INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-4, Issue-4, Oct-Dec-2013 Coden : IJABPT Copyrights@2013

ISSN : 0976-4550

Received: 09th August-2013 *Revised: 18th August-2013* *Accepted: 19th August-2013*

Research article

ANTIGLYCATION, ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF MATURE STRAWBERRY (*FRAGARIA* × *ANANASSA*) FRUITS

Pallavi Mandave#, Sandhya Rani#, Aniket Kuvalekar* and Prabhakar Ranjekar

Interactive Research School for Health Affairs, Bharati Vidyapeeth Deemed University, Pune, Maharashtra, India

*Corresponding Author E-mail: kuaniket@gmail.com

ABSTRACT: A comprehensive *in vitro* study involving antiglycation, antioxidant and anti-diabetic assays was carried out in mature fruits of strawberry. The effect of aqueous extract of mature strawberry fruits on glycation of guanosine with glucose and fructose with or without oxidizing entities like reactive oxygen species was analyzed. Spectral studies showed that glycation and/or fructation of guanosine was significantly inhibited by aqueous extract of strawberry. The UV absorbance of the glycation reactions was found to be maximum at 24 hrs. and decreased consecutively for 48, 72 and 96 hours. Inhibition of oxidative damage due to reactive oxygen species was also observed in presence of the plant extract. To our knowledge, antiglycation activity of strawberry fruit with reference to guanosine is being demonstrated for the first time. To determine the antioxidant activity of the plant extract, *in vitro* antioxidant enzymes assays (catalase, peroxidase, polyphenol oxidase and ascorbic acid oxidase) and antioxidant assays (DPPH, superoxide anion scavenging activity and xanthine oxidase) were performed. Maximum inhibition activity of 79.36%, 65.62% and 62.78% was observed for DPPH, superoxide anion scavenging and xanthine oxidase, respectively. In antidiabetic assays, IC_{50} value for alpha – amylase and alpha – glucosidase activity of fruit extract of strawberry was found to be 86.47 ± 1.12µg/ml and 76.83 ± 0.93 µg/ml, respectively. Thus, the aqueous extract of strawberry showed antiglycation, antioxidant and antidiabetic properties indicating that strawberry fruits, as a dietary supplement, may be utilized towards management of diabetes.

Keywords: Antiglycation, Antioxidant, Antidiabetic, Strawberry

INTRODUCTION

Strawberry (*Fragaria X ananassa* Duch.) is a hybrid species and an important berry crop which is cultivated world wide for its fruit. Strawberries have been reported to possess high antioxidant activity (Halvorsen, et. al., 2002) due to the presence of polyphenolic compounds like anthocyanins, flavonols and ellagitannins (Aaby, et. al., 2005). It has also been reported that strawberry contains vitamin C and glutathione, both of which are good antioxidants (Guo, et al., 1997). Strawberry extract also inhibits free radicals including superoxide radicals and hydrogen peroxide (Wang and Jiao, 2000). It has been reported that consuming polyphenols either in the form of fruits or vegetables increases insulin activity and decreases the risk of type 2 diabetes (Anderson and Polansky, 2002; Anderson, et. al., 2004; Landrault, et. al., 2003). Recently, it has been found that fistein, a polyphenol found in strawberry, activates glyoxalase 1, an enzyme which helps to remove advanced glycation end products (AGEs) (Maher, et. al., 2011). AGEs are formed under pathological conditions of oxidative stress due to hyperglycemia in patients with diabetes. AGEs have been implicated in chronic diabetic complications such as neuropathy, nephropathy, retinopathy and arthritis. Fistein, present in strawberry does not lower the blood glucose levels, but it helps in removal or destruction of AGEs (Maher, et. al., 2011) and thus, may prevent further complications of diabetes. Antioxidants also act as AGEs inhibitors by inhibiting or slowing down the free radical formation in many chronic diseases (Reddy and Beyaz, 2006). Significant antioxidant and anti-inflammatory functions have been associated with strawberries as they are good sources of phytochemicals, polyphenols and anthocyanins (Hannum, 2004). Anthocyanins also protect pancreatic beta cells from glucose induced oxidative stress (Al-Awwadi, et. al., 2005). On the basis of above background information, antiglycation, antioxidant and anti-diabetic potentials of strawberry were analyzed in the present study. To the best of our knowledge, this is the first comprehensive report on the antiglycation, antioxidant and anti-diabetic activities of mature strawberry fruit.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

MATERIALS AND METHODS

Chemicals

NBT (nitroblue tetrazolium), xanthine oxidase, allopurinol, EDTA, ascorbic acid, hypoxanthine, α-amylase, α-glucosidase, p-nitrophenyl-α-d-glucopyranoside, D-glucose, D-fructose and hydrogen peroxide were purchased from SRL (India). DPPH (2, 2′- diphenyl-1-picrylhydrazyl), quercetin, guanosine were purchased from Sigma Chemicals Co. All other chemicals and reagents were of analytical grade.

Plant material

Mature fruits of *Fragaria* × *ananassa* Duch. cv. *Sweet Charlie* were collected from commercial plantation (All India Strawberry Growers Association) located at Mahabaleshwar, Maharashtra, India, 1438 m above the mean sea level. They were immediately stored in liquid nitrogen till further analysis.

Preparation of plant extract

Fruits were crushed to a fine powder in liquid nitrogen, which was then extracted separately using phosphate buffer (pH 7, 100mM). Briefly, 1gm of fruit powder was added to 20 ml of solvent. Filtration was done using fine Muslin cloth and Whatman filter paper no. 1. Filtrates were centrifuged at 10,000 rpm, 4°C for 15 min and clear supernatant was used for further assays. The concentrated extracts were stored at -20°C for further analysis.

