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Original article

Amylase inhibitory and metal chelating effects of different layers of onion (*Allium cepa* L.) at two different stages of maturation *in vitro*

Nidhi Jaiswal* and Syed Ibrahim Rizvi
Department of Biochemistry, University of Allahabad, Allahabad-211002, Uttar Pradesh, India
**Department of Food and Nutrition, Lady Irwin College, University of Delhi, Delhi-110001, India*

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Abstract

Researches on traditional medicinal plants are consolidating the belief that this system of medicine provides a viable alternative to conventional drugs for various free radical mediated diseases, *e.g.*, diabetes. Onion (*Allium cepa* L.) is one of the oldest cultivated plants with multiple functional and biological properties. These are attributed to the presence of active phytomolecules, *e.g.*, phenolic acids, flavonoids (especially quercetin), cepaenes, thiosulfinates and anthocyanins. In the present study, outer layers (transitional layer with the first living cells below the dry onion peel) and innermost layers from two different sizes of onion were studied for their α-amylase inhibiting activity, flavonoid content, hydroxyl radical scavenging activity (HRSA) and metal chelating activity. Our results demonstrate that the outermost living layers had higher flavonoid content (ranging from 40.27 to 45.0 mg), followed by a continuous decrease towards the inner part of the bulb (ranging from 29.10 to 31.81 mg). Outermost living layers of the onion extracts were found most powerful hydroxyl radical scavenger and having higher ferrous ion chelating effects compared to the inner layers with higher HRSA of outer layers of smaller onion. Differences in % α-amylase inhibitory activity were also observed among different layers and sizes of onions, ranging 52.0 to 44.8% in case of outer layers and 36.00 to 39.12% in case of inner layers, showing high inhibitory activity in outer layers of onion ($p < 0.05$). Quercetin was a potent inhibitor with inhibitory activity ranging from 69.6 to 84.3% at different concentrations, respectively. The findings may explain the antioxidative and antidiabetic effect of onion and also foods containing quercetin.

Keywords: *Allium cepa* L., flavonoid content, hydroxyl radical scavenging activity, metal chelating activity, antidiabetic effect

1. Introduction

Reactive oxygen species (ROS) are produced in the cells by cellular metabolism and other exogenous environmental agents. They are generated by a process known as redox cycling and are catalysed by transition metals such as Fe^{2+} and Cu^{2+}. Increased physiological burden of free radicals and over production of ROS causes an imbalance between oxidants and antioxidants in the body leading to a state of oxidative stress, thereby damaging cellular biomolecules and resulting in various degenerative diseases including diabetes (Halliwell and Gutteridge, 1999; Martim *et al*., 2003).

In recent years, researches on traditional medicinal plants are gradually emerging as viable alternatives to conventional drugs for various free radical mediated diseases. Due to their pleiotropic biological effects, flavonoids, a class of phenolic compounds widely distributed in plants have received special attention as dietary constituents during the last few years (Pandey and Rizvi, 2009; Del Rio *et al*., 2010). Recent nutritional investigations have reported that polyphenols may be able to modulate nutrient availability through the inhibition of digestive enzymes involved in lipid and starch breakdown, which could lead to beneficial effects on calorie intake, obesity (McDougall *et al*., 2009) and blood glucose control (Grussu *et al*., 2011). α-amylase is one of the main enzymes in human that is responsible for the breakdown of starch to more simple sugars, thus the inhibitors of this enzyme can delay the carbohydrate digestion and reduce the rate of glucose absorption and obesity management by reducing sugar level in blood. The inhibition of α-amylase has been suggested as a strategy for diabetes control as they can decrease the attenuated postprandial plasma glucose levels by ultimately decreasing glucose release from starch and improve the glucose tolerance in diabetic patients (Ali *et al*., 2006; Kwon *et al*., 2006). In recent years, natural sources of α-amylase inhibitor have received a lot of interest due to search for alternative to synthetic enzyme inhibitors such as acarbose, metformin and orlistat, which have been found to exhibit adverse effects, mild efficacy and can cause gastrointestinal distress as a side effect (Randhir *et al*., 2008).

