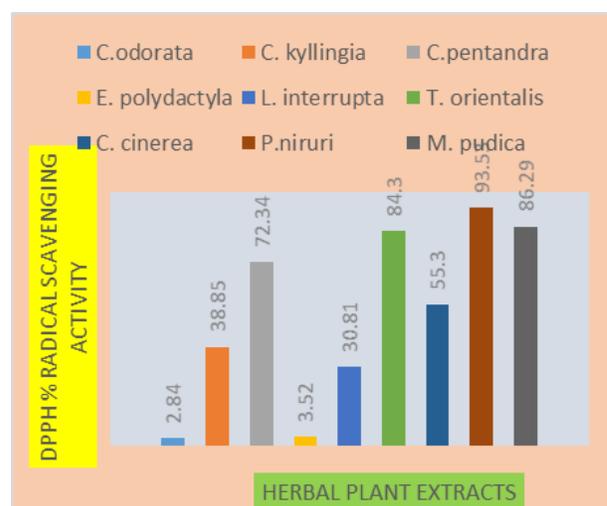


Figure 1. Percent DPPH scavenging activity of the nine plant extracts.

4.0 Conclusion

Among the nine selected medicinal plants *M. pudica* showed the highest total carotenoid content (1310.6 μmol/gram dried material) and the least is *T. amboinensis* (75.2 μmol/g). The free radical scavenging capacity also showed that all the selected medicinal plants has free radical scavenging activity however, their activity to scavenge varies from 2.84% to 93.53% depending on the medicinal plants. Analysis showed that the elevated values of total carotenoids and radical scavenging activity of the nine medicinal plants has a strong correlation on medicinal bioactivities of plant extracts, which could lead to the development of medications for clinical usage.

REFERENCES

- [1] Patel Chirag J, Satyanand Tyagi , Nirmala Halligudi , Jaya Yadav , Sachchidanand Pathak , Satya Prakash Singh , Ashish Pandey , Darshan Singh Kamboj , Pratap Shankar 2013. ANTIOXIDANT ACTIVITY OF HERBAL PLANTS: A RECENT REVIEW. Journal of Drug Discovery and Therapeutics 1 (8) 2013, 01-08 ISSN: 2320 - 4230
- [2] Valko M, Rhodes CJ, Moncol J, Izakovic M, et al. 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Mini-review. Chem. Biol. Interact. 160:140. [PubMed]
- [3] Cao G; Sofic ER; Prior RL 1996. Antioxidant capacity of tea and common vegetables. J. Agric. Food. Chem., 44: 3426- 3431.
- [4] Leonard SS, Cutler D, Ding M, Vallyathan V, Castranova V, Shi X. 2002. Antioxidant properties of fruit and vegetable juices. Ann Clin Lab Sci ;32:193-200.
- [5] Duha PD, Tu YY, Yen GC 1999. Antioxidants activity of aqueous extract of Harnjyur (Chrysanthemum morifolium Ramat). Lebensmwiss Technol. 32: 269-277.
- [6] Raja Sudarajan N, Ahamad H, Kumar V 2006. A natural antioxidant Cytisus scoparius Link-. 6: 1-7
- [7] Fiedor, Joanna and Burda, Kyetoslava 2014. Potential Role of Carotenoids as Antioxidants in Human Health and Disease . Nutrients. 6(2):466-488. Doi: 10.3390/nu6020466.
- [8] Martin H.D., Ruck C., Schmidt M., Sell S., Beutner S., Mayer B., Walsh R.1999. Chemistry of carotenoid oxidation and free radical reactions. Pure Appl. Chem. ;71:2253–2262. doi: 10.1351/pac199971122253. [Cross Ref]
- [9] Yamauchi R., Nobuyuki H., Inoue H., Kato K. 1993)Products formed by peroxy radical oxidation of β -carotene. J. Agric. Food Chem. ;41:708–713. doi: 10.1021/jf00029a005. [Cross Ref]
- [10] Edge R., Truscott T.G. 2010. Properties of Carotenoid Radicals and Excited States and Their Potential Role in Biological Systems. In: Landrum J.T., editor. Carotenoids: Physical, Chemical, and Biological Functions and Properties. CRC Press; Boca Raton, FL, USA: pp. 283–308.
- [11] Irondi, A.E., Obodh, G., Akintunde, J.K. 2012. Comparative and Synergistic Antioxidant Properties of Carica papaya and Azadarichta indica leaves. International Journal of Pharmaceutical Sciences and Research. Vol. 3(12): 4773-4eseerx.is779 Retrieved from <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.278.7380&rep=rep1&type=pdf>
- [12] Olson, JA. 1999. Carotenoids and human health. Archivos Latinoamericanos de Nutricion, 49(3 Suppl 1):7S-11S]
- [13] Krinsky, N., Johnson, E. 2005. Carotenoid actions and their relation to health and disease. Molecular Aspects of Medicine. Volume 26, Issue 6, Pages 459–516
- [14] Gupta,P.,Sreelakshmi,Y and Sharma,R. 2015. A rapid and sensitive method for determination of carotenoids in plant tissues by high performance liquid chromatography. Plant Method, doi: [10.1186/s13007-015-0051-0](https://doi.org/10.1186/s13007-015-0051-0)
- [15] Joseph,B.,George,J.and Mohan,J. 2013. Pharmacology and Traditional Uses of Mimosa pudica .International Journal of Pharmaceutical Sciences and Drug Research 2013; 5(2): 41-44, ISSN 0975-248X
- [16] Raghavendra, M., Reddy, A.M., Yadav, P.R., Raju, A.S. & Kumar, L.S. 2013. Comparative studies on the in vitro antioxidant properties of methanolic leafy extracts from six edible leafy vegetables of India. Asian Journal of Pharmaceutical and Clinical Research, 6(3), 96-99.
- [17] Stahl W.,Sies H., 2003. Antioxidant activity of carotenoids. Mol Aspects Med., 24(6):345-51
- [18] Giribabu N.,Visweswara R.,Kumar KP.,Muniandy S.,Rekha SS. And Salleh N. 2014. Aqueous Extract of Phyllanthus niruri Leaves Displays in Vitro Antioxidant Activity and prevents the elevation of Oxidative of Stress in the Kidney of Streptozotocin-Induced Diabetic Male rats. Evidence-Based Complementary and Alternative Medicine, 834815, doi.org/10.1155/2014/834815