ANTIGLYCATION ACTIVITY

Antiglycation activity of plant extract

Guanosine (100 µg) was incubated with plant extract (100 µg), glucose and fructose (600 mg) under sterile conditions for 24, 48 and 72 hours at 37°C. Solution of guanosine without sugar and plant extract served as a control. Following incubation for specified time, the absorbance of the solution was read at 254nm using Shimadzu UV-240 spectrophotometer.

Antiglycation activity of plant extracts under oxidative stress conditions *in vitro*

Hydrogen peroxide was used for the formation of Reactive Oxygen Species (ROS). Aqueous solution containing guanosine (100 µg), plant extract (100 µg), glucose or fructose (600 mg) and hydrogen peroxide (100 mM) was incubated for 24, 48 and 72 hours at 37°C. After the completion of reaction for different time periods tubes were irradiated under 254nm UV light for 30 minutes. The samples were then scanned for UV absorption spectra using Shimadzu UV-240 spectrophotometer.

ANTIOXIDANT ENZYMES

1. Catalase

Catalase activity was determined according to the method described by Bergmeyer (1974). The reaction mixture contained 1.5 ml 75mM sodium phosphate buffer (pH 7.0), 1.5 ml 25mM H_2O_2 and extract of strawberry (400µg). Decrease in absorbance at 240nm was recorded for 1 min. against blank. The protein concentration from the enzyme extract was determined by Bradford's method (Bradford, 1976). One unit of enzyme activity was defined as that amount of enzyme which causes decrease in absorbance of 0.001 in 1 min/mg protein under assay conditions.

2. Peroxidase Assay (POX)

The assay of POX was carried out as per the method described by Yuan and Jiang (2003). The reaction mixture contained 3 ml 0.1 M phosphate buffer (pH 7.0), 3 ml 20 mM guaiacol, 0.03 ml 1.7mM H_2O_2 and 400µg of fruit extract of strawberry. Increase in the absorbance at 436nm for 3 min. due to guaiacol oxidation, was recorded using spectrophotometer. The protein concentration from the enzyme extract was determined by Bradford's method (Bradford, 1976). The activity of POX was expressed in terms of the enzyme units. Unit activity of the enzyme was defined as that activity which increases the absorbance of the reaction mixture by 0.001 in 1 min/mg protein under assay conditions.

3. Polyphenol oxidase (PPO)

PPO assay was carried out using a method described by Jiang, et. al., (2002). The reaction mixture contained 1 ml 0.1 M methyl catechol, 2 ml 0.1 M phosphate buffer (pH 7.0) and 1 ml plant extract. Increase in absorbance at 495nm was recorded for 5 min. using a spectrophotometer. The protein concentration from the enzyme extract was determined using Bradford's method (Bradford, 1976). The activity of PPO was expressed in terms of the enzyme units. Unit activity of the enzyme was defined as that activity which increases the absorbance of the reaction mixture by 0.001 in 1 min/mg protein under assay conditions.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

4. Ascorbic acid oxidase

The ascorbic acid oxidase was determined by the method described by Oberbacher and Vines (1963). Briefly, 3ml of 8.8mg of Ascorbic acid in 0.1M phosphate buffer (pH 5.6) was taken in each of sample and reference cuvettes and 100 µg of fruit extract was added to sample cuvette. Increase in the absorbance was measured at 265 nm in 30 sec intervals for 5 min.

ANTIOXIDANT ASSAYS

1. DPPH- Free radical scavenging assay

DPPH (2,2′- diphenyl-1-picrylhydrazyl) is a stable free radical which is used to assess the scavenging activity of plant materials. DPPH assay was done as per the method described by Koleva, et. al., (2002). Various concentrations of plant extract (1-10µg/µl) were prepared before initiating the reaction. One twenty µl of DPPH solution in 0.2 mM absolute ethanol was then added to the reaction mixture which was then incubated at 25°C in the dark for 30 minutes. Absorbance was read at 517nm using a UV-visible spectrophotometer. Ascorbic acid was used as standard (1-10µg/µl). Blank was included for each sample dilution. The DPPH free radical scavenging activity was calculated using the following formula:

% scavenging = [(Absorbance of control – Absorbance of sample)/ (Absorbance of control)] x 100

2. Superoxide anion scavenging activity

Superoxide anion is the strongest reactive oxygen species among free radicals. It is important as it can form singlet oxygen and hydroxyl radical. Super oxide scavenging activity was determined by NBT reduction method as described by McCord and Fridovich (1969).

Different concentrations of plant extracts (0,10,20,50,100 and 500µg/µl) were mixed with 200µl 0.11mM Na_2EDTA in 66.67mM phosphate buffer (pH7.5), 100 µl 0.53mM riboflavin, 100 µl, 1.2mM NBT, 200 µl ethanol and 3 ml 51.5mM phosphate buffer (pH 7.4). The reaction mixture was kept under light for 15 minutes and the absorbance was monitored at 560nm. Antioxidant compounds present in plant extract inhibit NBT which results in decrease in absorbance. Percent inhibition was measured using the following formula:

% scavenging = [(Absorbance of control – Absorbance of sample)/ (Absorbance of control)] x 100

3. Xanthine oxidase assay

Xanthine oxidase is a terminal enzyme of purine catabolism found in liver and intestine (Battelli, et. al., 1972). It plays an important role in various diseases by generating superoxides during oxidation of substrates (Rohman, et. al., 2010).