Onion (*Allium cepa* L.), one of the oldest cultivated plants used for culinary, medicinal and spiritual purposes, has recently been gaining attention for its multiple functional properties which include free radical scavenging activity, immune stimulation, cardioprotective effects, anticancer and anti-infectious property (Jaiswal and Rizvi,

Author for correspondence: Dr. Syed Ibrahim Rizvi
Professor, Department of Biochemistry, University of Allahabad, Allahabad-211002, Uttar Pradesh, India
E-mail: sirizvi@gmail.com
Tel.: +91-9415305910

2014; Jaiswal and Rizvi, 2012; Desjardins, 2008). The various biological and pharmacological properties associated with onions have been attributed to the presence of active phytomolecules, *e.g*., phenolic acids, flavonoids (especially quercetin), cepaenes, thiosulfinates and anthocyanins (Corzo-Martýnez *et al*., 2007). From 25 different flavonols characterised in onions, the most prominent are the quercetin and quercetin conjugates, mainly quercetin-3,4'-O-diglucoside (QDG) and quercetin-4'-O-monoglucoside (QMG) (Price and Rhodes, 1997; Bonaccorsi *et al*., 2008; Slimestad *et al*., 2007). Amounts of this flavonoid in onions vary with bulb color and type, being distributed mostly in the outer skins and rings (Bonaccorsi *et al*., 2005; Beesk *et al*., 2010). It has been reported by various investigators that the dry outer scale extract of onion containing large amounts of quercetin, quercetin glucoside, which is generally considered as waste, possesses radical scavenging property and is an effective antioxidant against nonenzymatic lipid peroxidation (Nuutila *et al*., 2003; Bonaccorsi *et al*., 2008; Lee *et al*., 2012).

Although there are citations of antihyperglycemic and antidiabetic activity of some *Allium* species (Campos *et al*., 2003; Azuma *et al*., 2007; Nickavar *and* Yousefian, 2009), least attention was given on the activity of the different layers of onion extracts on *in vitro* α-amylase activity. The present study focuses on the study of amylase inhibition, antioxidant properties, antidiabetic and metal chelating effects of different layers of the edible part of the onion and during different stages of development.

2. Materials and Methods

2.1 Plant material and sampling

The red variety onion (Pusa red cultivar) was purchased from local market of Allahabad, Uttar Pradesh, India. The selected plants were collected and the herbarium sheets were sent to the herbarium in Botany Department of Allahabad University, Allahabad, Uttar Pradesh, India and the voucher specimen number (Specimen No.-25840) were obtained. Onion with two different sizes (15-20 g and 50-60 g), were then sub-divided into two different parts: the innermost layers and the outermost layers (the transitional layer with the first living cells). Onion extracts were prepared by the method as described by Stajner *et al*. (2008), with some modifications. 5 g of onion (inner layers and outer layers) was grounded with quartz sand in a cold mortar. The homogenized material was suspended in 10 ml solvent and centrifuged at 15,000 × g for 10 min at 4°C and the aliquots of the supernatants were collected. The solvent used was 100% aqueous methanol (pure methanol). Samples were re-extracted twice, the final volume made up to 100 ml with cold distilled water and stored at 4°C, before conducting experiments.

2.2 Chemicals and reagents

Alpha amylase from porcine pancreas and quercetin were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade procured from Merck, India or Himedia, India.

2.3 Determination of total flavonoid content

Total flavonoid content was determined by using a colorimetric method described previously (Dewanto *et al*., 2002; Jia *et al*., 1999). Briefly, 0.25 ml of diluted onion extracts was mixed with 1.25 ml of distilled water and, subsequently, with 0.075 ml of 5% sodium nitrite solution and allowed to react for 5 min. Then 0.15 ml of 10% aluminum chloride was added and allowed to further react for 6 min before 0.5 ml of 1 M sodium hydroxide was added. Distilled water was added to bring the final volume of the mixture to 3 ml. The absorbance of the mixture was immediately measured at 510 nm wavelength against a blank using a spectrophotometer. The flavonoid content was determined by a quercetin standard curve and expressed as mean (milligrams of quercetin equivalents per 100 g of fresh sample) ± SD for the triplicate extracts.

