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Research Article

Extraction of anthocyanin and analyzing its antioxidant properties from different onion (*Allium cepa*) varieties

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ABSTRACT

Onion waste is a renewable raw material rich in different molecular species of antioxidant compounds. To utilize this source as a raw material an environmentally sustainable procedure has been developed for the extraction of anthocyanin and to analyze its antioxidant properties. Three (Red, Small, Big) varieties of onion (*Allium cepa)* were studied for their total phenol contents, total flavanoid content, total anthocyanin content and antioxidant activities. The data represented in this study demonstrated that the amount of phenolic compound and anthocyanin content differ significantly between 3 varieties of onion peel, and determine the free radical scavenging activity of onion peel. The anthocyanin and phenol content were found to be high in red onion variety when compared to other two varieties. The unutilized outer layer of onion varieties were found to be a rich source of anthocyanin and significant antioxidant properties. The onion peel which is considered as waste can be used as a rich source for anthocyanins and antioxidants.

Keywords: *Allium cepa;* red; anthocyanin; total phenol; Flavanoid content; antioxidants; free radicals scavenging effect.

1. INTRODUCTION

Flavanoids are phenolic substances isolated from a wide range of vascular plants, and more than 8150 different flavanoids have been reported (Andersen and Markham, 2006) so far. They act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellents, and also for light screening (Pieatta, 2000). Many studies have suggested that flavanoids exhibit biological activities, including antiallergenic, antiviral, anti-inflammatory, and vasodilating effects. The 12 main classes of flavonoids differ in the level of oxidation and the substitution pattern on the C ring, while individual compounds within a class differ in the substitution pattern on the A and B rings.

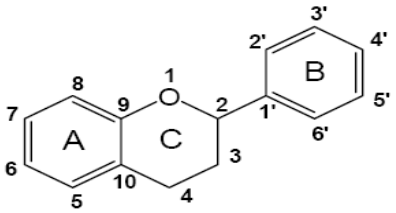

Figure 1: Basic flavanoids structure including the numbering system

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1.1 Anthocyanins

The word anthocyanin, derived from the Greek words (flower) and *kyanos* (blue) was originally used to describe the blue pigments of the cornflower, *Centaurea cyanus* (Marquart, 1835). Anthocyanins are polyphenolic compounds responsible for cyanic colors ranging from salmon pink through red and violet to dark blue of most flowers, fruits, leaves and stems. They comprise the largest group of the water-soluble pigments in the plant kingdom (Strack and Wray, 1994), and during the last few years it has been an exponential increase in the report of new anthocyanin structures (Andersen and Jordheim, 2006). This can be explained by the use of improved analytical techniques, but the potential use of anthocyanins as health beneficial compounds is another reason for the increased scientific interest in these pigments. They play a definite role in attracting insects in pollination and seed dispersal. They may also have a role in the mechanism of plant resistance to pest attack (Strack and Wray, 1989).

1.2 Structures

The anthocyanins consist of an aglycone (anthocyanidin), sugar(s), and, in many cases, acyl group(s). The classical anthocyanin aglycone is based on a C15 skeleton (C6–C3–C6 skeleton). (Andersen and Jordheim, 2006). Anthocyanins are positively charged at acidic pH. Even though there are around 30 different anthocyanidins, approximately 90% of all anthocyanins are based on the six most common anthocyanidins; pelargonidin, cyanidin, delphinidin, peonidin, petunidin and

malvidin which only differ by the hydroxylation and methoxylation pattern on their B-rings (Figure 2, left). The anthocyanins will differ with respect to glycoslyation of hydroxyl groups, nature of glycosyl units, substitution pattern, and potential aliphatic and aromatic acylation (Andersen and Jordheim, 2006). The 3- deoxyanthocyanidins (non glycosides) found in Sorghum. In plants spagnorubins and rosacyanin B are the only anthocyanidins (aglycones) found in their non glycosidated form. Andersen Jordheim, 2006 indicated the presence of cyanidin, peonidin and pelargonidin in black dried beans in glycosidated form (*Phaseolus vulgaris* L.). Pyranoanthocyanins (Figure 2, right) have been discovered in small amounts in wines and grape pomace (Bakker and Timberlake *et al.,* 1997; Fulcrand *et al.,* 1998; Mateus *et al.,* 2004; Cheynier, 2006.) More recently, glucosides of carboxypyranocyanidin have been isolated from red onion (Fossen and Andersen, 2003), and carboxypyranopelargonidin 3-glucoside from strawberry (Andersen *et al.,* 2004) extracts which are all in glycosidated form.