Xanthine oxidase assay was carried out as per the procedure reported by Cimanga, et. al., (1999). The inhibitory effect of xanthine oxidase was measured spectrophotometrically at 295nm. Allopurinol was used as a positive control. Each reaction mixture contained varying concentrations of plant extract (0, 10, 20, 50, 100 and 500µg/µl) mixed with 1.3ml 66.67mM phosphate buffer (pH 7.5) and 1.5ml 0.15mM xanthine solution. This mixture was then incubated at 30°C for 10 minutes. Xanthine oxidase solution 500 µl (0.28U) was added to the mixture to initiate the reaction. The change in absorbance at 295nm was measured each minute for 10 minutes using a microtiter plate reader. The % inhibition of this enzyme with plant extracts was calculated using the equation below:

% Inhibition= [(OD Control – OD Sample)/ (OD Control)] x 100

4. Determination of flavonoid content

Total flavonoid content was determined as per the method described by Ordonez (2006), using quercetin as standard. Each reaction mixture contained various concentration of plant extract (10-100 µg/ µl), 75 µl 5% $NaNO_2$ and 150 µl 10% $AlCl_3$. Final volume of the reaction was adjusted to 2.5 ml with distilled water. After 5 minutes, 500 µl 1M NaOH was added to the reaction. Absorbance was monitored at 510nm. Flavonoid content was calculated according to the following formula:

% Flavonoids= [{(OD extract * 0.05)/ OD Quercetin}/ Extract concentration] *100

ANTIDIABETIC ASSAYS

1. α-Amylase Inhibition Assay

The α-amylase inhibitory activity was determined by an assay from the Worthington Enzyme Manual (Worthington, 1993). Concentrations of plant extract ranging from (0,10, 20,40,60,80,100 µg/µl) were used for the assay. The reaction mixture contained 500 µl plant extract of desired concentration, 500 µl 0.02M phosphate buffer (pH 6.9) and 0.05U α-amylase. Reaction mixture was incubated at 25°C for 10 minutes.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

The reaction was initiated by adding 500µl 1% starch solution in 0.02M phosphate buffer. The reaction was stopped with 1ml 90 mM dinitrosalicylic acid (DNS), and the mixture was placed in a water bath at 85°C for 5 min. The reaction mixture was then diluted to 8 ml with distilled water, and the absorbance was measured at 540nm using UV-visible spectrophotometer. Controls were representative of the 100% enzyme activity. Acarbose, an α-amylase inhibitor, was used as a positive control. The % α-amylase inhibitory activity was calculated using the following formula:

% Inhibition= [(OD Control – OD Sample)/ (OD Control)] x 100

2. α- Glucosidase Inhibition Assay:

The α- Glucosidase inhibitory activity was determined as per the assay described by McCue et al. (2005). Various concentrations of plant extract were used (2, 4, 6, 8, 10, 20, 50, 100µg). The reaction mixture contained 50µl α-Glucosidase (5U/ml), plant extracts of various concentrations and 100 µl 0.1M phosphate buffer (pH 6.9). Paranitrophenyl- α-d-glucopyranoside was used as a substrate. After pre-incubation for 5 minutes, 50 µl 5mM substrate was added and the reaction mixture was further incubated for 5 min. at 25°C. Absorbance of the reaction mixture before and after addition of the substrate was measured at 405 nm on a microplate reader and was compared with control. Acarbose was used as a positive control. The α- glucosidase inhibitory activity was expressed as percentage inhibition and was calculated using the formula:

% Inhibition= [(OD Control – OD Sample)/ (OD Control)] x 100

Statistical Analysis

All samples were analyzed in triplicates. Data are presented as mean ± standard error. IC_{50} were calculated from the graphs for respective enzymes.

RESULTS AND DISCUSSION

Antiglycation activity of plant extract

It has been reported that nucleotides form advanced glycation end products (AGEs) *in-vitro* as well as *in-vivo* (Bucala, et. al., 1984). DNA nucleoside guanosine reacts with sugars in same way as with proteins and form DNA-AGEs complex (Seidel and Pischetsrieder, 1998) and it is known that 2-amino group of guanosine is most reactive. Some of the nucleotide AGEs are imidazopurinone derivative dG-G [3-(2'-deoxyribosyl)-6, 7-dihydro-6, 7-dihydroxyimidazo [2,3-b]purin-9(8)one] and CMdG (N (2)-carboxymethyldeoxyguanosine (Thornalley, 2003). These AGEs undergo auto-oxidation and have been implicated in Alzheimer's disease (Srikanth, et. al., 2011), cardiovascular disease (Simm, et. al., 2007) and also plays an important role in vascular complications of diabetes (Yan, et. al., 2007).

Table 1 summarizes the absorbance of control (guanosine + water), glycated, fructated and ROS-modified glycated and fructated guanosine. Maximum absorbance of glycated and fructated guanosine was observed at 24 hours with consecutive decrease from 48 to 96 hours as compared to control. Non-enzymatic glycation might block the free NH_2 group present in guanosine, which results in decreased absorbance at different time periods. Glycation or fructation reactions of guanosine under oxidative stress indicated increase in the absorbance consecutively with the time period which may be due to the breakage of bonds and bases as a result of oxidative damage.

