Research Article

Antioxidant and Free Radical Scavenging Activity of Anthocyanins from Two Forms of *Brassica oleracea*

P. Gomathi[1,2]*, R. Prameela[2] and A. Sanjeevakumar[2]

[1]Pharmacognosy Course and Research Unit, Department of Pharmacy, College of Health Sciences, Mekelle University, Mekelle, Ethiopia.
[2]Vaagdevi College of Pharmacy, Kishanpura, Hanamkonda, Warangal, Andhra Pradesh, India.

ABSTRACT

Anthocyanins are natural pigments which are responsible for the blue, purple, violet and red colours in fruits, flowers, stem and leaves. They belong to major flavanoid classes such as flavones, isoflavones, flavonones and anthocyanins which have strong antioxidant activity. The anthocyanins have a long history as part of the human diet. In the present scenario, there is a rising demand for natural sources of food colorants with nutraceutical benefits. Sources including anthocyanins are becoming increasingly important for this reason. Recent reports have demonstrated multiple benefits associated with the consumption of anthocyanins, which play a major role as free radical scavengers. Red cabbage is a model plant for anthocyanins. *Brassica oleracea* was selected based on its anthocyanin content. So, the present study was aimed to evaluate antioxidant and free radical scavenging activities of anthocyanin fraction of white (WCAAE) and red cabbage (RCAE). These were performed by using Nitric oxide radical scavenging, DPPH radical scavenging activity, Hydrogen peroxide radical scavenging and Reducing power methods. RCAE is more potent than standard used in both Reducing power method, Hydrogen peroxide radical scavenging methods. Total phenolic contents, Total flavonoid contents were also measured by using standard procedures. The results showed RCAE was more potent than WCAE.

Keywords: Anthocyanins, Antioxidants, DPPH, Nitric oxide, Quercetin.

INTRODUCTION

Anthocyanins are becoming increasingly important not only as a plant pigment but also as food colorants and antioxidants. An antioxidant is a compound that inhibits or delays the oxidation of substrate even if the compound is present in a significantly lower concentration than the oxidized substrate and can be recycled in the cells or irreversibly damaged[1]. This antioxidant property of the pigment arise from their high reactivity as hydrogen or electron donors and from the ability of the polyphenol derived radicals to stabilize and delocalize the unpaired electrons and from their ability to chelate transition metal ion[2].

These pigments are reported to have many therapeutic benefits including vasoprotective and anti-inflammatory properties, anti cancer, chemo protective and anti-neoplastic properties, reversing age related deficits[3]. It has also been suggested that anthocyanins has got the ability to stabilize DNA triple helical complexes[4] and can also protect the chloroplast against high light intensities[5].

Based on the colour of the head, two forms viz *Brassica oleracea* var. *capitata* f. *alba* (white cabbage) – white or greenish head, f. *rubra* (red cabbage) – red cabbage. The plant changes color according to the pH value of the soil, due to the pigment anthocyanin[6]. Acidic soils – grow reddish, alkaline soil-greenish colored cabbage.

MATERIALS AND METHODS

Plant Material

Brassica oleracea var. *capitata* f. *alba* (white cabbage) and *Brassica oleracea* var. *capitata* f. *rubra* (red cabbage) were purchased from the local market in Warangal and authenticated by Dr. V S Raju, Department of Botany, Kakatiya University, Warangal.

Extraction of Anthocyanins

Methanolic extraction is the classical method of extracting anthocyanins from plant materials. This procedure involves maceration or soaking of the plant material in methanol containing a small concentration (0.01%) of mineral acid (e.g., HCl). Methanol extraction is a rapid, easy, and efficient method for anthocyanin extraction. However, a crude aqueous extract with several contaminants is obtained, and methanol evaporation can result

in hydrolysis of labile acyl linkages, which is aggravated by the presence of HCl[7]. The anthocyanin extract obtained from white cabbage is designated as WCAE and from red cabbage is designated as RCAE.