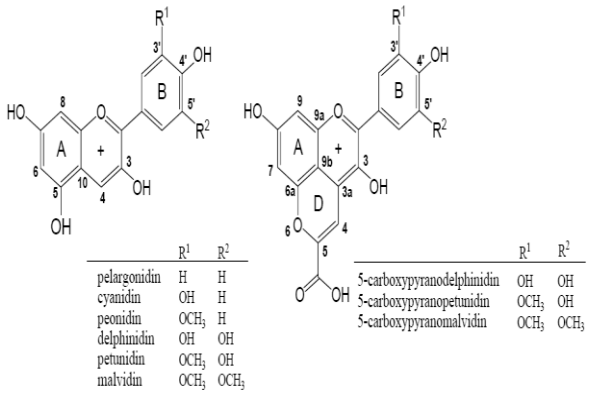

Figure 2: Left: Structures of the most common anthocyanidins occurring in nature; Right: Structures of some 5-carboxypyranoanthocyanidins

1.3 Functions in plants

Anthocyanins are involved in attraction of insects and animals for pollination and seed dispersal purposes as they constitute the chemical basis of flower color in angiosperms (Strack and Wray, 1994; Harborne and Williams, 1995). Their presence in young leaves, seedlings, roots and stems are not that obvious. There is increasing evidence that anthocyanins, particularly when they are located at the upper surface of the leaf or in the epidermal cells, have a role in the physiological survival of plants. The functions of anthocyanins in this context mainly been hypothesized as a compatible solute contributing to osmotic adjustment to drought and frost stress, and as antioxidant also as UV and visible light protectant.

1.4 Antioxidants

Are compounds that significantly delays or inhibits oxidation of a substrate. It may be considered as the scavengers of free radicals. Fruits and vegetables contain different antioxidant compounds,

(a) Chemical structure of quercetin,

(b) Chemical structure of quercetin-49-glycoside and

(c) Chemical structure of quercetin-3,4'-diglycoside

such as ascorbic acid, tocopherol, glutathione and carotinoids. It contributes to protect against oxidative damages. Antioxidant property of anthocyanin – high reactivity as hydrogen or electron donors, ability of the polyphenol derived radicals to stabilize and delocalize the unpaired electrons. Antioxidants are compounds with electron scavenging properties that may slow down or prevent the development of cancer. Quercetin, anthocyanins in grapes and red onions, and apples are found to have good antioxidant property. Furthermore, several recent studies have shown that different types of natural polyphenols may have neuroprotective effects both *in vitro* and *in vivo*, partly due to their electron scavenging properties. For example, it has been shown in a few studies that quercetin may prevent or slow down the development of Alzheimer's disease. Quercetin is a polyphenolic compound that occurs in vegetables and fruits mainly as different glycosides, although the skin of the fruit/vegetable commonly contains higher amounts of the quercetin aglycone.

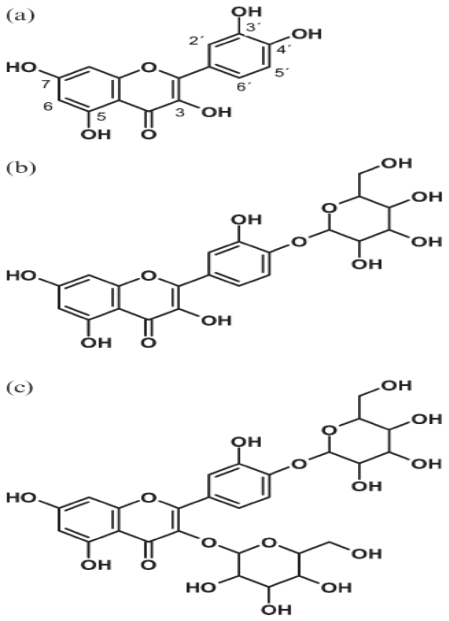

Figure 3: shows the chemical structure of quercetin and the two most common glycosides in onion, quercetin-49- glycoside and quercetin-3, 49-diglycoside

2. OBJECTIVE

The aim of the study is to determine the anthocyanin content in onion peel using different solvent system and analyze and to evaluate the antioxidants properties of anthocyanin from onion peel.

3. MATERIALS AND METHODS

3.1 Sample collection - Different varieties of onion peel were collected from the market and stored at -20˚C.

3.2 Extraction - 0.5 gm of onion peel were treated with 10 ml of 2 different solvents (methanol and acidified methanol). And the mixture was centrifuged at 10,000 rpm for 10 min and supernatant was taken for analysis.