Table 1. Absorbance of glycated, fructated and ROS-modified glycated and fructated guanosine at 260nm for various incubation time regimens

Incubation Time (hrs)	Absorbance at 260 nm				
	Glycated guanosine	Fructated guanosine	ROS-modified Glycated guanosine	ROS-modified Fructated guanosine	Guanosine + Water
24	3.2	3.3	3.1	3.3	1.2
48	2.9	3.2	3.22	3.5	1.1
72	2.87	2.9	3.39	3.64	0.9
96	2.78	2.8	3.41	3.7	0.6

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Table 2 shows the inhibition activity of plant extract of strawberry on glycated, fructated and glycation and fructation of guanosine under oxidative stress for different time periods (24, 48, 72 and 96 hours). Maximum inhibition of activity of 17% and 20% on glycated and fructated guanosine after 96 hours was observed. Similarly, maximum inhibition activity of 25% and 30% was observed after 96 hours of glycation/fructation reactions under oxidative stress and UV irradiation. Decrease in the absorbance and consecutive increase in inhibition was monitored as a result of oxidative damage of ROS-modified glycated and fructated guanosine. Incubation of 100µg of plant extract in these reactions for various time periods (48, 72 and 96 hours) results in increase in inhibition activity of glycation, fructation and glycation and fructation under oxidative stress conditions.

Reactive oxygen species (ROS) react with many macromolecules, causing damage to protein, lipids and DNA (Droge, 2002). Hydroxyl radical reacts with DNA by addition of double bonds. AGEs may form reactive oxygen species, which tend to form cross-links by binding to cell surface receptors (Brownlee, et. al., 1985, Schmidt, et. al., 1994; Singh, et. al., 2001). Further, AGEs have been associated with diabetes and its complications (Brownlee, 1995; Schmidt, et. al., 1995; Rojas and Morales, 2004; Garay-sevilla, et. al., 2005).

Formation of AGEs is slowest with glucose as compared to the natural sugar fructose (Rojas and Morales, 2004; Garay-sevilla, et. al., 2005). *In-vivo* fructose derived AGEs are formed ten times faster as compared to glucose derived AGEs (Hogan, et. al., 1992). In our work, maximum inhibition of 20% by plant extract at 96 hours is observed with fructated guanosine, indicating that fructose, compared with glucose, is a much more potent initiator of the Maillard reaction (Bunn and Higgins, 1981; McPherson, et. al., 1988). Maillard reaction is known to be involved in the aging process alongwith other physiological changes resulting in further increase in oxidative stress (Monnier, 1989). Plant extracts of strawberry delay non-enzymatic glycation reactions and further may delay the formation of advanced glycation end products in the form of fructated guanosine. Fawzi, et. al., (2012) have reported antiglycation activity of various medicinal plants used in folk medicine for management of diabetes. We have earlier reported the antiglycation and antioxidant activities of *Rubia cordifolia* for the management of diabetes (Rani, et. al., 2013).

These observations suggest that strawberry fruits possess antiglycation activity. This antiglycation activity of the plant extract can be co-related with the free radicals scavenging by antioxidant enzymes and high phenolic contents of the fruits.

Table 2. Antiglycation and inhibition percent data of glycated guanosine, fructated guanosine, ROS-modification with glucose and fructose by the plant extract of strawberry for various incubation time regimens

Incubation Time (hrs)	Antiglycation activity of plant extract with glycated guanosine	Antiglycation activity of plant extract with fructated guanosine	Inhibition by plant extract of glycation with ROS-modification/ UV/Guanosine	Inhibition by plant extract of Fructation with ROS-modification/ UV/Guanosine
24	12 %	13 %	17 %	18 %
48	13 %	15 %	20 %	23 %
72	16 %	17 %	23 %	28 %
96	17 %	20 %	25 %	30 %

Table 3. Enzyme activity of antioxidant assays (catalase, peroxidase, polyphenol oxidase and ascorbic acid oxidase). Each enzyme activity is expressed as a mean of triplicate experiments

Assays	Enzyme activity
Catalase Assay	0.896×10^2
Peroxidase Assay (POX)	0.260×10^2
Polyphenol oxidase (PPO)	1.613×10^2
Ascorbic acid oxidase	0.521×10^2

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

ANTIOXIDANT ENZYMES

Table 3 shows the activity of all antioxidant enzymes namely catalase, PPO, peroxidase and ascorbic acid oxidase. Fruit extract of strawberry displayed significant antioxidant activities depicting that ROS generation might lead to production of antioxidant defense systems in strawberry.

Catalase is an endogenous antioxidant enzyme which removes hydrogen peroxide in cells. Removal of hydrogen peroxide is necessary for protection against oxidative damage to cells and tissues. Amount of hydrogen peroxide in the reaction mixture is measured to calculate the rate of reaction. The Catalase activity of fruit extract of strawberry was found to be 0.896 X 10^2.

Polyphenol oxidase (PPO) catalyzes the oxidation of phenolic compounds using molecular oxygen. It catalyzes the oxidation of catechol to o-quinone, which is further used to synthesize melanins. PPO activity of the fruit extract of strawberry was found to be 1.613 X 10^2.

Peroxidases belong to a large family of enzymes and catalyze the reduction of hydrogen peroxide and oxidize various substrates. In biological processes, peroxidases play an important role in defense mechanism, immune response and pathogeny. The activity of peroxidase in fruit extract of strawberry was found to be 0.260 X 10^2.

Ascorbic acid oxidase catalyzes the oxidation of ascorbic acid to yield dehydroascorbic acid, followed by its decomposition. Ascorbic acid oxidase activity of the fruit extract was observed to be 0.521 X 10^2.