ANTIOXIDANT ASSAYS

Reducing Power Assay

The reducing power was determined according to the method of Oyaizu 1986[8]. Various concentrations of extracts (20-100µg/ml) were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50° C for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v) was added, the mixture was centrifuged at 3000 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% of ferric chloride, and the absorbance was measured at 700 nm: higher absorbance indicates higher reducing power. The assays were carried out in triplicate and the results were averaged. Ascorbic acid was used as standard[9,10].

Hydrogen Peroxide Radical Scavenging Activity

Plant extract prepared in various concentrations (20-100µg/ml) was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing the H_2O_2 without plant extract. All the analyses were performed in triplicate and results were averaged, and ascorbic acid used as a positive control treated in the same way with H_2O_2 solution. The percentage inhibition was measured by comparing the absorbance of control and test[11].

$$\textbf{H}_2\textbf{O}_2 \textbf{ scavenging activity} = \textbf{A}_{\textbf{control}} - \textbf{A}_{\textbf{test}} / \textbf{A}_{\textbf{control}} \times \textbf{100}$$

Where, $A_{control}$ = Absorbance of control reaction and A_{test} = Absorbance in the presence of the samples of extracts.

Nitric Oxide Radical Scavenging Activity

Using Sodium nitroprusside (SNP) in aqueous solution at physiological pH spontaneously generates NO which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess Reagent. Scavengers of NO compete with oxygen leading to reduced production of NO. SNP (10mM) in phosphate buffer saline (PBS) was mixed with 1ml of different concentration of extracts (20-100µg/ml) and incubated at 25°C for 150 minutes. To 1ml of incubated solution, 1ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 3% phosphoric acid) was added. The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine dichloride was read at 546 nm. All the analyses were performed in triplicate and results were averaged and ascorbic Acid used as a positive control treated in the same way with Griess reagent. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance of control and test[12].

$$\textbf{Nitric Oxide scavenged} = \textbf{A}_{\textbf{control}} - \textbf{A}_{\textbf{test}} / \textbf{A}_{\textbf{control}} \times \textbf{100}$$

Where, $A_{control}$ = Absorbance of control reaction and A_{test} = Absorbance in the presence of the samples of extracts.

DPPH Radical Scavenging Activity

The free radical scavenging activity of the different fractions of extract was measured using DPPH, employing the method of Blois, 1958[13]. One ml of extract and the reference compound in various concentrations (10, 25, 50, 75 and 100 µg/ml) were added to 1 ml of 0.1 mM solution of DPPH in methanol. After 30 minutes, absorbance was measured at 517 nm. A 0.1mM solution of DPPH in methanol was used as control, whereas ascorbic acid was used as a reference material. All tests were performed in triplicate. Percent inhibition was calculated using equation[10,13].

$$\textbf{Percentage Inhibition} = \textbf{A}_{\textbf{control}} - \textbf{A}_{\textbf{test}} / \textbf{A}_{\textbf{control}} \times \textbf{100}$$

Where, $A_{control}$ = Absorbance of control reaction and A_{test} = Absorbance of sample

RESULTS AND DISCUSSION

Brassica oleracea var. *capitata* f. *rubra* and *Brassica oleracea* var. *capitata* f. *alba* containing anthocyanins were extracted with maceration of the plant material in methanol containing a small concentration of mineral acid (e.g., HCl). Percentage yield was found to be 2.8%, 2.2% respectively.

Reductive ability

The reducing power increased as the extract concentration increased, indicating some compounds in both anthocyanin extracts were both electron donors and could react with free radicals to convert them more stable. In the present study reductive ability of RCAE was

more than that of standard i.e., ascorbic acid. Figure 1 shows the reducing power potentials of the RCAE, WCAE in comparison with a standard Ascorbic acid.

Hydrogen peroxide scavenging activity

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. The scavenging activity of hydrogen peroxide by ascorbic acid and the red cabbage anthocyanin extract exhibited scavenging activity higher than ascorbic acid and white cabbage anthocyanin extract exhibited lower than ascorbic acid. IC_{50} values of ascorbic acid, RCAE, WCAE were listed in the table 1. Figure 2 illustrates the Effect of RCAE and WCAE on Hydrogen peroxide radicals.