3.3. Analytical procedures

3.3.1 Flavanoids conformation test

A. Fecl$_3$:1 ml of sample extraction was added with a small amount of $FeCl_3$**,** and results were observed.

B. Alcl$_3$: 1 ml of sample extraction was added with 5% of $AlCl_3$ solution, and results were observed.

3.3.2 Total phenolic assay

Total phenolic compounds in anthocyanin samples were quantified by using Folin-ciocalteu's method described by Ronald et.,al (1998).50 µl of Folin-ciocalteu's reagent (50% v/v) were added to 10µl of sample extract. It was incubated for 5 min. After incubation 50µl of 20 % (w/v) sodium carbonate and water was added to final volume of 400 µl. Blank was prepared by replacing the reagent by water to correct for interfering compounds. After 30 min of incubation, the absorbance was measured using spectrophotometer at 760 nm.

3.3.3 Stability at variable P^H

The anthocyanin stability was tested by treating 1 ml of sample with 1 ml of P^H 1.0 and 4.5 solutions. The color change was observed. (Strack, 1909).

3.3.4 Determination of total anthocyanin

The total amount of anthocyanin content was determined by using p ^{H} differential method. A spectrophotometer was used for the spectral measurements at 210 nm and 750 nm.(Fuleki & Francis,1968). The absorbance of the samples (A) was calculated as follows:

A= (Absorbance λ vis-max-A750) pH 1.0- (Absorbance λ vis-max-A750) pH 4.5 Anthocyanin pigment content (mg/liter) = (A X MW X DF X 1000)/ (ε X 1).

Where,

Molecular weight of anthocyanin (cyd-3-glu) = 449

Extraction coefficient (ε) =29,600

DF=Diluted factor

3.3.5 Total flavonoid content

The flavonoid content was determined according as the aluminum chloride colorimetric method described by Chang, Yang and Chern (2002).Briefly, aliquots of 0.1g of onion peel sample was dissolved in 1 ml of deionized water. This solution (0.5 ml) was mixed with 1.5 ml of 95% alcohol, 0.1 ml of 10 % aluminium chloride hexahydrate (AlCl3), 0.1 ml of 1 M potassium acetate (CH3COOK), and 2.8 ml of deionized water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415 nm against a deionized water blank on a spectrophotometer. Quercetin was used as a standard. Using a seven point standard curve (0-50mg/l), the levels of total flavonoid contents in onion peel was determined in triplicate, respectively. The data was expressed as milligram quercetin equivalents (QE)/100 g fresh matter from fresh the onion peel.

3.4 ANTIOXIDANT ASSAYS

3.4.1. Quantification of Ascorbic acid

This assay was carried out by the method of Sadasivam and Manickam (1997). 0.1 ml of brominated sample extract was added with 2.9 ml of distilled water. Then 1 ml of 2 % DNPH reagent and 1-2 drops of Thiourea was added with sample. After incubation at 37^{0}c for 3 hours, the range-red osazone crystals that were formed were dissolved by the addition of 7 ml of 80% Sulphuric acid. Again incubated for 5 minutes. After incubation absorbance was measured at 450 nm. Vitamin C concentration was expressed in terms of mg/g of sample.

3.4.2. Scavenging activity of DPPH radical

Scavevging activity of Anthocycanins against DPPH radicals was assessed according to the method of Larrauri, Sanchez-Moreno, and Saura-Calixto (1998) with some modifications. Briefly, 0.1 mM DPPH-methanol solution was mixed with 1 ml of 0.1mM DPPH methanol solution. After the solution was incubated for 30 min at 25º C in dark, the decrease in the absorbance at 517nm was measured. Control contained methanol instead of antioxidant solution while blanks contained methanol instead of DPPH solution in the experiment. Ascorbic acid and BHT were used as positive controls. The inhibition of DPPH radicals by the samples was calculated according to the following equation:

DPPH-scavenging activity (%) = [1-(absorbance of the sample-absorbance of blank)/absorbance of the control] ×100

3.4.3. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined according to the methods described by Singh, Murthy and Jayaprakash (2002). 0.1 ml of the different extracts of anthocyanin samples extract was taken in different test tubes. 1.0 ml of iron-EDTA solution (0.1% ferrous ammonium sulfate and0.26% EDTA), 0.5 ml of DMSO (0.85% v/v in 0.1 M Phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.5 ml 0f 0.22% ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80-90 ^{0}c for 15 min. The reaction was terminated by the addition of 1 ml of ice cold TCA (17.5 %w/v).3 ml of Nash reagent (75 g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added to all of the tubes and left at room temperature for 15 min for the color development. The intensity of the yellow color formed was measured spectrophotometrically at

