Int J Pharm Bio Sci 2015 April; 6(2): (B) 1071 - 1076

Research Article **Allied science**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EVALUATION OF ANTIOXIDANT, CAROTENE AND ALKALOID CONTENT IN *SPINACH OLERACEA* AND *ANNONA SQUAMOSA*

V.KAVITHA[*1] AND V.SARADHA RAMADAS[2]

*[*1]Department of Food Service Management and Dietetics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamilnadu, India*
[2] Department of Food Service Management and Dietetics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamilnadu, India

ABSTRACT

The aim of the present study is to determine the antioxidant, carotene and alkaloids content of the selected plant leaves namely *spinach oleracea* and *Annona squamosa.* The antioxidant screening was done by DPPH free radical scavenging assay using shade dried leaf extracts. The results showed that IC_{50} for *Annona squamosa* and *Spinach oleracea* was 68.24 (µg/ml), 42.57 µg/ml respectively. Annona squamosa shows highest free radical scavenging activity at the concentration 20µg/ml. *Annona squamosa* was found to have a maximum amount of carotene (173.24 mg/100gm) followed by *Spinach oleracea* (58.88 mg/100gm). Regarding alkaloid estimation, *Annona squamosa* has the highest alkaloid content of 0.48%, while *Spinach oleracea* had the lowest content (0.03%). Plant antioxidants are believed to play a role in protection against various diseases and delaying ageing processes. Hence the present investigation suggests that medicinal plants which possess good antioxidant potential are the best supplements for the diseases associated with oxidative stress.

KEYWORDS: ***Spinach oleracea*, *Annona squamosa,* Antioxidant, Carotene, Alkaloid**

*Corresponding author

V.KAVITHA
Department of Food Service Management and Dietetics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamilnadu, India

INTRODUCTION

In traditional societies nutrition and health care are strongly interconnected and many plants have been consumed both as food and for medicinal purposes[1].Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds. Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications[2]. Diabetes is usually accompanied by increased production of free radicals[3]or impaired antioxidant defenses[4]. An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may therefore have health promoting effects in the prevention of degenerative diseases[5]. *Annona squamosa*belongs to the family *Annonaceae.* This plant is commonly called custard apple in English and sharifa in Hindi and Sitapazham in Telugu in India[6]**.** It is considered beneficial for cardiac disease, diabetes, hyperthyroidism and cancer. *Spinacia oleracea*is an edible flowering plant in the family of Amaranthaceae. Spinach has a high nutritional value and is extremely rich in antioxidants, especially when fresh, steamed, or quickly boiled. It is a rich source of vitamin A (lutein), vitamin C, vitamin E, vitamin K, magnesium, manganese, folate and iron. It has been also credited with various biological activities like virus inhibitor[7]**.** The study revealed that the leaves of Annona squamosa contain a considerable quantity of phenolic compounds that were found to be the major contributor for their antioxidant an antibacterial activites[8].Based upon their medicinal properties and literature review the present study was conducted to evaluate the antioxidant, carotene and alkaloid content of selected shade dried leaf powder of *Annona squamosa* and *Spinach oleracea.*

MATERIALS AND METHODS

Collection of Medicinal Plants

The spinach *(Spinach oleracea*) leaves were purchased at market in Thiruchirappalli district, Tamilnadu. Random selection of leaves was done by purchasing from randomly selected sellers in the market. The custard apple leaves (*Annona squamosa*) were collected from the households. The leaves were identified and authenticated by the botanists, Jamal Mohamed College, Thiruchirappalli. The green leafy vegetables were sorted to remove from the stalk, trimmed and washed. Washing was done with water to remove dirt and other contaminants. The cleaned leaves were allowed to shade dried for a week before analysis was carried out.

Determination of Antioxidant Activity

DPPH Free Radical Scavenging Activity

The antioxidant activity was determined by DPPH Method using standard procedure[9] proposed by Patel Rajesh and Patel Natwar (2011).

