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Research Article

NUTRITIONAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF PUMPKIN WASTES

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ABSTRACT

The waste generated during processing of pumpkin is in the form of peel and pulp. The present study aims to evaluate the proximate parameters and antioxidant potential of processing wastes from pumpkins. The peel and pulp wastes were oven dried, powdered and analysed for moisture, protein, ash, crude fibre, dietary fibre, β-carotene and minerals like phosphorus and iron. The methanol extracts prepared from the powdered peel and pulp was used for determining total polyphenols and antioxidant activity. The peel was found to be a good source of minerals like phosphorus (319.33 mg/100 gm) and iron (42.99 mg/100 gm) and dietary fibre (28.81%). The pulp was found to be a rich source of β-carotene (142.38 mg/100 gm). The peel and pulp samples presented almost similar values of polyphenols and antioxidants. Also, the peel and pulp exhibited a similar dose-dependent inhibition of DPPH activity. Overall, the results show that pumpkin wastes can be exploited for the nutrient and antioxidant components and used for value addition in food formulations. Hence, this study can pave the way for utilisation of bio-wastes from the pumpkin.

Keywords: Pumpkin, Total Phenolics, Antioxidant activity.

INTRODUCTION

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. They are compounds when added to food products, especially to lipids and lipid-containing foods, can increase the shelf-life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food products during processing and storage. They can be synthetic or natural. However, some important restrictions on the use of synthetic antioxidants are being imposed due to their potential carcinogenicity[1]. Many synthetic antioxidants, such as butylated hydroxytoluene (BHT), Butylated hyroxyanisole (BHA) and tertiary butyl hydroquinone (TBHQ) are commonly used for preservation of fats and oils. However, growing scientific evidences have shown adverse effects of synthetic antioxidants[2]. Therefore, there has been an upsurge of interest for exploitation of natural antioxidants, especially of plant origin. Also, there has been growing interest in the beneficial health effects of consuming fruits and vegetables. Mainly, the presence of phenolic antioxidants is believed to have the protective mechanisms. Polyphenols are naturally occurring substances in all plant materials, particularly in fruits, vegetables, seeds and herbs, and also in plant products such as beverages, wines or cocoa[3]. Phenolic compounds from natural sources have attracted great attention during the last two decades, because they are potent antioxidants that play an important role in protecting the body tissues against oxidative stress, and thus contribute to human health[4]

Pumpkins (Cucurbit sp) belonging to the *Cucurbitaceae* family are grown widely around the world. It is cultivated from Northern Mexico to Argentina and Chile and has spread to Europe (France and Portugal), Asia (India and China) and Western America. Production statistics reveal that India is one of the leading producers of pumpkin in the world (Anon 2008)[5]. The immature fruit is cooked as a vegetable, while

the mature fruit is sweet and used to make confectionery and beverages. Pumpkins are seasonal crop that have been used traditionally both as human food and animal feed. The fruit has good β-carotene content and has a moderate content of carbohydrates, vitamins and minerals[6]. Norfezah Md Nor[7] in the study on the development of expanded snack foods containing pumpkin flour categorized the pumpkin fractional composition as 10-12% peel, 3-4% pulp, 79-82% flesh and 4-6% seed. It was found that the food processing mostly used only the fleshy part of pumpkin, which could generate 18-21% of pumpkin waste.

Antioxidants are of interest to both food scientists and health professionals and there has been a convergence of interest among researchers in these fields as the role of antioxidants in the diet and their impact on human health has come under attention. Various extracts of pumpkin have potential antioxidant activity which might play an important role in pre-diabetics, diabetics and individuals with vascular injury. Xia & Wang[8] demonstrated the hypoglycaemic action of pumpkin (fruit) extract as well as its role as an antioxidant to reveal a mechanism for its cytoprotective (cell-protecting) action in streptozotocin-induced diabetic animals. Pumpkin seeds have high content of vitamin E (tocopherol; an antioxidant), and pumpkin seed oil has been considered to provide a significant source of vitamin E in Japanese diets[9]. The antioxidant activities of pumpkin[10], male and female flower extracts[11], oil[12] and cold-pressed pumpkin seed flour[13] have been reported. The objective of the present study was to evaluate the total polyphenols and antioxidant activity of pumpkin peel and pulp, generated as processing waste.

2.0 MATERIALS AND METHODS

2.1 Raw material

Pumpkin was procured from local market in Hyderabad, Telengana, India. It was washed and peeled. The peel and pulp were separated manually and dried in a tray drier at 55 ± 5°C for 8 hrs. They were powdered in a domestic mixer. The powdered samples were packed in MPE pouches until the time of analysis.