412 nm against the reagent blank. The percentage of hydroxyl radical scavenging activity is calculated by using the formula:

% of hydroxyl radical scavenging activity=1-absorbance of sample/absorbance of blank×100

3.4.4. Determination of reducing power

The reducing power was determined according to the method of Oyaizu (1986). A 0.25ml aliquot of various concentrations of anthocyanins was mixed with 2.5 ml of 200mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50 º C for 20 min. after 2.5 ml of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 650g for 10 min. a 5ml aliquot of the upper layer was mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride at 700nm was measured. A higher absorbance a higher reducing power.

3.4.5 Determination of superoxide radical-Scavenging activity

Superoxide radicals were generated by the method of Ginnopolites and Ries (1977), described by Siddhurajuna, Mohab and Beckera (2000), with some modifications all solutions were prepared in 0.05 M phosphate buffer (pH 7.8). The photo induced reactions were performed in aluminium foil-lined box with two 30W fluorescent lamps. The distance between the reaction solution and the lamp was adjusted until the intensity of illumination reached about 4000 lux. A 30µL aliquot of various concentrations of anthocycanins was mixed with 3ml of reaction buffer solution (1.3 mm riboflavin, 13 mM methionine, 63 µM nitro blue tetrazolium and 100µM EDTA, pH 7.8). The reaction solution was illuminated for 15 min at 25 º C. The reaction mixture, without sample, was used as a control. The scavenging activity was calculated as follows: scavenging activity (%) = (1-absorbance of the sample/absorbance) ×100

3.4.6 Metal chelating activity

The chelation of ferrous ions by the extract was estimated by the method of Dinis *et.,al.(*1994) with slight modification and compared with that EDTA, BHT and that of ascorbic acid. The chelation test initially includes the addition of ferrous chloride. The antioxidants present in the samples chelates the ferrous ions from the ferrous chloride. The remaining ferrous combine with ferrozine to form ferrous-ferrozine complex. The intensity of the ferrous-ferrozin complex formation depends on the chelating capacity of the sample and the colour formation was measured at 562 nm (Shimadzu UV-Vis 2450).

Different concentrations of standard and extracts (100-500 µg/ml) were added to a solution of 100 µl FeCl2 (1mM).The reaction was initiated by the addition of 250 µl ferrozine (1 Mm). The mixture was finally quantified to 1.3 ml with methanol, shaken vigorously and left standing at room temperature for 10 min. after the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically. All the test and analysis were done in duplicate and average values were taken. The percentage inhibition of ferrous-ferrozine complex formation was calculated using the formula; %= 1-As/Ac X 100**.**

Where, 'Ac' is the absorbance of the control, 'As' is the absorbance of the sample.

3.4.7 β-carotene-linoleic acid test

Antioxidant activity of the samples was determined using the β-carotene-linoleic acid test. Approximately 10mg of β-carotene (type I synthetic, sigma-Aldrich) was dissolved in chloroform (10ml). The carotene-chloroform solution, (0.2ml) was pipette into a boiling flask containing linoleic acid (20 mg, sigma-Aldrich) and 200 mg Tween 40 (sigma-Aldrich). Choloroform was removed using a rotary evaporator at 40 C for 5min, and distilled water (50ml) was added to residue slowly with vigorous agitation, to form an emulsion. A portion of the emulsion (5ml) was added to a tube containing the sample solution (0.2ml) and the absorbance was immediately measured at 470 nm against a blank, consisting of an emulsion without β-carotene. The tubes were placed in a water bath at 50 C and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm over a 60min period. Control samples contained 200µL of water instead. Butylated hydroxytoluene (BHT, Sigma-Aldrich), a stable antioxidant, was used as a synthetic reference. The antioxidant activity was expressed as inhibition percentage with reference to the control after a 60 min incubation using the following equation: AA=100(DRc-DRs)/DRc.

Where,

AA=antioxidant activity; DRc=degradation DRs=degradation rate in presence of the sample = (1n (a/b)/60); a=absorbance time; rate of the control= (1n (a/b)/60); at 0;b=absorbance at 60 min.