2.4 Metal (Fe^{2+}) chelating activity assay

The chelating activity of onion extracts for ferrous ions Fe^{2+} was measured according to the method previously described (Dinis *et al*., 1994). Briefly, to 0.5 ml of extract in deionized water, 1.6 ml of deionized water and 0.05 ml of $FeCl_2$ (2 mM) were added. After 30s, 0.1 ml ferrozine (5 mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe^{2+}-ferrozine complex was measured at wave length 562 nm. The chelating activity of the extract for Fe^{2+} was calculated using the following equation:

$$\text{Chelating rate} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

2.5 α-amylase inhibitory activity

The α-amylase inhibition assay method was performed using the method previously described (Tundis *et al*., 2007). Briefly, a starch solution (0.5% w/v) was obtained by stirring 0.125 g of potato starch in 25 ml of 20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9 at 65°C for 15 min. The α-amylase (EC 3.2.1.1) solution was prepared by mixing 0.0253 g of α-amylase in 100 ml of cold distilled water. The colorimetric reagent was prepared mixing a sodium potassium tartrate solution (12.0 g of sodium potassium tartrate, tetrahydrate in 8.0 ml of 2 M NaOH) and 96 mM 3, 5- dinitrosalicylic acid solution. Control and onion extracts were added to starch solution and left to react with α-amylase solution at 25°C for 5 min. The reaction was measured over 3 min. The generation of maltose was quantified by the reduction of 3, 5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid, the product being detectable at 540 nm. In the presence of an α-amylase inhibitors, less maltose will be produced and the absorbance value would decrease. The inhibition percentage of α-amylase was assessed by the following formula:

$$\text{Inhibition } \alpha\text{-amylase } \% = 100 \times (A_{Control} - A_{Sample}) / A_{Control}$$

2.6 Statistical analysis

Values were mean ± SD of 5-6 separate experiments done in triplicates. Statistical analyses were conducted using the software PRISM version 5.01 for Windows (Graph Pad Software, San Diego, California, USA). Statistical differences were analyzed with Student's *t* test and the differences were considered to be significant when $p < 0.05$.

3. Results and Discussion

The present study was undertaken to evaluate the antioxidant activity, antidiabetic activity and also its correlation with flavonoid content, as a function of 1. Size of onion (big and small), 2. In inner layers and outer layers.

3.1 Total flavonoid content

Figure 1 shows the quantitative data of total flavonoid content of onion extracts expressed as mg quercetin /100 g fresh sample. Results showed a higher flavonoid content of outer living layers (ranging from 40.27 to 45.0 mg), followed by a continuous decrease towards the inner part of the bulb (ranging from 29.10 to 31.81 mg). Difference was also observed between flavonoid content of outer layers with respect to different stages of maturation, being higher in smaller onion (p <0.05).

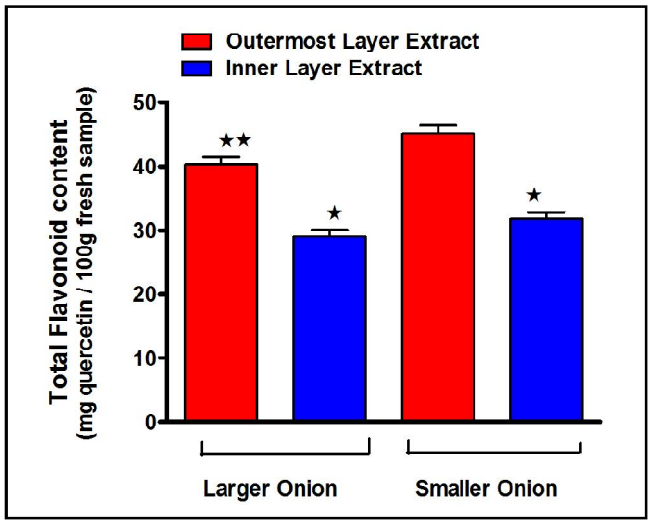

Figure 1: Total flavonoid content of outermost living layers and innermost layers of onion at two different stages of maturation. Concentration of total flavonoid content is expressed as mg quercetin/ 100 gm fresh weight. Values represent mean ±SD. Significant difference * (p < 0.01) in flavonoid content of outer layers as compared to their respective inner layers of different sizes. **(p < 0.05) between both the outer layer extracts of onion.

Our results are in good agreement with earlier reports, where difference in phenolic content in different layers and flavonoid content was observed in the different parts of the onion bulb (Jaiswal and Rizvi, 2012; Beesk *et al*., 2010; Bilyk *et al*., 1984; Prakash *et al*., 2007). In general, a higher flavonoid content of onion extracts is mainly due to the presence of various phenolic compounds (quercetin, kaempferol and luteolin), among them, QDG and QMG are the main flavonols, which make up to 80-85% of the total flavonoid content of onion extracts (Desjardins, 2008; Price and Rhodes, 1997; Rhodes and Price, 1996; Sellappan and Akoh, 2002). Quercetin and kaempferol and their glycosides have been reported to be the most abundant flavonoids in the acid hydrolysed samples of onion (Chu *et al*., 2000; Price and Rhodes, 1997).