Processing of Plants for Extract Preparation

The shade dried plant leafs were powdered using a grinder. The extraction was done at room temperature. About 100 g of dried, ground plant materials were soaked in methanol (1 L of 98%) for 5-7 days separately. The soaked material was stirred every 18 h using a sterilized glass rod. The final extracts were passed through Whatman filter paper No.1 (Whatman Ltd., England). The filtrates obtained were concentrated under vacuum on a rotary evaporator at 40˚C and stored at 4˚C for further use. The stock solution of crude extracts (5 mg/ml) was prepared by dissolving a known amount of dry extract in 98% methanol. The working solutions (1, 2, 4, 6, 8, 10, 15, 25, 50, 75, 100, 250, 500 and 750 µg/ml) of the extracts were prepared from the stock solution using suitable dilution.

Procedure for DPPH Free Radical Scavenging Activity

Dissolved 4.3mg of DPPH (1,1- Diphenyl 1-2-picrylhydrazyl) in 3.3 ml methanol; it was protected from light by covering the test tubes with aluminium foil. Then 150µl DPPH solution was added to 3ml methanol and absorbance was taken immediately at 517nm for control reading. 50 µl of various concentrations of aqueous leaf extract as well as standard compound (Ascorbic acid) were taken and the volume was made uniformly to150 µl using methanol. Each of the samples was then further diluted with methanol up to 3 ml and to each 150µl DPPH was added. Absorbance was taken after 15 min at 517nm using methanol as blank on UV- visible sphectrometer. The IC_{50}values for each sample leaf extracts as well as standard preparation were calculated. The DPPH free radical scavenging activity was calculated using the following formula:

Percent scavenging = [Absorbance of control-Absorbance of test sample/ Absorbance of control] x 100.

***Carotene Analysis*:** Carotene content was analyzed using the standard method[10].

Analysis of alkaloids

The alkaloid content was determined gravimetrically[11]. A measured weight of the sample was dispersed in 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4hour at 28˚C. It was later filtered via what man No 42 grade of filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of concentrated aqueous NH_4OH until the alkaloid was precipitated. The alkaloid precipitated was received in a weighed filter paper, washed with one percent ammonia solution dried on the oven at 80˚C. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

Statistical analysis

Samples were analyzed in triplicate and the results were given as Mean ± S.D.

RESULTS AND DISCUSSION

Antioxidants from plant materials terminate the action of free radicals thereby protecting the body from various diseases[12].Hence the present study was aimed to measure the antioxidant activity using DPPH method. The results were summarized in Table-1 and represented in Figure-1.

DPPH Free Radical Scavenging Activity

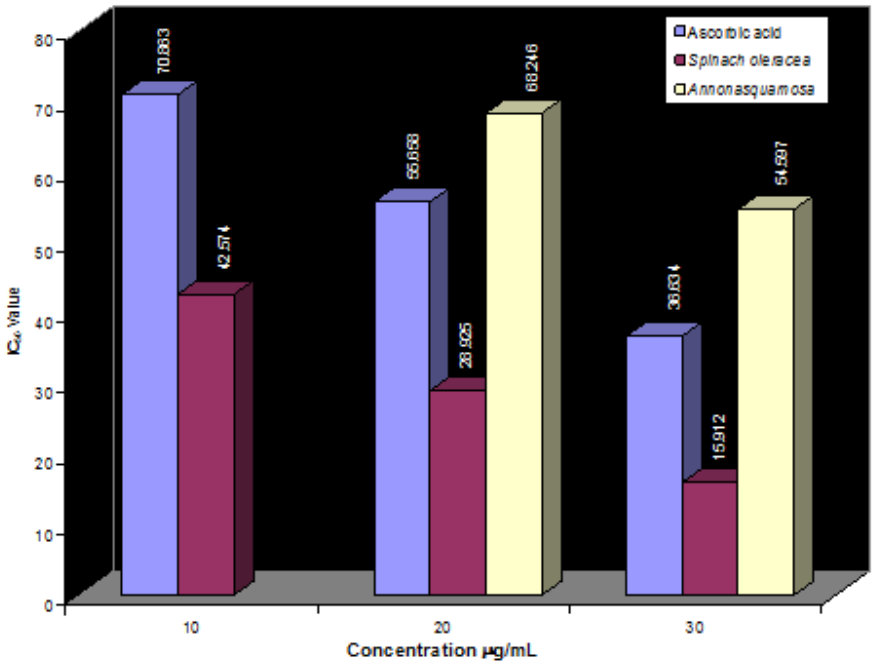
One of the quick methods to evaluate antioxidant activity is the scavenging activity on DPPH, a stable free radical and widely used index. In the DPPH Free radical scavenging activity, the selected shade dried plant leaves were evaluated for their free radical scavenging activity with ascorbic acid as standard compound. The selected shade dried plant extracts exhibited moderate to high antioxidant activities. The IC_{50} was calculated for each leaf samples as well as ascorbic acid as standard.