4. RESULTS AND DISCUSSION

1. Anthocyanin extraction and Quantification

The extraction of anthocyanin from onion peel was done by using methanol and acidified methanol solvent systems. A spectrum of the extract, especially the peek in the visible region was recorded at 400 nm, where a single peek was observed in acidified methanol extract and the absorbance was also increasing in the case of methanol extract (Virachnee *et.al.,* 2004)

2. Flavonoid confirmation test

In the presence of $FeCl_3$ the methanol and acidified methanol extracts showed brown color which confirms the presence of Flavanoids. In the presence of $AlCl_3$ dark color was observed in methanol and acidified methanol extracts.

Table 1: Total anthocyanin, flavanoid and phenol content in different onion (*Allium cepa*) varieties

Different onion (*Allium cepa*) varieties				
Assays	**Solvents**	**Red onion (mg/g)**	**Small onion(mg/g)**	**Big onion (mg/g)**
Total anthocyanin	Methanol	80	30	40
	1% methanol Hcl	109	58	35
Total flavanoid content	Methanol	0.362	0.322	0.174
	1% methanol Hcl	1.276	0.988	0.922
Total phenol	Methanol	530	376	420
	1% methanol Hcl	505	358	380

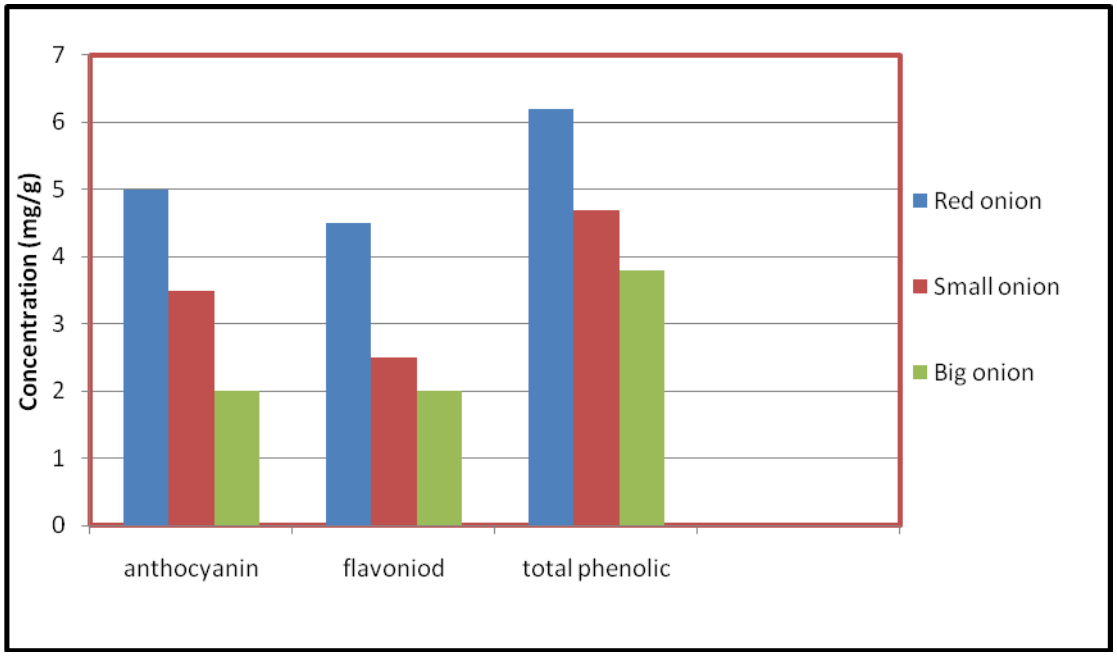

Figure 4: Total anthocyanin, flavonoid and phenolic content's of *Allium cepa*

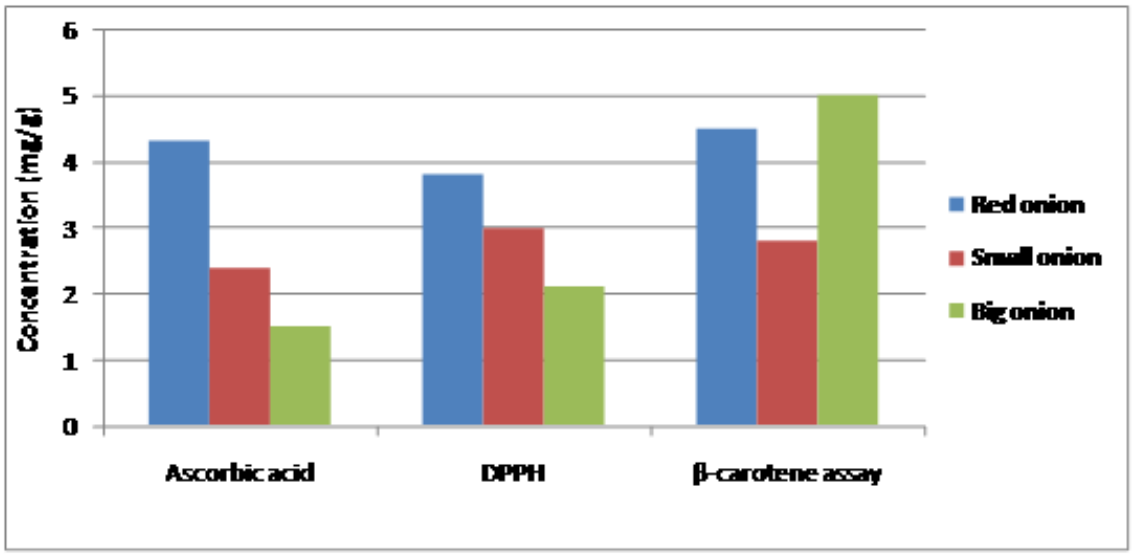