Nitric oxide scavenging activity

NO is an important chemical mediator generated by endothelial cells, macrophages etc., Excess concentration of NO is associated with several diseases. Oxygen reacts with excess NO generate nitrite and peroxynitrite anions, which act as free radicals. In the present study the extract competes with oxygen to react with NO and thus inhibits the generation of the anions. IC_{50} values of ascorbic acid, RCAE, were listed in the table 1. Figure 3 illustrates the Effect of RCAE and WCAE on nitric oxide radicals.

DPPH radical scavenging activity

DPPH is a relatively stable free radical and the assay determines the ability of anthocyanin extract to reduce DPPH radical to the corresponding hydrazine by converting the unpaired electrons to paired once. In the present study dose dependent inhibition of DPPH radical indicates that anthocyanin extract cause reduction of DPPH radical. IC_{50} values of ascorbic acid, RCAE, WCAE were listed in the table 1. Figure 4 illustrates the Effect of RCAE and WCAE on DPPH radicals.

CONCLUSION

Many plant phenolic compounds exhibiting antioxidant properties have been studied and proposed for production against oxidation. Extracts from plants which contribute health benefits to consumers, arising from protection from free radical – mediated deteriorations had stronger antioxidant activity than that of synthetic antioxidants. Anthocyanins represent a class of important antioxidants, as they are so common in human foods. The present study clearly points out the total flavonoid content, total phenolic content, total anthocyanin content and its antioxidant potential of the selected fruits emphasizing the importance of incorporating these vegetables as a regular component in diet. The data presented in this study demonstrates Antioxidant and free radical scavenging activities were performed by using Nitric oxide radical scavenging, DPPH radical scavenging activity, Hydrogen peroxide radical scavenging and Reducing power methods. RCAE is more potent than standard used in both Reducing power method, Hydrogen peroxide radical scavenging methods. The results showed RCAE was more potent than WCAE.

Table 1: Antioxidant activity of RCAE and WCAE

Method	IC_{50} values		
	Ascorbic Acid	RCAE	WCAE
Hydrogen peroxide scavenging activity	30.858	19.903	39.123
Nitric oxide scavenging activity	41.905	47.549	52.093
DPPH radical scavenging activity	8.129	6.016	8.254