The difference in the distribution of flavonoids being higher in the outer layers, could be explained by the increased activity of light-induced enzyme phenylalanine ammonia lyase which catalyses the biosynthesis of flavonoids in the outermost localizing living cells of the whole onion bulb due to more exposure of sunlight than the outermost dry peel and innermost layers of the onion bulb portion (Friedman, 1997; Hirota *et al*., 1999). The cells of outermost dead dried peel of onion are not exposed to sunlight, resulting in lesser biosynthesis of flavonoids, thus having flavonoid content lesser than the outermost living cells layer (Beesk *et al*., 2010).

3.2 Hydroxyl radical scavenging activity (HRSA)

Hydroxyl radicals can be produced in cells by a variety of processes: such as phagocytosis, prostaglandin biosynthesis, ionizing, irradiation and decomposition of lipid hydroperoxides. Hydroxyl radicals, an extremely reactive in biological systems, generated by the Fenton reaction are known to cause oxidatively induced breaks in DNA strands to yield its open circular or relaxed forms and contribute to significant biological effects such as carcinogenesis, mutagenesis and cytotoxicity. They are reported to mediate the lethal cell injury in cultured hepatocytes (Halliwell *et al*., 1999). All the onion extracts were studied for their HRSA and compared with quercetin of different concentrations (10^{-4}mol/l and 10^{-6}mol/l), Figure 2. Outermost living layers of the onion extracts were found to be most powerful hydroxyl radical scavenger compared to the inner layers with higher HRSA of outer layers of smaller onion. Quercetin also shows a high HRSA activity at different concentrations. The scavenging of hydrogen peroxide and reducing capacity of whole onion extracts has been recently documented by various investigators (Benkeblia, 2005; Shon *et al*., 2004). Variation in free radical scavenging activity and H_2O_2 scavenging activity in different layers of onion has already been documented by Jaiswal and Rizvi (2012).

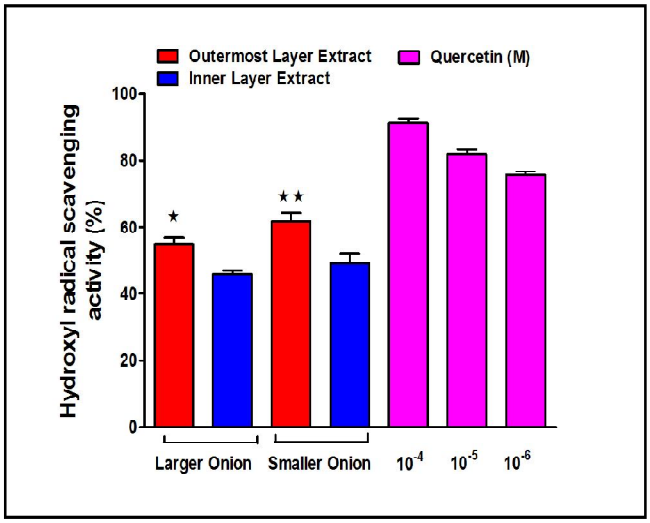

Figure 2: Hydroxyl radical scavenging activity (HRSA) (%) of outermost living layers and innermost layers of onion at two different stages of maturation, compared with quercetin (10^{-4} to10^{-6} M).Values represent mean ±SD. Significant difference *(p < 0.05) in the scavenging activity of outer layers of larger onion with its respective inner layers and outer layers of smaller onion. Significant difference **(p < 0.01) in outer layers of smaller onion with its respective inner layers.

3.3 Metal chelating activity

Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, iron is an extremely reactive metal and is known as effective lipid oxidation pro-oxidant, which catalyses various oxidation reactions in biological systems (Shon *et al*., 2004). It causes lipid peroxidation through the Fenton and Haber-weiss reaction (Halliwell and Gutteridge, 1990) and decomposes the lipid hydroxide into peroxyl and alkoxyl radicals that can perpetuate the chain reactions

(Halliwell, 1991). Metal ion-chelating activity of an antioxidant molecule prevents oxyradical generation and the consequent oxidative damage (Kumar *et al*., 2008). Thus, metal chelation is an important antioxidant property because it reduces the concentration of the catalyzing transition metal in lipid oxidation.