Figure 5: Antioxidant activity of *Allium cepa* varieties

3. Total phenolic assay

Phenolic content in onion peel varies considerably particularly with cultivar (Bajaj *et.,al*., 1980). Total extracted phenol from onion peel varied in different solvent system.ie, In red onion (530 mg/g) followed with big onion (420 mg/g) and small onion (376 mg/g).In acidified methanol the extracted phenol content was found to be less (Table-1).When methanol is used as a solvent the extracted phenol content was high in red onion it was very low in small onion.

Table 2: Antioxidant analysis of different solvent extract in different onion peel (*Allium cepa*) varieties

Different onion peel (*Allium cepa*) varieties				
Antioxidant Assay's	**Solvents**	**Red onion**	**Small onion**	**Big onion**
Ascorbic acid (mg/g)	Methanol	0.49	0.09	0.18
	1% methanol Hcl	0.75	0.14	0.09
DPPH radicals (%)	Methanol	66	76	78
	1% methanol Hcl	62	24	21
Hydroxyl radical scavenging activity (%)	Methanol	29	20	28
	1% methanol Hcl	14	20	23
Reducing power (%)	Methanol	1.178	0.956	1.15
	1% methanol Hcl	1.18	0.968	1.168
Superoxide radical-Scavenging activity (%)	Methanol	95	48.4	53.12
	1% methanol Hcl	97	57.5	68
Metal chelating activity (**%**)	Methanol	67	70	69
	1% methanol Hcl	91	93	97
β -Carotene assay (%)	Methanol	15	9	3
	1% methanol Hcl	19	28	15