2.2 CHEMICAL AND REAGENTS

Chemicals and solvents used in the study were of laboratory grade and were procured from SD Fine-Chem Ltd. (Mumbai, India).

2.3 Proximate analysis of Peel and Fibrous matter

Dried powder of pumpkin peel and pulp was analysed for proximate parameters such as moisture, ash, crude fibre and protein. Moisture was estimated by oven method, Protein by Kjeldahl nitrogen method, Crude fibre by acid alkali digestion method and crude fat was extracted with Hexane in a soxhlet apparatus. Total ash content was determined by igniting the samples in a muffle furnace, at 550°C, for 3-4 h. The samples were also analysed for total dietary fibre as per AOAC 16th Edition[14].

2.4 Estimation of phosphorus and Iron

Phosphorus and iron were analyzed by colorimetric method according to Ranganna[15]. To the ash solution, molybdate reagent was added and mixed followed by addition of aminonaphthosulphonic acid solution with constant mixing, and making up to volume. Similarly a blank was prepared using water instead of the sample. It was allowed to stand for 10 mins and colour measured at 650 nm setting the blank at 100% transmission. The iron was determined by converting the iron to ferric form using oxidising agents like potassium persulphate or hydrogen peroxide and treating thereafter with potassium thiocyanate to form the red ferric thiocyanate which was measured colorimetrically at 480 nm.

2.5 Estimation of Ascorbic acid

Ascorbic acid was estimated by 2, 6 dichlorophenol indophenol titration method, which is based on reduction of the dye colour from blue to pale pink by ascorbic acid [15]

2.6 Extraction and Quantification of β-Carotene by HPLC in pumpkin peel and fibrous matter

Standard β-carotene preparation- β-carotene standard (5 mg/100 ml) was freshly prepared in hexane and stored in an amber coloured volumetric flask prior to use. 1 ml of β-carotene from this stock solution was further diluted with hexane to yield a final concentration of 1 mg/ml. The extraction of β-carotene in the peel and pulp powder was carried out with acetone and further purification with hexane and distilled water. The sample (1.0 g) was extracted with acetone until the residue became colourless. The extracts were transferred to a separating funnel followed by addition of hexane and water. The Hexane extract was collected and its volume adjusted. The solution was then filtered with anhydrous sodium sulphate. The β-carotene in the pumpkin flour was identified using the HPLC system software by comparing retention time (RT) of unknown peak with reference standard. The system consisted of Shimadzu prominence LC-20AD binary gradient fitted with an ultra restek HPLC C_{18} analytical column (25 cm x 4.6

mm ID) 5µ particle size. Detection was done by SPD 20A series variable wavelength detector at wavelength of 450.0 nm. The gradient mobile phase consisted of acetonitrile and chloroform with a flow rate of 1ml/min. The elution program involved a linear gradient from 80 to 20% of acetonitrile for 0-5 min and 20 to 80% of chloroform from 5-15 min and again 80% of acetonitrile for 15-20 min followed by 5 min equilibrium. Total programme time was of 25 min, compounds were quantified using LC solution software. 10µL samples were injected after dissolving the same in hexane.

2.7 Preparation of sample extracts

For estimation of polyphenols and antioxidant activity, the powdered samples were extracted in methanol. 5g of sample was suspended in 100 ml methanol, allowed to extract for 3 hours with agitation, centrifuged at 3000 rpm and filtered[16]. The extracts were analyzed for polyphenols and antioxidant activity.

2.8 Determination of total phenolics

Total polyphenol content (TPC) was determined using the Folin-Ciocalteu's reagent[17]. To the methanolic extracts of pumpkin peel and pulp, 0.5 ml of Folin-Ciocalteu reagent was added. The contents were mixed and added with 1 ml of saturated sodium carbonate solution, followed by adjusting the volume to 10 ml with distilled water. The mixture in the tubes were thoroughly mixed by vortexing. Tubes were allowed to stand at ambient temperature for 1 hr until the characteristic blue color developed. The control was prepared with methanol. Absorbance of the clear supernatants was measured at 675 nm using a spectrophotometer. Gallic acid was used as a standard and total phenolic content was calculated and expressed as mg gallic acid equivalent (GAE) per g sample. All analyses were performed in triplicate.