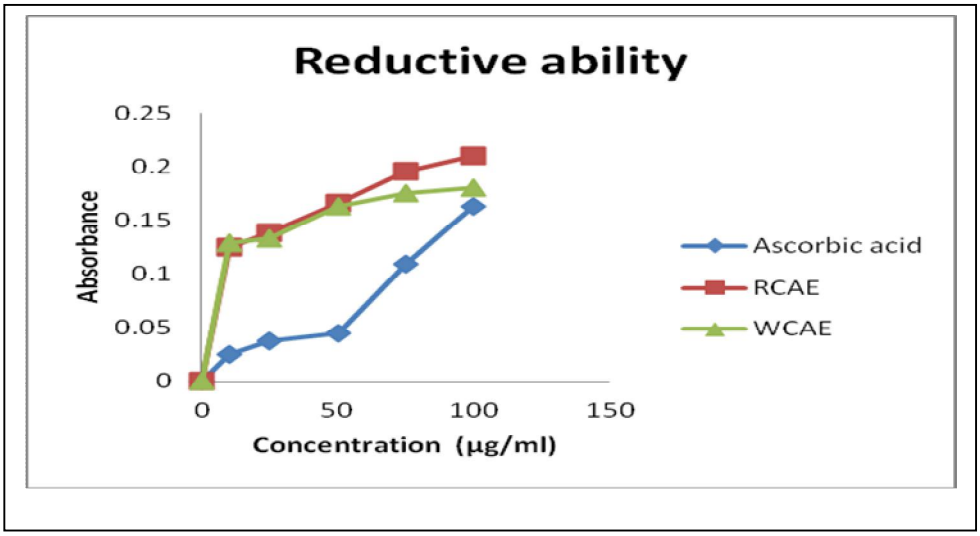

Fig. 1: Effect of RCAE and WCAE on reducing power

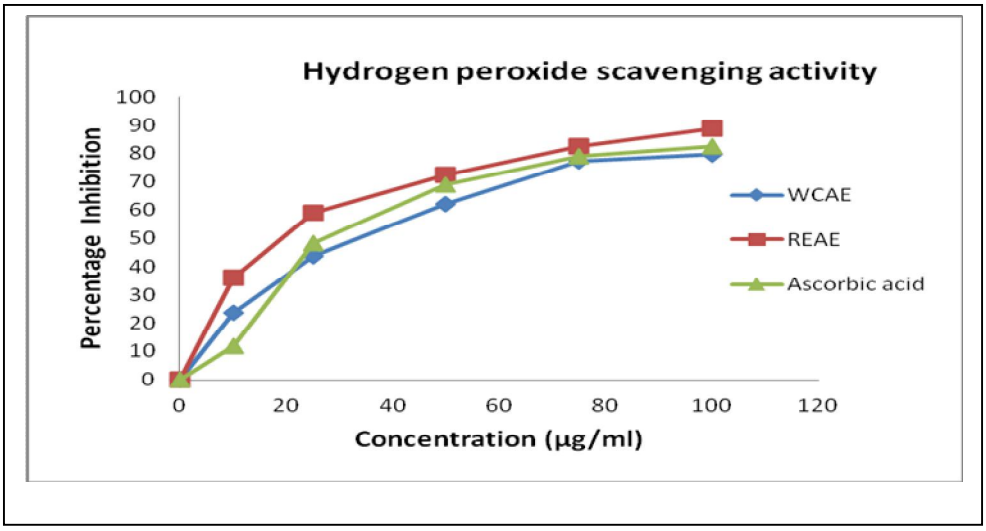

Fig. 1. Effect of RCAE and WCAE on hydrogen peroxide radicals

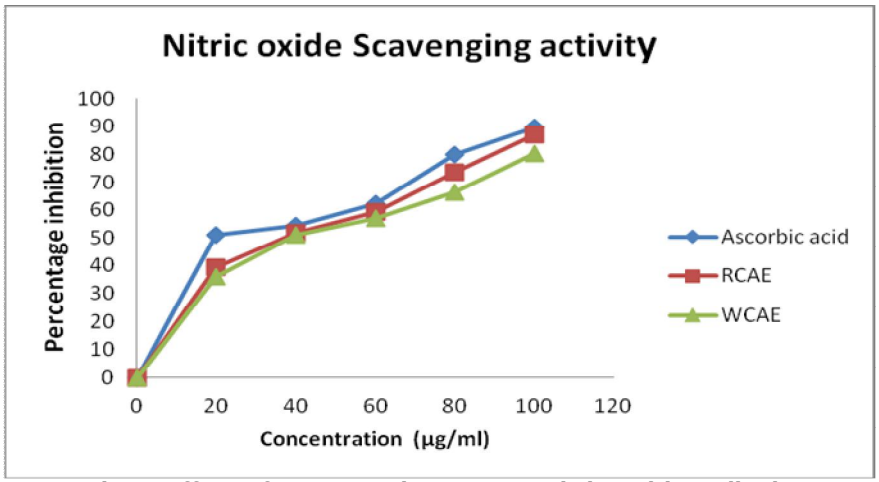

Fig 1: Effect of RCAE and WCAE on nitric oxide radicals

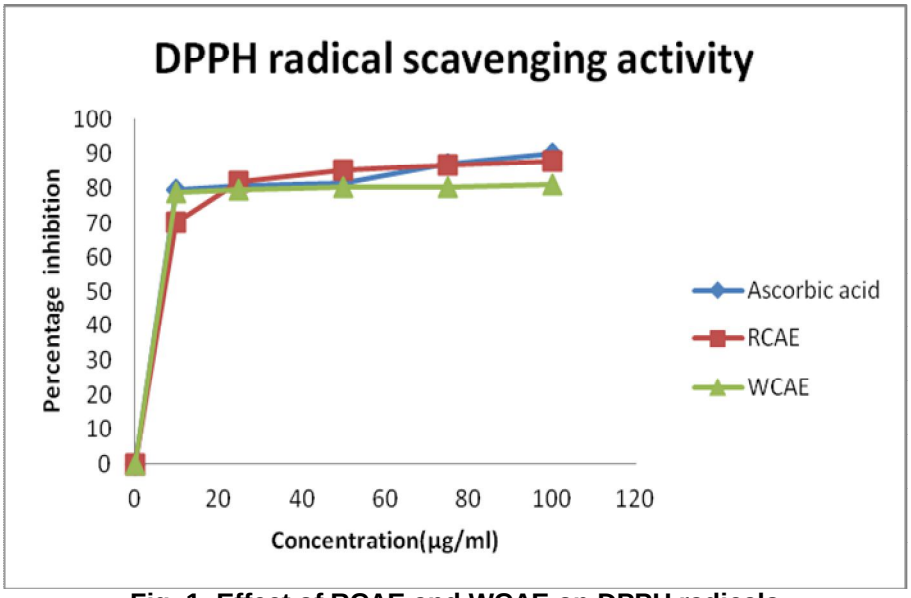

Fig. 1: Effect of RCAE and WCAE on DPPH radicals

REFERENCES

1 Halliwell B and Gutteridge. Free redicals in biology and medicine, 4th edition. Oxford: Oxford university press. . 2007;258.

2 Rice-Evans C, Miller NJ and Paganga G. Antioxidant properties of phenolic compounds. Trends plant sci. 1997;2:152-159.

3 Joseph O Kuti. Antioxidant compounds from four Opuntia cactus pear fruit varieties. Food chem. 2003;85:527-583.

4 Mas T, Susperregui J, Berké B, Chèze C, Moreau S, Nuhrich A and Vercauteren J. DNA triplex stabilization property of natural anthocyanins. Phytochem. 2000;53:679-687.

5 Pietrini, Lanelli and Massacci. Anthocyanin accumulation in the illuminated surface of maizeleaves enhances protection from photo inhibitory risk at low temperature without further limitation to photosynthesis. Plant, cell environ. 2002;25:1251-1259.