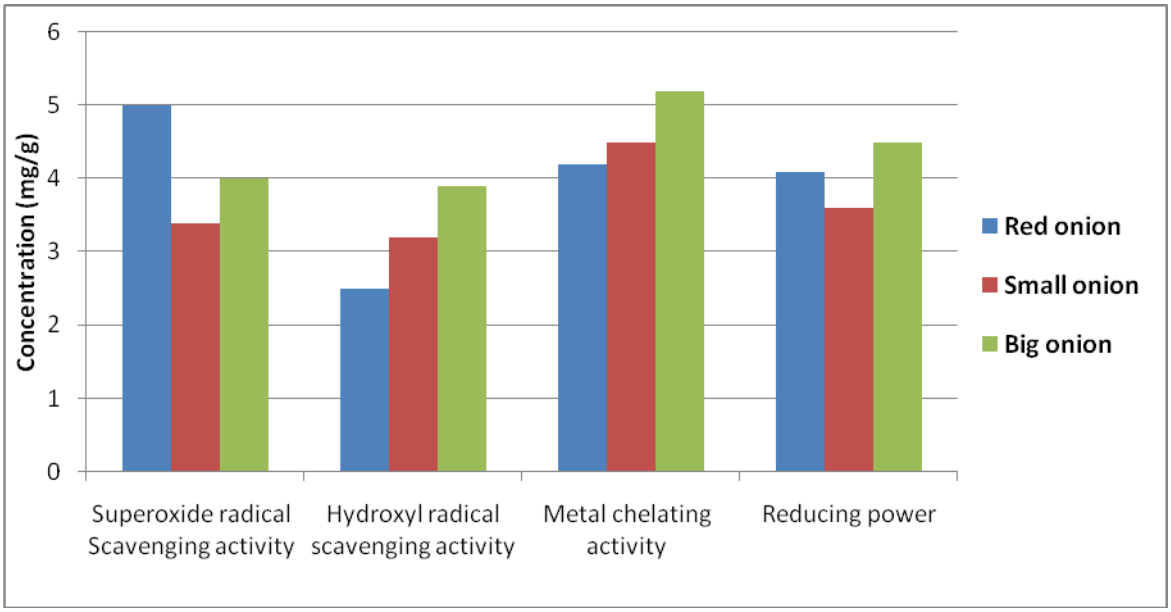

Figure 6: Antioxidant activity of *Allium cepa* varieties

4. Stability at variable pH

The sample appears red color at pH 1 and color disappeared at pH 4.5, Giusti, 2003 reported that, the anthocyanins are stable in low pH. The results were found to be same in the extracts of methanol and acidified methanol.

5. Determination of total anthocyanin

The total anthocyanin content in methanol extracts of red, small, big onion peel were 80 mg/g,

30 mg/g, 40 mg/g respectively and in acidified methanol extracts the total anthocyanin contents were 109 mg/g, 58 mg/g, and 35 mg/g respectively. (Table-1).Acidified methanol extract resulted significantly higher values i.e., 17 % highest than the methanol extract. Several authors reported that acidified methanol preserves the extracted anthocyanin in their original form better. It should be the solvent of choice for quantification and analysis of anthocyanins.

6. Total Flavanoids content

This study shows that total flavanoid content extracted from onion peel using acidified methanol is high in concentration. Amoung the varieties red onion has given highest yield of flavanoid content (1.276 mg/g), followed by small onion (0.988mg/g) and big onion (0.922mg/g) (Table-1). Our results suggest that when the acidified methanol is used as a solvent system, the total phenolic and flavanoid content is highest in red onion followed by small and big onion. Hakkinen and Torronen (2000) reported that flavonoid and selected phenolic acid contents in strawberry and *Vaccinium* species are influenced by cultivar, cultivation site and technique (Hakkinen and Torronen (2000) reported that flavonoid and selected phenolic acid contents in strawberry and *Vaccinium* species are influenced by

cultivar, cultivation site and technique (Hakkinen and Torronen).In our results correlation was observed between total phenolic, flavanoid content and anthocyanin content. Jin *et.,al.,* 2006 have reported that high correlation was observed with phenolic and flavanoid content from red onion. Similar results were observed in this experiment.

7. ANTIOXIDANTS

7.1 Ascorbic acid assay

It was observed that the vitamin C content was very low in big onion peel extract (0.1 mg/g) where as in red onion and small onion extracts. It was observed as 0.98 and 1.5 mg/g. (Table-1) Foyer, 1993 has reported that ascorbate has been found in chloroplast, vacuole and extracellular compartments of plant cell and shown to function as a reluctant for many free radicals.

7.2 Scavenging activity of DPPH radical

The ability of phenolic compound quench reactive species by hydrogen donation was measured through the DPPH radical scavenging activity assay. Activity is measured as the reactive decrease in absorbance at 517nm as the reaction between DPPH and antioxidant progresses. (Huang *et,al.,* and Singh & Ragini, 2004). Antioxidant activity was evaluated with percentage inhibition values, the concentration and the radical scavenging activity as listed in the table-2.The result indicated the percentage inhibition values of onion peel extract ranging from 21 to 78 %.

Methanol extract of big onion peel had highest radical scavenging activity, while in acidified methanol extract very low activity was observed. Genotype and growth conditions such as water availability and temperature affect the synthesis and accumulation of phenolic compounds in some parts of the plant consequently, antioxidant activity. (Reyes, 2005) and (Kalt, 2005).

7.3 Hydroxyl radical scavenging activity

The hydroxyl radical is extremely reactive free radicals formed in biological system and has been implicated as a higher damaging species in free radical pathology. Capable of damaging almost every molecule found in living cells. This species is considered to be one of the quick initiators of the lipid peroxidation process, abstracting hydrogen atoms from unsaturated fattyacids. (Kappus, 1991). (Figure-6). The hydroxyl radical scavenging activity of the different varieties of onion peel extract was given in table 2. Methanol extract of red onion peel exhibited a highest activity of 29 % followed by big onion peel extract with activity of 28 % and small onion extract with 20 % activity. This is similar to the observations of several others who have reported, a dose dependent activity in other fruits such as pomegranate peel and grape pomace.(Chang *et.,al.,2002,*). The ability of methanol extract of anthocyanin to quench hydroxyl radical seems to be directly related in to prevention of propagation of the process of lipid peroxidation. The methanol extract seems to be a good scavenger of active oxygen species, thus reducing rate of chain reaction. A high positive correlation was observed between the polyphenol content and hydroxyl radical scavenging activity of total phenolic content of onion peel extracts.