2.9 Radical scavenging activity by DPPH assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured following the method of Nanjo et al[18]. Different concentrations of pumpkin peel and pulp extracts were taken in test tubes and the volume made up to 1ml. 4 ml of 0.1 mM methanolic solution of DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand at room temperature for 30 min. The control was prepared as above without any extract. The changes in the absorbance of the samples were measured at 517 nm. Radical Scavenging activity was expressed as the inhibition percentage and was calculated using the following formula

% Radical scavenging activity = (Control OD - Sample OD/Control OD) x 100

The IC_{50} is defined as the concentration of antioxidant necessary to decrease the initial DPPH concentration by 50%. The IC_{50} of the samples was derived from the % scavenging activity vs concentration plot and is expressed as mg/ml.

2.10 ABTS assay

The method of Re et al.[19] was adopted for the determination of ABTS activity of the extracts. This assay is based on decolorization that occurs when the radical cation ABTS+ is reduced to ABTS' (2, 2'azino-bis (3-ethylbenzthiazoline-6-sulphonic acid). In brief, the radical was generated by reaction of ABTS in water with potassium persulphate ($K_2S_2O_8$) (1:1). The mixture was held in dark at 27°C for 16 h (time needed to obtain stable absorbance at 734 nm). After incubation, the solution was further diluted with water (1 ml of ABTS reagent made upto 40 ml with DW) to obtain an initial absorbance value of 0.7 ± 0.005 at 734 nm. For the assay, the extracts of peel and pulp were allowed to react with 2 ml of ABTS solution for 30 min and absorbance was measured at 734 nm. The percentage of scavenging inhibition capacity of the extract was calculated using the following equation.

$$\% \text{ Inhibition} = [(Abs_{control} - Abs_{sample})] / (Abs_{control}) \times 100$$

2.11 Ferric ion reducing antioxidant power assay (FRAP)

Ferric ions reducing power was measured according to the method reported by Rohan and Anup[20]. The methanolic extracts of peel and pulp were mixed with 2.5 ml of phosphate buffer and 2.5 ml of 1% potassium ferricyanide and the mixture was incubated at 50°C for 30 min. After incubation time, 2.5 ml of 10% Trichloroacetic acid (TCA) and 0.5 ml of 1% ferric chloride was added to the mixture, which was kept aside for 10 mins. Finally, the absorbance was measured at 700 nm. Ascorbic acid was used as a positive reference standard.

3.0 RESULTS AND DISCUSSION

3.1 Proximate analysis of peel and pulp

The nutritional composition of the powdered pumpkin peel and pulp is presented in Table 1. The moisture content at the time of analysis was found to be around 7.235 and 11.45% respectively in peel and pulp (Table 1). Protein content was more or less similar in both peel (12.5%) as well as pulp (12.28%). The ash content of pulp was high (10.17%) as compared

to peel (6.04%) indicating high mineral content. Both the wastes were found to be good source of phosphorus and iron. The vitamin C content of the peel and pulp samples was around 18.90 mg/100 g and 14.67 mg/100 g respectively. These results indicate that if the peel and pulp wastes were to be used for value addition, they would contribute considerable amounts of nutrients to products. Identification and quantification of β-carotene in pumpkin peel and pulp was carried out by HPLC method. The ß-carotene is eluted after 8 mins and was identified with standard β-carotene (Fig 1). The pulp waste (142.38 mg/100g) was found to be a rich source of β-carotene (Fig 2 & 3) than peel (11.89mg/100 g)

3.2 Total polyphenols

Phenolic moieties present in the molecular structure of natural antioxidants often help in enhancing their antioxidant activity [21,22]. Phenolic compounds such as tannins, flavonoids are considered to be the major contributors to the antioxidant capacity of plants. These antioxidants also possess diverse biological activities, such as anti-inflammatory, anti-atherosclerotic and anti-carcinogenic activities[23]. The total phenolic contents of peel and pulp extracts of pumpkin as determined by Folin-Ciocalteu method are reported as gallic acid equivalents (Table 2). The phenolic content was similar for peel (5.21 mg GAE/g) and pulp (5.19 mg GAE/g) (Table 2).