Figure 3 shows the obtained ferrous chelating abilities of onion extracts, which was compared with that of quercetin and EDTA. The values demonstrates that EDTA which serves as the positive control showed the highest percentage of the chelating effect and the chelation rate reached 90.71 ± 3.76% at 100 µg/ml. The chelating ability of the onion extracts were 34-35.6% in case of outer layers and 21.6-28.5 % in case of inner layers at 100 µg/ml concentration. The results suggest that the ferrous ion chelating effects of outer layers of onion are higher than inner layers ($p < 0.05$) and can be considered as moderate metal chelator since its activities are approximately two times lesser than EDTA. Quercetin also showed metal chelating activity with an inhibition of 43.6 % at 100 µg/ml, suggesting that flavonoids possess strong affinity toward iron. Quercetin is known to exert multiple mechanisms including antioxidant activity, anti-inflammation, modification of signal transduction pathways, and interactions with receptors and other proteins. *In vitro* and *in vivo* studies have proven that antioxidant properties of quercetin protect the erythrocytes from oxidation induced damage (Jaiswal and Rizvi, 2014; Jaiswal and Rizvi, 2011) and also protect DNA of normal cells against oxidative damage (Papiez *et al*., 2008).These compounds contain aromatic hydroxyl groups which exert its antioxidative effects in cells by chelating transition metal ions, catalyzing electron transport and scavenging free radicals (Hu *et al*., 1995).

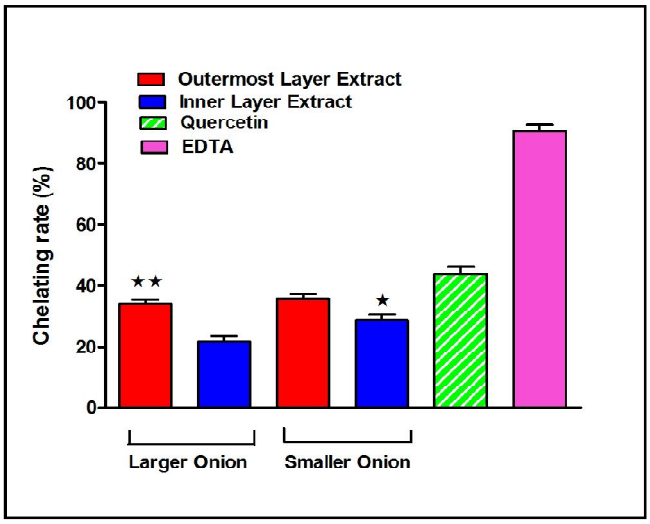

Figure 3: Metal chelating activity (%) of outermost living layers and innermost layers of onion at two different stages of maturation, compared with quercetin (100 µg/ml) and EDTA (100 µg/ml).Values represent mean ±SD. Significant difference * ($p < 0.05$) and ** ($p < 0.01$) in the reducing power of outer layers with their respective inner layers of different sizes.

Besides phenolic compounds, organosulfur compounds such as S-propenylcysteine sulfoxide (major component), S-propylcysteine sulfoxide and S-methylcysteine sulfoxide and non-flavonoid compounds have been reported in onion to show alkylperoxyl radical scavenging activity and also being responsible for most of its biological properties (Sawa *et al*., 1999; Corzo-Martinez *et al*., 2007). The triple synergistic action of phenols from onion in scavenging ROS, repairing DNA radicals and metal chelation has been reported (Prakash *et al*., 2007).

3.4 α-amylase inhibitory activity

In the present study, inhibitory activity of different sizes and layers of onion against porcine pancreatic α-amylase were studied and compared with quercetin (Figure 4). Results showed that quercetin was potent inhibitor against porcine pancreatic α-amylase, with inhibitory activity ranging from 69.6 to 84.3% at different concentrations, respectively. Differences in % inhibitory activity were also observed among different layers and sizes of onions, ranging from 52 to 44.8 % in case of outer layers and 36 to 39.12 % in case of inner layers, showing high inhibitory activity in outer layers of onion ($p < 0.05$). Since, phenolics play a role in mediating amylase inhibition and have the potential for type II diabetes management (McCue and Shetty, 2004, Chethan *et al*., 2008).