Table 1

DPPH Free Radical Scavenging Activity of Samples and IC_{50}Values

Samples	Concentration µg/ml	IC_{50} value
Ascorbic acid	10	70.863
	20	55.658
	30	36.634
Spinach oleracea	10	42.574
	20	28.925
	30	15.912
Annonasquamosa	20	68.246
	30	54.597

Figure 1
DPPH Free Radical Scavenging Activity of Samples and IC_{50} Values



The IC_{50} value for *Spinach oleracea* was 42.57 µg/ml respectively which were comparatively lower than the IC_{50} (70.86µg/ml) of ascorbic acid. The IC_{50} value for *Annona squamosa* was 68.24(µg/ml) which was near to the standard. The scavenging effect increased with the increasing concentrations of test compounds. From the results of DPPH, *Annona squamosa* shows highest scavenging effect at the concentration 20 µg/ml. The scavenging effect was seen in appreciable amount at various concentrations in both selected leaves and it support the earlier studies carried out in an isolate compound from *Annona squamosa*[13]. It showed that all compounds are equally effective as antioxidant compared to ascorbic acid. The flavonoids from plants are reported to possess antidiabetic[14] and free radical scavenging activity[15].

Table 2
Mean Carotene Content (mg/100g) and Alkaloid Content (%) of the Samples

Samples	Carotene	Alkaloids
Spinach oleracea	58.88 ± 0.85	0.0392 ± 0.000
Annona squamosa	173.24 ±1.29	0.4893 ±0.002

Values are expressed in means ±SD for three determinations.

Carotene content in selected plants

The results of carotene content in selected plant leafs were shown in Table-2 .Among the selected plant leaves, *Annona squamosa* was found to have a maximum amount of carotene (173.24mg/100gm) followed by *spinach oleracea* (58.88 mg/gm). Kowsalya & Vidhya, (2004) reported that β-carotene content of Arai keerai in the form of sun dried and shade dried leaves contain 52.75 mg/100g , 66.56mg/100g respectively[16]. Lakshmi and Vimala (2000) evident that drying of green leafy vegetables can retain substantial amounts of β – carotene[17].

Alkaloid content in selected plants

The determination of alkaloids in the detected shade dried plant leafs were carried out by employing previously reported techniques[11] and the results were reported in Table -2. The

alkaloid content of *Spinach oleracea* and *Annona squamosa* was 0.0392% and 0.4893%respectively which were found to be lower than the*Moringa oleifera* in the form of extract[18] (0.07%) and shade dried leaf powder[19] (1.18%). Phytochemicals contained in plant foods have been linked to many positive effects on human health, including coronary heart diseases, diabetes, high blood pressure, cataracts, degenerative diseases, and obesity[20].

CONCLUSION

The present investigation shows that the selected shade dried plant leaf powder exhibited the greatest antioxidant activity. *Annona squamosa* shows good antioxidant activity as compared with the standard ascorbic acid and also found with higher amount of alkaloids and carotene followed by *Spinach oleracea*. Since the selected leafy vegetable samples are rich in carotene and varying levels of antioxidants, inclusion of these in the diet is likely to reduce oxidative stress. These findings provide basis for developing a valuable food recipes to enhance human nutrition via their phenolic composition and antioxidant activity.