3.3 IC_{50} of DPPH scavenging activity

DPPH radical scavenging activity is one of the most widely used method for screening the antioxidant activity of plant extract. The radical scavenging activities of the extracts were determined by using DPPH a stable free radical. 1, 1-diphenyl-2-picrylhydrazyl is a nitrogen-centered free radical, colour of which changes from violet to yellow on reduction. The degree of discoloration indicates the scavenging potentials of the extracts. The results are often expressed as IC_{50} which is the concentration of the sample required to scavenge 50% of the free radicals present in the system. A lower IC_{50} value indicates a high radical scavenging ability. Fig 4 shows the antioxidant activity of methanol extracts of peel and pulp assessed using the DPPH radical scavenging. The methanol extract of pumpkin peel and pulp exhibited a similar dose-dependent inhibition of DPPH activity, with a 50% inhibition (IC_{50}) at a concentration of 18 mg/ml. At a concentration of 50 mg/ml, the scavenging activity of peel and pulp extract reached around 80%. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability[24].

3.4 IC_{50} of ABTS scavenging activity

ABTS is also frequently used by the food industry and agricultural researchers to measure the antioxidant capacities of foods. In this assay, ABTS is converted to its radical cation by addition of potassium persulfate. This radical cation is blue in color and absorbs light at 734 nm. The ABTS radical cation is reactive towards most antioxidants including phenolics, thiols and ascorbic acid. During this reaction, the blue ABTS radical cation is converted back to its colorless neutral form. Fig 5 shows ABTS radical scavenging activity of methanolic extracts of pumpkin peel and pulp. The IC_{50} value for pumpkin peel and pulp was around 6 mg/ml and 10 mg/ml respectively.

3.5 Reducing ability (FRAP)

The FRAP assay is a method of measuring the ability of reductants (antioxidants) to reduce Fe^{3+} to $[Fe^{2+}]$[25]. The formation of blue coloured Fe^{2+} –TPTZ complex (Fe^{2+} tripyridyltriazine) increases the absorbance at 700 nm. In the present study, the trend for ferric ion reducing activities of pumpkin peel and pulp is shown in Fig 6. The absorbance of peel (0.30, 0.53, 0.76, 0.89, 1.04, 1.04) and pulp (0.35, 0.51, 0.73, 0.88, 1.05, 1.12) clearly increased, due to the formation of the Fe2+-TPTZ complex with increasing concentration indicating a similar reducing power of both peel and pulp. Change in absorbance is directly related to antioxidant present in reaction mixture. Higher absorbance indicates higher reducing potency

CONCLUSIONS

The nutritional compositional analysis of pumpkin wastes indicate that both peel and pulp are good source of dietary fibre and minerals like phosphorus and iron and exhibited almost similar antioxidant activity which could be due to the presence of polyphenol compounds. Work on the incorporation of pumpkin peel and pulp in expanded snacks and bakery products have been reported. Thus, these wastes of the pumpkin could be utilised as a source of supplement or further exploited for value addition as they are rich in nutrients and antioxidant components. This study has also showed the ability of the extracts to scavenge different free radicals. However, as extract is not pure compound, to study the presence of active compounds in total extract and effect of these compounds in various activities, further investigations are needed.

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Table 1: Proximate composition of Pumpkin peel and pulp

Parameters	Peel	Pulp
Moisture (%)	7.23±0.07	11.45±0.08
Ash (%)	6.04±0.05	10.17±0.02
β-carotene (mg/100 gm)	11.89±0.10	142.38±0.80
Protein (%)	12.5±0.08	12.28±0.04
Crude fibre (%)	13.91±0.04	5.42±0.05
Dietary fibre (%)	28.81±0.05	13.29±0.04
Phosphorous (mg/100 gm)	319.33±0.05	292.23±0.05
Iron (mg/100 gm)	42.99±0.10	37.94±0.04
Ascorbic acid (mg/100 gm)	18.90±0.13	14.67±0.01

#Average of triplicate analysis ± SD

Table 2: Phenolic content (as gallic acid equivalents) in Pumpkin peel and pulp extracts