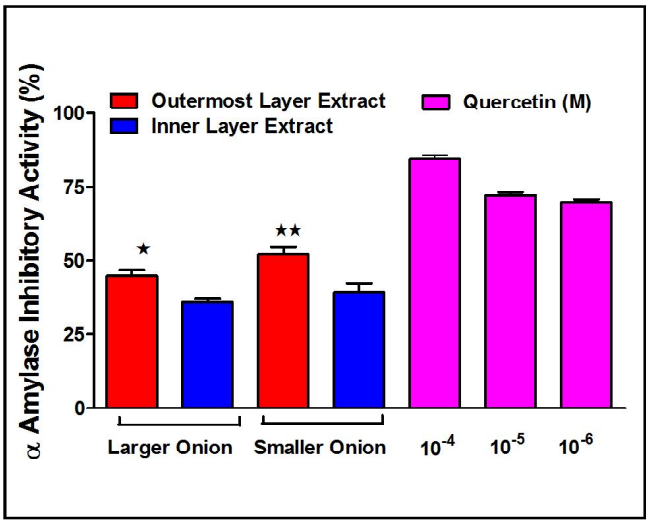

Figure 4: α-amylase inhibitory activity(%) of outermost living layers and innermost layers of onion at two different stages of maturation, compared with quercetin (10^{-4} to10^{-6} M). Values represent mean ±SD. Significant difference* ($p < 0.05$) in the scavenging activity of outer layers of larger onion with its respective inner layers and outer layers of smaller onion. Significant difference **($p < 0.01$) in outer layers of smaller onion with its respective inner layers.

Phenolics are able to bind with the reactive sites of α-amylase and alter its catalytic effects, hence, the presence of phenolics and flavonoids in onion extracts would have contributed toward α-amylase inhibition. Our results corroborates with the report of Tadera *et al*., (2006), showing several flavonoid compounds especially quercetin for their inhibitory activity against α-amylase and that inhibitory activity increased appreciably with an increase in the number of hydroxyl group on the B ring. Epidemiological studies have also shown that the intake of certain types of flavonoids, including quercetin and myricetin is inversely associated with the risk of incident type II diabetes (Griffiths *et al*., 2002).

4. Conclusion

In conclusion, the outer living layer (the transitional layer with the first living cells below the dry onion peel) is expected to be a good resource for food ingredients and easily accessible source for nutraceutical compounds. However, difference in flavonoid content, metal chelating activity and HRSA of outer layers with respect to different sizes of onion could be explained on the basis of lesser moisture content in smaller onion as reported earlier by the same authors. We provide enough evidence for the strong biological antioxidant activity of the onion extracts and a good source of α - amylase inhibitors, therefore motivating the use of the natural products against oxidative stress and thereby against various degenerative diseases especially diabetes. We consider that this fraction deserves more intensive study, including its *in vivo* antioxidant activity in order to understand its potential as nutraceutical and for better extraction of flavonoids.

Conflict of interest

We declare that we have no conflict of interest.

References

Ali, H.; Houghton, P.J. and Soumyanath, A. (2006). alpha-amylase inhibitory activity of some Malaysian plants used to treat diabetes with particular reference to *Phyllanthus amarus*. J. Ethnopharmacol., **107**(3):449-455.

Azuma, K.; Minami, Y.; Ippoushi, K. and Terao, J. (2007). Lowering effects of onion intake on oxidative stress biomarkers in streptozotocin-induced diabetic rats. J. Clin. Biochem. Nutr., **40**(2):131-40.

Beesk, N.; Perner, H; Schwarz, D.; George, E.; Kroh. L.W. and Rohn, S. (2010). Distribution of quercetin-3,4'-O-diglucoside, quercetin-4'-Omonoglucoside, and quercetin in different parts of the onion bulb (*Allium cepa* L.) influenced by genotype. Food Chem., **122**(3): 566-571.

Benkeblia, N. (2005). Free-Radical scavenging capacity and antioxidant properties of some selected onions (*Allium cepa* L.) and garlic (*Allium sativum* L.) extracts. Braz. Arch. Biol. Technol., **48**(5): 753-759.

Bilyk, A.; Cooper, P.L. and Sapers, G.M. (1984). Varietal difference in distribution of quercetin and kaempferol in onion tissue. J. Agric. Food Chem., **32**(2):274-276.

Bonaccorsi, P.; Caristi, C.; Gargiulli, C. and Leuzzi, U. (2005). Flavonol glucoside profile of southern Italian red onion (*Allium cepa* L.). J. Agric. Food Chem., **53**(7):2733-2740.