6 Ram P R, Mehrotra B N. Compendium of Indian Medicinal Plants, volume 5. 1998; 137-138.

7 Rodriguez S E, Ronald E W. Extraction, Isolation and Purification of Anthocyanins. Current Protocol Food Analyt Chem. 2001;F1.1.1-F1.1.11.

8 Oyaizu M. Studies on product of browning reaction prepared from glucoseamine. Jap. J. Nut. 1986;44:307-315.

9 Ghosh T, Maity T K, Bose A. In-vitro free radical scavenging activity of ethanolic extract of leaves of Cajanus cajan (L.) Millsp. J Nat Rem. 2009;9(2):228-234.

10 Gupta M, Mazumdhar U K, Gomathi P. Evaluation of anti-oxidant and free radical scavenging activities of Plumeria acuminata leaves. J Biol Sci. 2007;7(8):1361-1367.

11 Akinpelu D A, Aiyegoro O A, Okoh A I. The in vitro antioxidant property of methanolic extract of Afzelia africana (Smith.). J Med Plant Res. 2010;4(19):2021-2027.

12 Susuanta K M, Goutham C, Gupta M, Mazumdar U K. In vitro anti- oxidant activity of Diospyros malabarica kostel bark. Indian J Exp Biol.2006;44:39-44.

13 Nagulendran K R, Velavan S, Mahesh R, Hazeena begum V. In Vitro Antioxidant Activity and Total Polyphenolic Content of Cyperus rotundus Rhizomes. E-J Chem. 2007;4(3):440-449.