Parameter	Concentration mg GAE/g	
	Peel	Pulp
Total Phenols	5.19±0.05	5.21±0.06

#Average of triplicate analysis ± SD

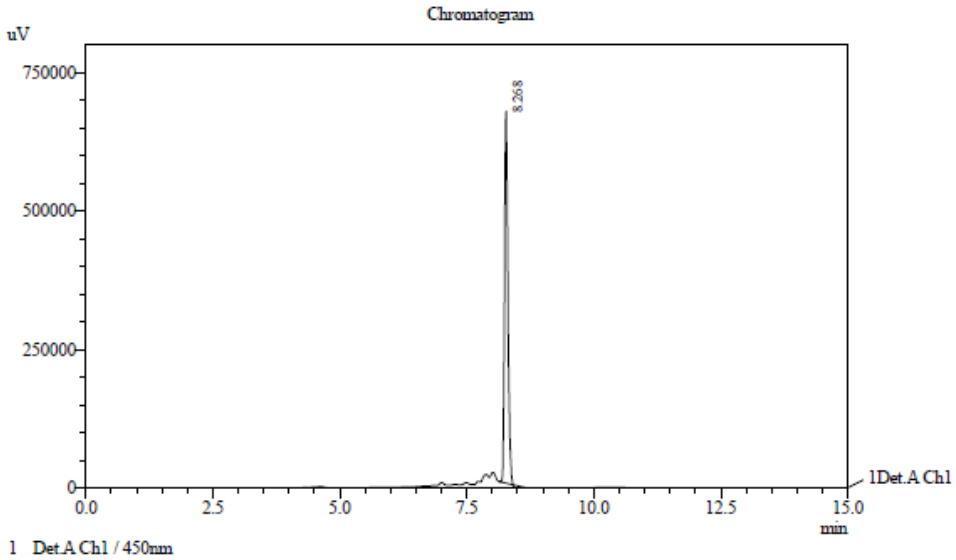

Fig. 1: HPLC separation of standard β-carotene

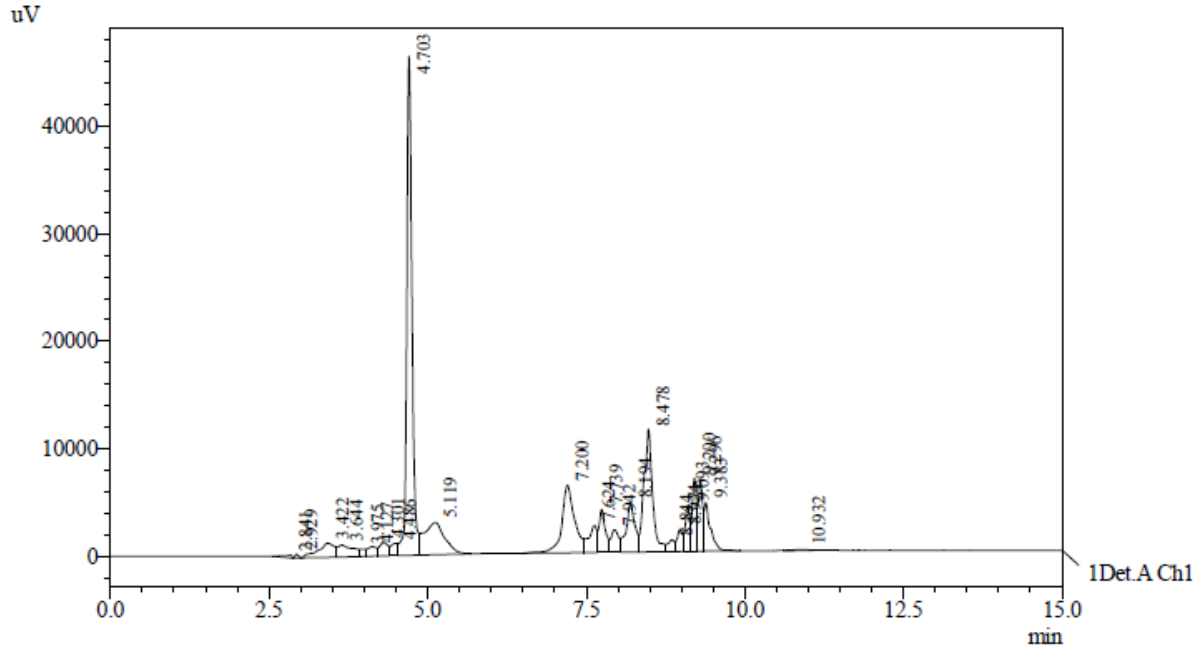

Fig. 2: HPLC of β-carotene in Pumpkin Peel

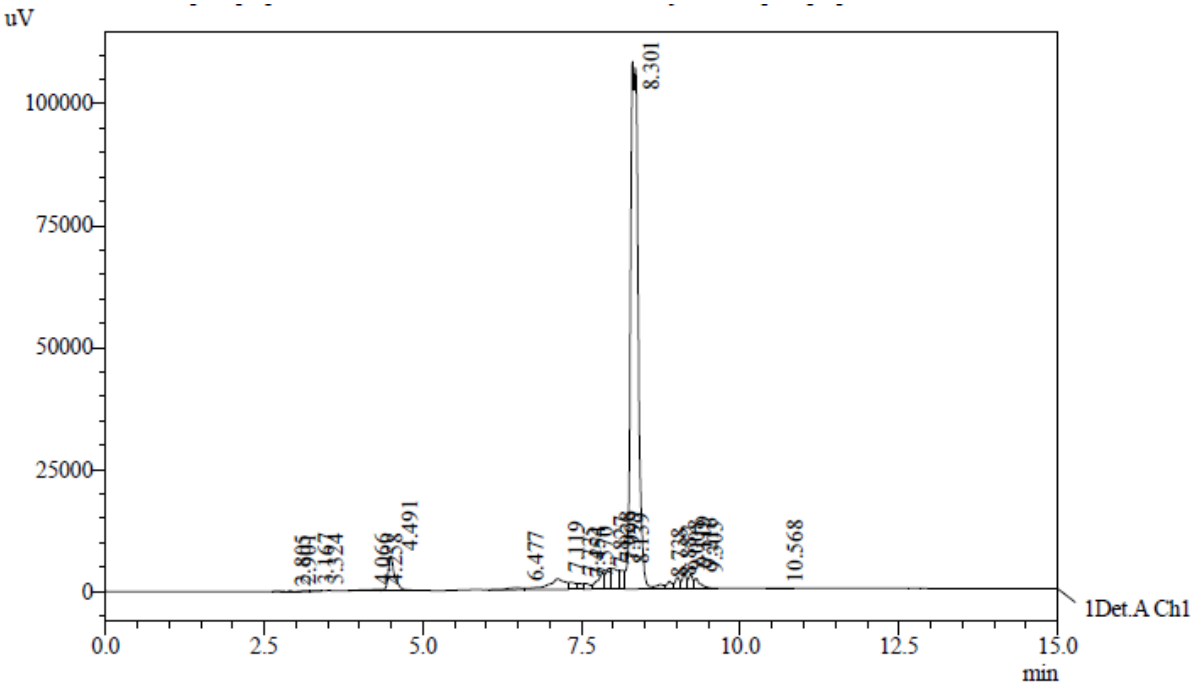

Fig. 3: HPLC of β-carotene in Pumpkin pulp

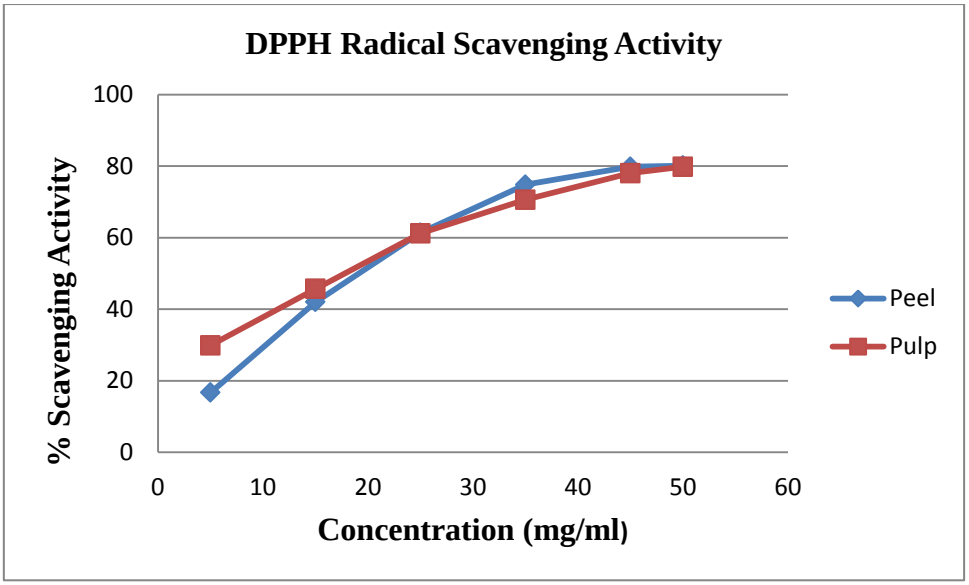

Fig. 4: DPPH radical scavenging activity of pumpkin peel and pulp

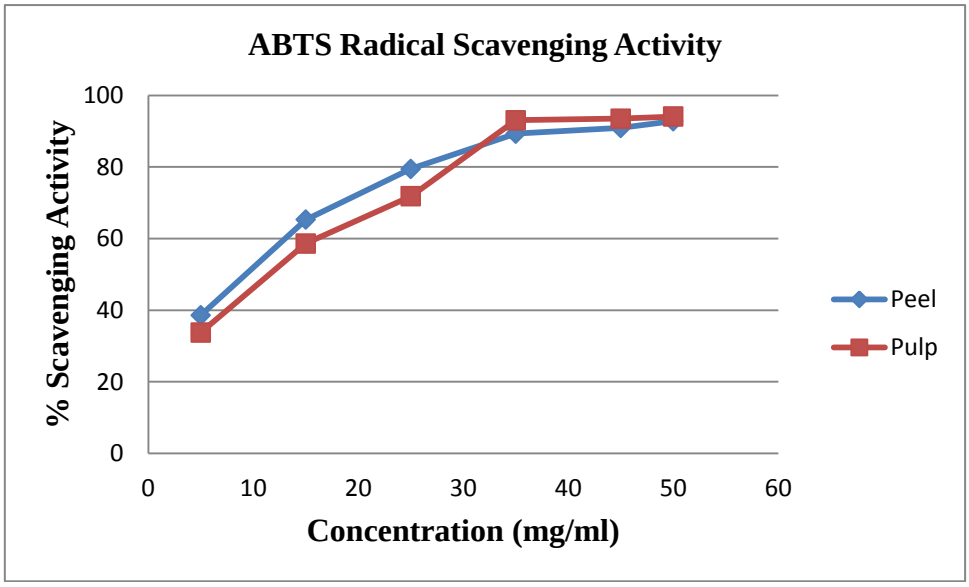

Fig. 5: ABTS radical scavenging activity of pumpkin peel and pulp

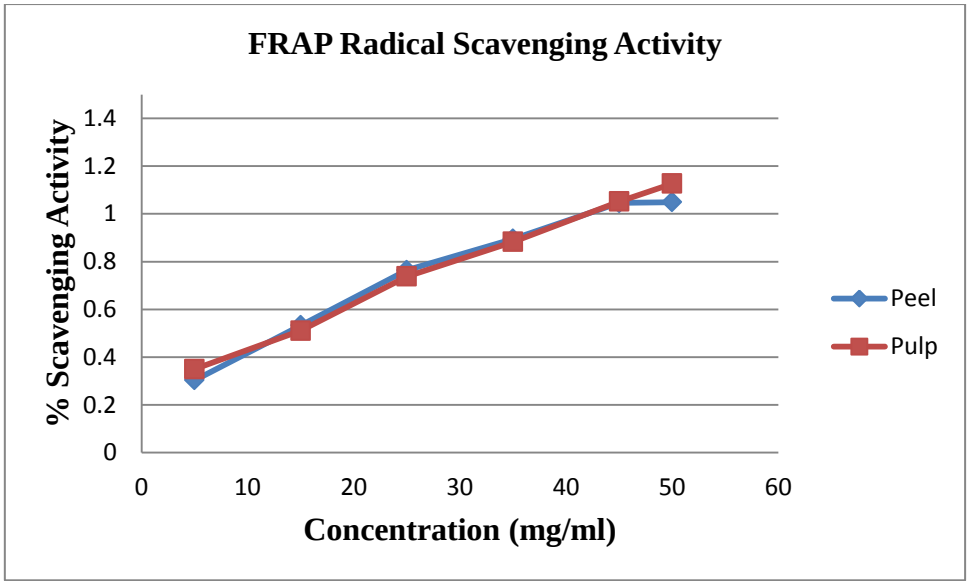

Fig. 6: FRAP activity of Pumpkin peel and pulp

REFERENCES

1. Velioglu YS, Mazza G, Gao L and Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. Journal of Agriculture Food Chemistry. 1998;46:4113-4117
2. Yesilyurt V, Halfon B, Ozturk M and Topcu G. Antioxidant potential and phenolic constituents of Salvia cedronella. Food Chemistry. 2008;108(1): 31-39
3. Aouidi F, Ayari S, Ferhi H, Roussos S and Hamdi M. Gamma irradiation of air-dried olive leaves: Effective decontamination and impact on the antioxidative properties and on phenolic compounds. Food Chemistry. 2011;127:1105-1113.