Changes in the activities of antioxidant enzymes under different environmental conditions has been studied in many plants (Li, et. al., 2000; Wu, et. al., 2004; Zhang, 2004). Catalase, PPO, peroxidase and ascorbic acid oxidase play an important role in the scavenging process. The actions of peroxidase and polyphenol oxidase enzymes predominantly affect the ability of fresh and processed fruits and vegetables to maintain their characteristic flavor and color (Vamos-Vigyazo, 1981; Burnette, 1977). Peroxidases are involved in many physiological processes in plants, involving responses to biotic and abiotic stresses and the biosynthesis of lignin.

ANTIOXIDANT ASSAYS

Antioxidant properties of the fruit extract of strawberry were evaluated to assess its ability to scavenge DPPH, superoxide anion free radical and xanthine oxidase activity. It is well known that antioxidants present in fruits and vegetables can seize the free radical chain of oxidation and can form stable free radicals, which would not initiate or propagate further oxidation.

Results of all the antioxidant assays (DPPH, superoxide anion free radical and xanthine oxidase) are summarized in Table 4.

Table 4. Activity of antioxidant assays (DPPH, Superoxide anion free radical, xanthine oxidase) of the fruit extract of strawberry. Results were expressed as percent of inhibition as compared to controls. IC_{50} Value represents the mean ±SD of three separate experiments.

Antioxidant assays	Standard Compound	Standard		strawberry extract	
		IC_{50} (µg/µl)	Maximum Inhibiton activity (conc. µg)	IC_{50} (µg/µl)	Maximum Inhibition activity (conc.)
DPPH- Free radical scavenging assay	Ascorbic acid	1.56±1.2	79.36% (9.27 µg/µl ±0.70)	7.6±2.1	75.23% (9.76 µg/µl ±0.25)
Superoxide anion free radical	Quercetine	174.41±11.2	65.62% (486 µg/µl ±0.28)	453.45±0.34	53.12 % (494 µg/µl ±1.51)
Xanthine Oxidase activity	Allopurinol	128.96±2.1	62.78% (420 µg/µl ±0.912)	334.62±1.34	51.99 % (498 µg/µl ±1.42)

DPPH is one of the fastest methods to determine the antioxidant potential of plant extracts. DPPH assay depends on the electron or hydrogen atom-donating properties of antioxidants. IC_{50} value of fruit extract of strawberry and control (ascorbic acid) was found to be 7.6µg/µl and 1.56µg/µl, respectively. Maximum inhibition by the extract was observed to be 75.23% at a concentration of 9.76 ± 0.25 µg/µl as compared to ascorbic acid which was observed to be 79.36% at a concentration of 9.267 ± 0.702 µg/µl.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Superoxide is quite toxic and is produced in large quantities by the enzyme NADH oxidase. Antioxidants present in the fruit extract of strawberry consume superoxide anion which is revealed by the decrease in absorbance at 560nm. Scavenging activities of the plants are known to be associated with their polyphenolic content (Kimura, et. al., 1985; Hatono, et. al., 1989). Maximum percent inhibition of fruit extract of strawberry on superoxide free radical was found to be 53.12% at a concentration of 494µg/µl. Standard quercetine showed a maximum inhibition of 65.62% at a concentration of 486µg/µl. The IC_{50} value for superoxide scavenging activity of fruit extract of strawberry was found to be 453.45 ± 10.34 µg/µl whereas, the IC_{50} value of the standard quercetine was 174.41 µg/µl ± 11.2.

Xanthine oxidase generates reactive oxygen species and synthesizes uric acid which plays an important role as an oxidative stress marker and antioxidant (Glantzounis, et. al., 2005). Inhibition of xanthine oxidase has been associated with cardiovascular health. Maximum xanthine oxidase activity (51.99%) was observed at 498µg/µl plant extract. Standard Allopurinol (420 µg/µl) showed a maximum inhibition of 62.78%. The IC_{50} value for xanthine oxidase activity of fruit extract of strawberry was found to be 334.62 ± 1.34 µg/µl whereas, the IC_{50} value of the standard Allopurinol was 128.96 ± 2.1 µg/µl.

Flavonoid content

Flavonoids and phenolics are antioxidant compounds present in fruits and vegetables (Huang, et. al., 1998) and have been shown to work as antioxidants *in-vitro* (Kubo, et. al., 1999). As an antioxidative agent, these compounds help in slowing down of oxidation reaction. Flavonoid content of strawberry in terms of quercitine equivalents was found to be 316.1mg/gm. Natural antioxidants from plants are safe for the body and can be taken orally, even in large doses (Green, et. al., 2004). Therefore, studies to identify natural antioxidants from plant gained much importance (Gulcin, et. al., 2004). The present study indicates that mature fruits of strawberry have significant antioxidant activity and flavonoid contents. More studies are required to understand the mechanism of action of these antioxidants.