Bonaccorsi, P.; Caristi, C.; Gargiulli, C. and Leuzzi U. (2008). Flavonol glucosides in *Allium* species: A comparative study by means of HPLC-DAD-ESI-MS-MS. Food Chem., **107**(4):1668-1673.

Campos, K.E.; Diniz, Y.S.; Cataneo, A.C.; Faine, L.A.; Alves, M.J. and Novelli E.L. (2003). Hypoglycaemic and antioxidant effects of onion, *Allium cepa*: Dietary onion addition, antioxidant activity and hypoglycaemic effects on diabetic rats. Int. J. Food Sci. Nutr., **54**(3):241-246.

Chethan, S.; Sreerama, Y.N. and Malleshi, N.G. (2008). Mode of inhibition offinger millet malt amylases by the millet phenolics. Food Chem., **111**(1):187-191.

Chu, Y.H.; Chang, C.L. and Hsu, H.F. (2000). Flavonoid content of several vegetables and their antioxidant activity. J. Sci. Food Agric., **80** (5):561-566.

Corzo-Martínez, M.; Corzo, N. and Villamiel, M. (2007). Biological properties of onions and garlic. Trends Food Sci. Technol., **18**(12):609-625.

Del Rio, D.; Borges, G. and Crozier, A. (2010). Berry flavonoids and phenolics: bioavailability and evidence of protective effects. Br. J. Nutr., **104** (3):67-90.

Desjardins, Y. (2008). Onion as a nutraceutical and functional food. Chron. Horticult., **48**:8-14.

Dewanto, V.; Wu, X.; Adom, K.K. and Liu, R.H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agric. Food Chem., **50**(10):3010-3014.

Dinis, C.P.; Madeira, V.M.C. and Almeida, L.M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys., **315**(1):161-169.

Friedman, M. (1997). Chemistry, biochemistry, and dietary role of potato polyphenols. A review. J. Food Chem., **45**(5):1523-1540.

Griffiths, G.; Trueman, L.; Crowther, T.; Thomas, B. and Smith, B. (2002). Onions-a global benefit to health. Phytother. Res., **16**(7):603-615.

Grussu, D.; Stewart, D. and McDougall, G.J. (2011). Berry polyphenols inhibit alpha-amylase *in vitro*: Identifying active components in rowanberry and raspberry. J. Agric. Food Chem., **59**(6):2324-2331.

Halliwell, B. and Gutteridge, J.M.C.(1990). Role of free radicals and catalytic metal ions in human diseases; an overview. Methods Enzymol., **186**:1-85.

Halliwell, B. and Gutteridge, J.M.C. (1999). Fifth edition. Free radicals in biology and medicine. New York: Oxford University Press.

Halliwell, B. (1991). Reactive oxygen species in living systems: source, biochemistry, and role in human disease. Am. J. Med., **91**(3):14-22.

Hirota, S.; Shimoda, T. and Takahama, U. (1999). Distribution of flavonols and enzymes participating in the metabolism in onion bulbs: Mechanism of accumulation of quercetin and its glucosides in the abaxial epidermis. Food Sci. Technol., **5**(4):384-387.

Hu, J.P.; Calomme, M.; Lasure, A.; De Bruyne, T.; Pieters, L.; Vlietinck, A. and VandenBerghe, D.A. (1995). Structure-activity relationships of flavonoids with superoxide scavenging ability. Biol. Trace Elem. Res., **47**(1-3):327-331.

Jaiswal, N. and Rizvi, S.I. (2011). Protective effect of different layers of onion extracts (*Allium* cepa L.) on markers of oxidative stress in erythrocytes. Cell Membranes Free Rad. Res., **3**(2):147-153.

Jaiswal, N. and Rizvi, S.I. (2012). Variation of antioxidant capacity in different layers of onion (*Allium* cepa L.) at two different stages of maturation. Curr. Nutr. Food Sci., **8**(2):126-130.

Jaiswal, N. and Rizvi, S.I. (2014). Onion extract (*Allium cepa* L.), quercetin and catechin up-regulate paraoxonase 1 activity with concomitant protection against low-density lipoprotein oxidation in male Wistar rats subjected to oxidative stress. J. Sci. Food Agric., **94**(13):2752-2757.

Jia, Z.; Tang. M. and Wu, J. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., **64**(4):555-559.

Kumar, S.K.; Ganesan, K. and Rao, P.V.S. (2008). Antioxidant potential of solvent extracts of *Kappaphycus alvarezii* (Doty) Doty - An edible seaweed. Food Chem., **107**(1):289-295.