4. Chiou A, Karathanos VT, Mylona A, Salta FN, Preventi F and Andrikopoulos NK. Currants (Vitis vinifera L.) content of simple phenolics and antioxidant activity. Food Chemistry. 2007;102:516-522
5. Anon 2008 FAOSTAT Food and Agriculture organization of United Nations. http://faostat.fao.org/site/56//default.aspx#ancor (Accession date 30.08.2008)
6. Mukesh Yadav, Shalini Jain, Radha Tomar, Prasad GBKS and Hariom Yadav. Medicinal and biological potential of pumpkin: an updated review. Nutrition Research Reviews. 2010;23:184 – 190.
7. Norfezah Md Nor . Development of expanded Snack foods containing pumpkin flour and corn grits using Extrusion Technology, Ph.d thesis. 2013.
8. Xia T and Wang Q. Antihyperglycemic effect of Cucurbita ficifolia fruit extract in streptozotocin-induced diabetic rats. Fitoterapia. 2006;77:530 – 533.
9. Imaeda N, Tokudome Y, Ikeda M, Kitagawa I, Fujiwara N and Tokudome S. Foods contributing to absolute intake and variance in intake of selected vitamins, minerals and dietary fiber in middle-aged Japanese. J Nutr Sci Vitaminol. 1999;45:519 – 532.
10. Kwon YI, Apostolidis E, Kim YC and Shetty K. Health benefits of traditional corn, beans, and pumpkin: In vitro studies for hyperglycemia and hypertension management. Journal of Medicinal Food. 2007;10(2):266–275.
11. Tarhan L, Kayali HA and Urek RO. In vitro antioxidant properties of Cucurbita Pepo L. male and female flowers extracts. Plant Foods for Human Nutrition. 2007;62:49–51.