7.4 Reducing power

The potassium ferric cyanide reduction method was used to measure the ability of phenolic compounds to quench radicals through electron donation. The activity of total antioxidants of onion peel is measured by spectrophotometer and in which the change in absorbance is measured when the antioxidant reduces the ferric ion/cyanide complex to the ferrous form and resulted with higher absorbance value(Chou, *et.al.,* 2003). Table -2 shows reducing power of the different varieties onion peel extract. In the present study, the acidified methanol extracts of red onion reveals the highest reducing power as 1.188 mg/ml followed by big onion and small onion peel varieties. The reducing property are generally associated with the presence of reductones (Pin-per, Duh, 1998) which have been shown to exert antioxidant action by breaking free radical chain by donating a hydrogen atom (Crondon, 1990).

7.5 Superoxide radical-Scavenging activity Superoxide anion radicals are produced by a number of cellular reactions, including various enzymes systems such as lipooxygenase, peroxidase, NADPH oxidase and xanthain oxidase. Superoxide anions place an important role in plant tissue and are involved in the formation of other cell damaging free radicals. (Bloknina *et.al*., 2003). In the present study, superoxide radical can be generated by illuminating a solution containing riboflavin. Based on the results obtained as represented in the table-2, it is clear that methanol extract of red onion peel has better super oxide scavenging activity as compare to the other two sample extracts, which may be again due to the higher amount of total phenol content.

As with the reducing power, the anthocyanins exhibited an excellent superoxide anion scavenging activity, which was much higher than those of ascorbic acid and BHT. Further superoxides are also known to indirectly initiate lipid peroxidation as a result of H_2O_2 formation, creating precursors of hydroxyl radicals (Meyar and Isakser, 1995). Our result clearly shows that antioxidant activity of anthocyanins extracted from different varieties of onion peel is also related to its ability to scavenge superoxides.

7.6 Metal chelating activity

The ability of antioxidants to form insoluble metal complexes with ferrous ion or to generate steric hindrance that prevent interaction between metal and lipid is evaluated using the ion chelating capacity assay (Hsu *et.al.,* 2003). The activity is measured by monitoring the decrease in absorbance of the red ferric (Fe2+)

- ferrozin complex as antioxidants complete with ferrozin in chelating ferrous ion (Elmastas *et.al.,* 2003). Figure-6 shows the metal chelating power of different varieties of onion peel. From the table it is clear that chelating power of methanol extract of big onion peel was higher as compared with two varieties, while acidified methanol extracts of red onion peel showed lowest metal chelating activity among the varieties. Correlation was found between ion chelating capacity and phenolic content in all the 3 varieties of onion peel. This may indicate the presence of antioxidants responsible for metal chelation.

Non phenolic metal chelators include phosphoric acid, ascorbic acid, carnosin, some amino acids, peptides and proteins such as transferrin ovotransferrin are also responsible for metal chelation (Lee *et.,al* 2004). In our above results of ascorbic acid assay, indicated that acidified methanol extracts of onion peel showed higher activity. So the metal chelating activity of different onion peel extracts is due to phenolic and non phenolic compounds. So the results of the above analysis indicated that both phenolic and nonphenolic metal chelators like ascorbic acid are responsible for metal chelating activity in onion peel. (Al-Saikhan *et.,al.,* 1995) reported that potato contains ascorbic acid and reduced tripeptides.

7 β -Carotene assay

In the β- Carotene bleaching assay, oxidation of linoleic acid releases linoleic acid peroxide as free radicals that oxidize β-Carotene resulting in discolorization, (Figure-5) thus decreasing the absorbance value (Talcott *et.,al* (2002)). A linear relationship was found between the ability of a sample extract which inhibit oxidation and antioxidant capacity. In this study it was observed that the highest value of β carotene in small onion (28 mg/g) and lowest in other 2 varieties.Salunkhe and Desai (1988) reported that the same genera of fruit from different locations exhibited a variation in antioxidant capacity. These finding agree with our results also.

8. CONCLUSION

The onion peel could be evaluated as a major source of anthocyanin, Flavanoids and polyphenol antioxidants. Antioxidant activity of onion peel is well known and reported in the literature. The data represented in the study demonstrated that the amount of anthocyanin and phenolic compounds differ significantly between different varieties and determine the free radical scavenging activity of different varieties of onion peel.

9. AKNOWLEDGEMENT

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