Kwon, Y.I.; Jang. H.D. and Shetty, K. (2006). Evaluation of Rhodiolacrenulata and Rhodiolarosea for management of type 2 diabetes and hypertension. Asia Pac. J. Clin. Nutr., **15**(3):425-432.

Lee, B.; Jung, J.H. and Kim, H.S. (2012). Assessment of red onion on antioxidant activity in rat. Food Chem. Toxicol., **50**(11):3912-3919.

Martim, AC.; Sanders, R.A. and Watkins, J.B. (2003). Diabetes, oxidative stress, and antioxidants: A review. J. Biochem. Mol. Toxicol., **17**(1):24-38.

McCue, P. and Shetty, K. (2004). Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase *in vitro*. Asia Pac. J. Clin. Nutr., **13**(1):101-106.

Mcdougall, G.J. and Stewart, D. (2009). The inhibitory effects of berry polyphenols on digestive enzymes. BioFactors., **23**(4):189-195.

Nickavar, B. and Yousefian, N. (2009). Inhibitory effects of six Allium species on α-amylase enzyme activity. Iranian J. Toxicol., **8**(1):53-57.

Nuutila, A.M.; Puupponen-Pimia, R.; Aarni, M. and Oksman-Caldentey, K.M. (2003). Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. Food Chem., **81**(4):485-493.

Pandey, K.B. and Rizvi, S.I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. Oxid. Med. Cell. Longev., **2**(5):270-278.

Papiez, M.A.; Cierniak, A.; Krzysciak, W.; Bzowska, M.; Taha, H.M.; Jozkowicz, A. and Piskula, M. (2008). The changes of antioxidant defense system caused by quercetin administration do not lead to DNA damage and apoptosis in the spleen and bone marrow cells of rats. Food Chem. Toxicol., **46**(9):3053-3058.

Piparo, E.L.; Scheib, H.; Frei, N.; Williamson, G.; Grigorov, M. and Chou, C.J. (2008). Flavonoids for controlling starch digestion: Structural requirements for inhibiting human α-Amylase. J. Med. Chem., **51**(12):3555-3561.

Prakash, D.; Singh, B.N. and Upadhyay, G. (2007). Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). Food Chem., **102**(4):1389-1393.

Price, K.R. and Rhodes, M.J.C. (1997). Analysis of the major flavonol glycosides present in four varieties of onion (*Allium* cepa) and changes in composition resulting from autolysis. J. Sci. Food Agric., **74**(3):331-339.

Randhir, R.; Kwon, Y.I. and Shetty, K. (2008). Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of selected grain sprouts and seedlings. Innov. Food Sci. Emerg. Technol., **9**(3):355-364.

Rhodes, M.J.C. and Price, K.R. (1996). Analytical problems in the study of flavonoid compounds in onions. Food Chem., **57**(1):113-117.

Sawa, T.; Nakao, M.; Akaike, T.; Ono K. and Maeda, H. (1999). Alkylperoxyl radical-scavenging activity of various flavonoids and other polyphenolic compounds: Implications for the antitumor-promoter effect of vegetables. J. Agric. Food Chem., **47**(2):397-402.

Sellappan, S. and Akoh, C. (2002). Flavonoids and antioxidant capacity of Georgia-grown Vidalia onions. J. Agric. Food Chem., **50**(19): 5338-5342.

Shon, M.Y.; Choi, S.D.; Kahng, G.G.; Nam, S.H.; and Sung, N.J. (2004). Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions. Food Chem. Toxicol., **42**(4):659-666.

Slimestad, R.; Fossen, T. and Vagen, I.M. (2007). Onions: A source of unique dietary flavonoids. J. Agric. Food Chem., **55**(25):10067-10080.

Stajner, D.; Igic, R.; Popovic, B.M. and Malencic, D.(2008). Comparative study of antioxidant properties of wild growing and cultivated *Allium* species. Phytother. Res., **22**(1):113-117.

Tadera, K.; Minami, Y.; Takamatsu, K. and Matsuoka, T. (2006). Inhibition of α-glucosidase and α-amylase by flavonoids. J. Nutr. Sci. Vitaminol., **52**(2):149-153.

Tundis, R.; Loizzo, M.R.; Statti, G.A. and Menichini, F. (2007). Inhibitory effects on the digestive enzyme α-amylase of three *Salsola* species (Chenopodiaceae) *in vitro*. Pharmazie., **62**(6):473-475.