ANTI-DIABETIC ASSAYS

Alpha–amylase and Alpha–glucosidase

Alpha-amylase catalyzes the breakdown of starch into sugars. Alpha amylase inhibitors like acarbose, voglibose are considered effective for the treatment of diabetes as they delay carbohydrate absorption. These synthetic inhibitors are associated with various undesirable side effects (Scott and Spencer, 2000) and hence natural inhibitors from plant sources are in demand. IC_{50} value for Alpha - amylase activity of fruit extract of strawberry was found to be 86.47 ± 1.12µg/µl (Fig.1a) whereas, the IC_{50} value of the standard acarbose was 14.67 ± 1.2 µg/µl. Maximum percent inhibition of fruit extract of strawberry was found to be 52.35% at a concentration of 97µg/µl. Standard acarbose showed a maximum inhibition of 72.10% at a concentration of 92µg/µl. α-glucosidase hydrolyzes the terminal, non-reducing 1, 4-linked α-D-glucose residues with release of α-D-glucose. Inhibitors like acarbose antagonize the activity of α-Glucosidase, thus delaying carbohydrate absorption and helping in lowering down the blood sugar levels (Koyasu, et. al., 2010). These inhibitors have various side effects and therefore there is an urgent need for the inhibitors from natural resources. IC_{50} value for Alpha- glucosidase activity of fruit extract of strawberry was found to be 76.83 ± 0.93 µg/µl whereas, the IC_{50} value of the standard acarbose was 45.49 ± 0.78 µg/µl. Maximum percent inhibition of fruit extract of strawberry was found to be 58.83% at a concentration of 91µg (Fig. 1b). Standard acarbose showed a maximum inhibition of 69.43% at a concentration of 96µg/µl.

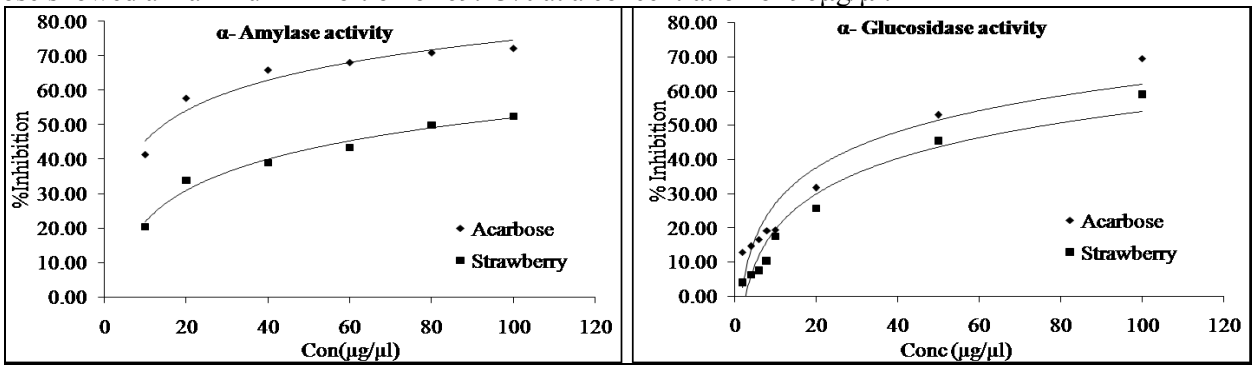

Figure 1 Alpha amylase (a) and Alpha glucosidase (b) inhibitory activity by plant extract of strawberry. Each value represents mean ± standard deviation of triplicate experiments.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Inhibitors of Alpha-amylase and alpha-glucosidase are considered effective for the control of blood glucose. Inhibitors from natural sources offer an attractive approach for the treatment of hyperglycemia, as they delay carbohydrate absorption by inhibiting the activity of carbohydrate hydrolyzing enzymes. They have low side effects and the therapies are well tolerated as compared to other hypoglycemic oral agents. Our present research suggests that strawberry may play a potential role in inhibiting α-amylase and α-glucosidase enzyme activities. This inhibiton activity may be due to the presence of polyphenolic compounds present in the strawberry.

CONCLUSION

Strawberry is a fruit of nutritional and economical importance. To the best of our knowledge, this is a first comprehensive report on strawberry where antiglycation, antioxidant and anti-diabetic properties are studied. This study indicates that strawberry possesses antiglycation, antioxidant and anti-diabetic activity. Dietary consumption of strawberry and its antioxidant effects may be potentially helpful for prevention of diabetes and its complications. Further studies to demonstrate the *in vivo* potential of these activities of strawberry are warranted.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Bharati Vidyapeeth Deemed University, Pune, India for providing the financial assistance for this work.

REFERENCES

Aaby K, Skrede G. and Wrolstad R.E. (2005). Phenolic composition and antioxidant activities in flesh and achenes of strawberries (*Fragaria ananassa*). J Agric Food Chem: Vol.53, 4032–4040.

Al-Awwadi N.A, Araiz C, Bornet A, Delbosc S, Cristol J.P, Linck N, Azay J, Teissedre P.L. and Cros G. (2005). Extracts enriched in different polyphenolic families normalize increased cardiac NADPH oxidase expression while having differential effects on insulin resistance, hypertension, and cardiac hypertrophy in high-fructose-fed rats. J Agric Food Chem: Vol.53, 151-157.

Anderson R.A, Broadhurst C.L, Polansky M.M, Schmidt W.F, Khan A, Flanagan V.P, Schoene N.W. and Graves D.J. (2004). Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. J Agric Food Chem: Vol.52, 65-70.

Anderson R.A. and Polansky M.M. (2002). Tea enhances insulin activity. J Agric Food Chem: Vol.50, 7182-7186.

Battelli M.G, Corte E.D. and Stirpe F. (1972). Xanthine oxidase type D (dehydrogenase) in the intestine and other organs of the rat. Biochemical Journal: Vol.126,747-749.

Bergmeyer H.U. (1974). *Methods of Enzymatic Analysis, Volume 1*. 2nd edition. Edited by Bergmeyer HU. New York: Academic press: pp: 438.

Bradford M.M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry: Vol.72, 248-254.