REFERENCES

1. Pieroni A. Medicinal plants and food medicines in the folk traditions of the upper Lucca province, Italy.J.Ethnopharmacol,70:235-237, (2000).
2. Ceriello A. Oxidative stress and glycemic regulation. Metabolism; 49(2, Suppl 1):27–29, (2000).
3. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. Diabetes; 48:1–9,(1999).
4. Saxena AK, Srivastava P, Kale RK, Baquer NZ. Impaired antioxidant status in diabetic rat liver. Effect of vanadate. Biochem Pharmacol; 45(3):539–542,(1993).
5. Shahidi, F. Natural antioxidants: chemistry, health effects and applications. Urbana,IL: AOCS press, (1997).
6. Raj Sobiya. D., Vennila Jannet. J.,Aiyavu. C., Panneerselvam. "The heapatoprotective effectof alcoholic extract of Annona squamosa leaves on experimentally induced liver injury in Swiss albino mice". International Journal of Integrative Biology, 5(3):182-186, (2009).
7. Adam G., Mundry KW and Straub P. Isolation and Characterization of a Virus Inhibitor from Spinach *(Spinacia oleracea L.),*Journal of Phytopathology, 115: 357 – 367, (2008).
8. Gowdhami M., Sarkar BL., and Ayyasmy.Screening of Phytochemicals and Antibacterial Activity of *Annona Squamosa* Extracts, International Journal of Pharmaceutical Science Invention ISSN (Online), 2319-6718, ISSN (Print):2319-670X, Vol.3, Issue7:30-39,(2014).
9. Patel Rajesh M. and Patel Natwar J. In vitro antioxidant activity of coumarin compounds by DPPH, Super oxide and nitric oxide free radical scavenging methods. Journal of advanced pharmacy education and research, 1:52-68, (2011)
10. Sadasivam.S, Manickam. A.Biochemical methods,second edition, New Age International (P) Limited Publishers,187-188, (2004).
11. Harbone, J.B. Phytochemical methods A Guide to Modern Techniques of Plant Analysis, 2nd Edition, New York, Chapman and Hall,88-185, (1973).
12. Lai LS., Chou ST. etal. Studies on the antioxidative activities of Hsiantsao (Mesona procumbens.Hemsl.) leaf gum, *J.Agric*. Food Chem, 49: 963-968, (2001).
13. Chandrasekhar C., Kulkarni VR.Isolation, Characterizations and Free radical Scavenging Activity of *Annona Squamosa*

Leaf, Journal of Pharmacy research, 4(3):610-611,(2011)
14. Lukacinova A., Mojzis J., Benacka R., Keller J., Kurila P., Vasko L., Racz O. and Nistiar F. Preventive Effects of Flavonoids on Alloxan –Induced Diabetes Mellitus Rats. Acta vet. Brno,77: 175-82, (2008).
15. Desai P.V., Wadekar R.R., Kedar G.H. and Patil K.S. Free radical scavenging activity of aqueous extract of roots of *Baliospermum montanum* Moell-Arg, Int. Journal of Green Pharmacy, 2(1): 31-33 (2008).
16. Kowsalya, S. and Vidhya, M.R. Nutritive value of selected dehydrated green leafy vegetables, Ind.J.Nutr.Dietet, 41:279-286, (2004).
17. Lakshmi. B and Vimala. V. Nutritive Value of Dehydrated Green Leafy Vegetable Powders, Journal of Food Science and Technology, Vol 37:465-471, (2000).
18. Madukwe E.U., Ugwuoke A. L,and Ezeugwu J. O. Nutrient Composition and Sensory Evaluation of Dry *Moringa Oleifera* Aqueous Extract, International Journal of Basic & Applied Sciences IJBAS-IJENS, Vol:13 No:03:100-102, (2013).
19. Madukwe E. U., Ugwuoke A. L and Ezeugwu J. O**.**Effectiveness of dry *Moringa oleifera* leaf powder in treatment of anaemia, International Journal of Medicine and Medical Science, Vol.5(5):226-228, (2013).
20. Liu S., Manson J.E., Lee I.M., Cole S.R., Hennekens C.H., Willett W.C. and Buring J.E. Fruit and Vegetable Intake and Risk of Cardiovascular Disease: the women's health study, American Journal of Clinical Nutrition, 72:922–8, (2000).