12. Fruhwirth GO, Wenzl T, El-Toukhy R, Wagner FS and Hermetter A. Fluorescence screening of antioxidant capacity in pumpkin seed oils and other natural oils. European Journal of Lipid Science and Technology. 2003;105:266–274.
13. Parry JW, Cheng Z, Moore J and Yu LL. Fatty acid composition, antioxidant properties, and anti-proliferative capacity of selected cold-pressed seed

flours. Journal of the American Oil Chemists' Society. 2008;85:457–464.
14. Official Methods of Analysis of AOAC International. 1997; 16th Edn, Volume II, Section 45.4.07, Method 985.29
15. Ranganna S. Handbook of Analysis and Quality Control for Fruit and Vegetable Products. Tata McGraw-Hill Education Pvt. Ltd, New Delhi. 2010.
16. Shyamala BN and Jamuna P Nutritional Content and Antioxidant Properties of Pulp Waste from D carota and B vulgaris 397 Mal. J Nutr. 2010;16(3):397-408.
17. Sadasivam S and Manickam A. Vitamins. In: Biochemical methods, Edited by New Age International (P) Limited, New Delhi. 1997, 2nd Edn: 185-186.
18. Nanjo F, Goto K, Seto R, Suzuki M, Sakai M and Hara Y. Scavenging effects of tea catechins & their derivatives on 1, 1-diphenyl-2-picrylhydrazyl radical. Free Radic Biol Med. 1996;21(6): 895-902.
19. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C. Antioxidant activity: applying an improved ABTS radical cation decolorization assay. Free Rad Biol Med. 1999; 26:1231-1237.
20. Rohan Sharadanand and Anup Subhash Hendre. Total Antioxidant capacity of fresh leaves of Kalanchoe pinnata. Journal of Pharmacognosy and Phytochemistry. 2014;2(5):32-35.
21. Ramamoorthy PK and dan A Bono. Antioxidant activity, total phenolic and flavonoid content of morinda citrifolia fruit extracts from various extraction processes. Journal of Engineering Science and Technology. 2007;1(2):70-80.
22. Karimi E, Jaafar HZE and Ahmad S. Phenolics and Flavonoids Profiling and Antioxidant Activity of Three Varieties of Malaysian Indigenous Medicinal Herb Labisia pumila Benth. Journal of Medicinal Plants Research. 2011;5(7):1200-1206.
23. Chung KT, Wong TY, Huang YW and Lin Y. Tannins and human health: a review. Critical Reviews in Food Science and Nutrition. 1998;38:421–464.
24. Baumann J, Wurn G and Bruchlausen FV. Prostaglandin synthetase inhibiting O-2 radical scavenging properties of some flavonoids and related phenolic compounds. Deutsche Pharmakologische Gesellschaft Abstracts of the 20th spring meeting, Naunyn–Schmiedebergs Abstract No: R27 cited in Arch Pharmacol. 1979;307: R1 –R77.
25. Benzie IFF and Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239:70-76.