Brownlee M, Vlassara H. and Cerami A. (1985). Nonenzymatic glycosylation products on collagen covalently trap low-density lipoprotein. Diabetes: Vol.34, 938 –941.

Brownlee M. (1995). Advanced protein glycosylation in diabetes and aging. Annu Rev Med: Vol.46, 223-234.

Bucala R, Model P. and Cerami A. (1984). Modification of DNA by reducing sugars: a possible mechanism for nucleic acid aging and age-related dysfunction in gene expression. Proc. Natl. Acad. Sci: Vol.81, 105-109.

Bunn H.F. and Higgins P.L. (1981). Reaction of monosaccharides with proteins: possible evolutionary significance, Science: Vol.213, 222-224.

Burnette F.S. (1977). Peroxidase and its relationship to food flavor and quality: A review. J Food Sci: Vol.42, 1-6.

Cimanga K, De Bruyne T, Hu J.P, Cos P, Apers S, Pieters L, Tona L, Kambu K, Vanden Berghe D. and Vlietink A.J. (1999). Constituents from *Morinda morindoides* leaves as inhibitors of xanthine oxidase and scavengers of superoxide anions, Pharm Pharmacol Comm: Vol.5, 419–424.

Droge W. (2002). Free radicals in the physiological control of cell function. Physiol. Rev: Vol.82, 47–95.

Garay-Sevilla M.E, Regalado J.C, Malacara J.M, Nava L.E, Wrobel-Zasada K, Castro-Rivas A. and Wrobel K. (2005). Advanced glycosylation end products in skin, serum, saliva and urine and its association with complications of patients with type 2 diabetes mellitus. J Endocrinol Invest: Vol.28, 223–230.

Glantzounis G.K, Tsimoyiannis E.C, Kappas A.M. and Galaris D.A. (2005). Uric acid and oxidative stress. Curr Pharm Des: Vol.11, 4145-4151.

Green K, Brand A. and Murphy M. (2004) Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes. Diab:Vol.53, S110–S118.

Gulcin I, Kufrevioglu O.I. and Oktay M. (2004). Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). J Ethnopharmacol: Vol. 90,205–215.

Guo C, Cao G. and Sofic E. (1997). High-performance liquid chromatography coupled with coulometric array detection of electroactive components in fruits and vegetables. Relationship to oxygen radical absorbance capacity. J AgricFood Chem: Vol.45, 1787–1796.

Halvorsen B.L, Holte K. and Myhrstad M.C. (2002) A systematic screening of total antioxidants in dietary plants. J Nutr: Vol.132, 461–471.

Hannum S.M. (2004) Potential impact of strawberries on human health: a review of the science. Crit Rev Food Sci Nutr: Vol.44, 1-17.

Hatono T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y. and Yasuhara T. et al. (1989). Effects of the interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. Chemical & Pharmaceutical Bulletin: Vol.37, 2016–2021.

Hogan M, Cerami A. and Bucala R. (1992). Advanced glycosylation end products block the antiproliferative effect of nitric oxide: role in the vascular and renal complications of diabetes mellitus. J Clin Invest: Vol.90, 1110–1115.

Huang Y, Tan A, Shen Y. and Lu J. (1998). Scavenging effect of total flavonoids of *Lycium barbarum* L on active oxygen radicals and inhibitory effects on heat output from L 1210 cells. Wei Sheng Yen Chiu: Vol.27, 109–111.

Jiang Y.M, Zhang Z.Q, Joyce D.C. and Ketsa S. (2002). Postharvest biology and handling of longan fruit **(***Dimocarpus longan* Lour.**).** Postharvest Biol Technol: Vol. 26**,**241-252.

Kimura Y, Okuda T, Hatono T, Agata I. and Arichi S. (1985). Effects of extracts of leaves of *Artemisia* species and caffeic acid and chlorogenic acid on lipid metabolic injury in rats fed peroxidized oil. Chemical & Pharmaceutical Bulletin: Vol.33, 2028–2034.

Koleva II, van Beek TA, Linssen JP, de Groot A, Evstatieva LN. (2002). Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem Anal: Vol.13, 8–17.

Koyasu M, Ishii H, Watarai M, Takemoto K, Inden Y, Takeshita K, Amano T, Yoshikawa D, Matsubara T. and Murohara T. (2010). Impact of acarbose on carotid intima-media thickness in patients with newly diagnosed impaired glucose tolerance or mild type 2 diabetes mellitus: A one-year prospective, randomized, open-label, parallel-group study in Japanese adults with established coronary artery disease. Clin. Ther: Vol.32, 1610-1617.

Kubo A, Aono M, Nakajima N, Saji H, Tanaka K. and Kondo N. (1999). Differential responses in activity of antioxidant enzymes to different environmental stresses in *Arabidopsis thaliana*. J. Plant Res: Vol.112, 279-390.

Landrault N, Poucheret P, Azay J, Krosniak M, Gasc F, Jenin C, Cros G. and Teissedre P.L. (2003). Effect of a polyphenols enriched chardonnay white wine in diabetic rats. J AgricFood Chem: Vol.51, 311-318.

Li J, Yan X.F. and Zu Y.G. (2000). Generation of activated oxygen and change of cell defense enzyme activity in leaves of Korean pine seedling under low temperature. Acta Botanica Sinica, 2:148-152.

Maher P, Dargusch R, Ehren J.L, Okada S. and Sharma K. et. al., (2011). Fisetin lowers methylglyoxal dependent protein glycation and limits the complications of diabetes. PLoS ONE: Vol.6, 1-11.

Mahomoodally F.M, Subratty A.H, Fakim A.G. and Choudhary M.I. (2012). Antioxidant, antiglycation and cytotoxicity evaluation of selected medicinal plants of the Mascarene Islands. BMC Complementary and Alternative Medicine: Vol.12,165-176.

McCord J.M. and Fridovich I. (1969). Superoxide Dismutase: An enzymic function for erythrocuprein (hemocuprein) J. Biol. Chem: Vol.244, 6049-6055.

McCue P, Kwon Y.I. Shetty K. (2005). Anti-amylase, anti-glucosidase and anti angiotensin I converting enzyme potential of selected foods, J Food Biochem: Vol. 29,278–294.

McPherson J.D, Shilton B.H. and Walton D.J. (1988). Role of fructose glycation and cross-linking of proteins, Biochemistry: Vol.27, 1901–1907.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Monnier V.M. (1989) Toward a Maillard reaction theory of aging. In: The Maillard Reaction in Aging, Diabetes, and Nutrition. (Baynes, J.W., and Monnier, V.M., eds.), Alan R. Liss, New York, pp:1-22.

Oberbacher M.F. and Vines H.M. (1963). Spectrophotometric Assay of Ascorbic Acid Oxidase. Nature: Vol.197, 1203-1204.

Ordonez A.A.L, Gomez J.D, Vattuone M.A. and Isla M.I. (2006). Antioxidant activities of Sechium edule (Jacq.) Swartz extract. Food Chemistry: Vol.97, 452–458.

Rani S, Mandave P, Khadke S, Jagtap S, Patil S. and Kuvalekar A. (2013). Antiglycation, Antioxidant and Antidiabetic activity of traditional medicinal plant: *Rubia cordifolia* Linn. for management of hyperglycemic effect. International Journal of Plant Animal and Environmental Sciences: Vol.3, 42-49.

Reddy V.P. and Beyaz A. (2006). Inhibitors of the Maillard reaction and AGE breakers as therapeutics for multiple diseases, Drug Discov Today: Vol.11, 646-654.

Rohman A, Riyanto S, Yuniarti N, Saputra W.R, Utami R. and Mulatsih W. (2010). Antioxidant activity, total phenolic, and total flavonoid of extracts and fractions of red fruit (*Pandanus conoideus* Lam). International Food Research Journal: Vol.17, 97-106.

Rojas A. and Morales M.A. (2004). Advanced glycation and endothelial functions: a link towards vascular complications in diabetes. Life Sci: Vol.76, 715–730.

Schmidt A.M, Hori O, Brett J, Yan S.D, Wautier J.L. and Stern D. (1994). Cellular receptors for advanced glycation end products: implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. Arterioscler Thromb: Vol.14, 1521–1528.

Schmidt A.M. Yan S.D. and Stern D.M. (1995). The dark side of glucose. Nat Med: Vol.1, 1002–1004.

Scott L.J. and Spencer C.M. (2000). Miglitol: a review of its therapeutic potential in type 2 diabetes mellitus, Drugs: Vol.59, 521-549.

Seidel W. and Pischetsrieder M. (1998). DNA-glycation leads to depurination by the loss of N2-carboxyethylguanine in vitro. Cell Mol Biol:Vol.44, 1165-1170.

Simm A, Wagner J, Gursinsky T, Nass N, Friedrich I, Schinzel R, Czeslik E, Silber R.E. and Scheubel R.J. (2007). Advanced glycation endproducts: a biomarker for age as an outcome predictor after cardiac surgery? Exp Gerontol: Vol.42, 668-675.

Singh R, Barden A, Mori T. and Beilin L. (2001). Advanced glycation end-products: a review. Diabetologia: Vol.44, 129 –146.

Srikanth V, MacZurek A, Phan T, Steele M, Westcott B, Juskiw D. and Münch G. (2011). Advanced glycation endproducts and their receptor RAGE in Alzheimer's disease. Neurobiology of Aging: Vol.32, 763–777.

Thornalley P.J. (2003). protecting the genome: defense against nucleotide glycation and emerging role of glyoxalase I overexpression in multidrug resistance in cancer chemotherapy. Biochem Soc Trans: Vol.31, 1372-1377.

Vamos-Vigyazo L. (1981). Polyphenol oxidase and peroxidase in fruits and vegetables. Crit. Rev Food Sci Nutr. Vol.15, 49-127.

Wang S.Y. and Jiao H. (2000). Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. J Agric Food Chem: Vol.48, 5677-5684.

Worthington V. (1993). Alpha amylase: Worthington Enzyme Manual. Worthington Biochemical, Corp. Freehold, NJ, pp: 36-41.

Wu J.H, Yang L. and Sun G.R. (2004) Generation of activated oxygen and change of cell defense enzyme activity in leaves of maize seeding under stress of low temperature. Bulletin of Botanical Research. Vol. 4,456-459.

Yan S.F, D'Agati V, Schmidt A.M. and Ramasamy R. (2007). Receptor for Advanced Glycation Endproducts (RAGE): a formidable force in the pathogenesis of the cardiovascular complications of diabetes & aging. Current molecular medicine: Vol.7, 699–710.

Yuan Z.Y. and Jiang T.J. (2003). Horseradish peroxidase**.** In Handbook of Food Enzymology**.** Edited by Whitaker JR, Voragen A, Wong DWS. New York: Marcel Dekker Inc. pp: 403-411.

Zhang GX (2004). Effect of low-temperature stress on physiological reaction of cineraria. Journal of Nanjing Forestry University (Natural Sciences): Vol.5, 89-92.