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Assessment of Strawberry Polyphenols Aqueous Extract for Major Compositional and Biofunctional Attributes

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ABSTRACT

The current study was conducted to assess the major compositional parameters like total phenolic , anthocyanin and total flavonoid content and also to assess the effect of strawberry polyphenol extract on the antioxidant potential using DPPH and ORAC assay, carbohydrate hydrolyzing enzymes i.e α-amylase, α-glucosidase inhibition activity and ACE inhibition activity spectrophotometrically. The total phenolic content in the strawberry polyphenol extract was observed to be 10 mg GAE per ml of extract. Total flavonoid content was estimated approximately 526±0.88 µg quercetin per ml of extract and total anthocyanin content was observed to be 164±1.15 µg cyaniding-3- glucoside equivalent per ml of extract. These major compositional components have a contribution towards the biofunctional properties like antioxidant, ACE inhibitory and antidiabetic properties. The antioxidant activity measured by DPPH and ORAC assays was observed to be 2.76±0.01 mM, 8.3±0.15 mM, respectively at the polyphenol concentrations 0.5 mg/ml. The percentage inhibition activity of carbohydrate hydrolyzing enzyme i.e α-amylase, α- glucosidase and ACE (angiotensin converting enzyme inhibitory activity) of polyphenol extract was observed to be 6.33±1.45, 25.33±0.88 and 24.16±0.44 at 0.5 mg/ml polyphenol concentration. Results predicted the health promoting attributes of strawberry polyphenol extract. Hence, these can be supplemented into the diet of human beings, as the synthetic drugs leads to bad effects on the human health.

Key Words: Phytonutrients, Strawberry, Flavonoid, Anthocyanin, Fruit, Diseases

INTRODUCTION

In the recent years it has been observed that high intake fruits and vegetables by the human population can lead to the prevention of several life threading diseases (15). Among the fruits strawberry is a rich source of phenolic phytochemicals, consumed either fresh fruit or processed (1). It contains a good quantity of phenolic compounds which are beneficial for the health (5, 25). Polyphenol content and flavoinoid content are the major contributing factor for providing health benefits (19). Polyphenols are the secondary metabolites of plants and considered to possessed several health promoting attributes (20). Present scenario indicates that strawberries consumption as a natural source of bioactive components is related to the prevention of several life threatening diseases such as hypertension and other cardiovascular diseases (1). Apart this, previously reported findings suggested the anti-inflammatory, anticarcinogenic and antiproliferative activities of strawberry consumption (1). Strawberries are also rich in other nutritive compounds such as vitamins, fatty acids, minerals, fibres and secondary metabolites (12). Flavonoids are the major components, followed by ellagitannins, flavonols and phenolic acids contributes towards bioactivity (23). Anthocyanin present in strawberry contributes towards color and sensory properties (2). Another important phenolic compound is tannins i.e ellagitannins but it is present in a few berries and nuts (9). Strawberry is also rich in high proportion of condensed tannins i.e proanthocyanidin (1). The estimation of these condensed tannins is quite difficult, as lacking of the optimum methodology for their extraction and determination (7). The health benefits of these strawberry polyphenolic compounds can be delivered to human being without being consumed any synthetic drug or better utilization of natural source of health promoting components, their content and beneficial level need to be understand. Hence the present study was aimed with the examining the major phenolic compounds content and their level of bioactivities.

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MATERIALS AND METHODS

Materials

Strawberry fruit pulp was procured from the M/s Delta nutritive Pvt. Ltd., Mumbai, Folin-Ciocalteu's reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS)gallic acid was procured from the Sigma chemicals.

Preparation of strawberry polyphenols extract from strawberry fruit pulp

The strawberry fruit pulp was collected from the M/s Delta nutritive Pvt. Ltd., Mumbai. The water soluble polyphenol extract was prepared according to the procedure described by Cossu *et al*. (2009). The water and fruit pulp was mixed and homogenized using mechanical homogenizer in 1:3. The mixing process was continued for about 30 min. Then centrifugation was done at 4025 g for 15 min (Kubota Tokyo, Japan). Polyphenols extracted in supernatant were concentrated by freezing and lyophillization technique. The prepared polyphenol extract was stored at -20ºC for further analysis.

Determination of total monomeric anthocyanin pigments

The anthocyanin content was estimated using the method of AOAC; 2005. The difference in the absorbance of pigments at 520nm at pH 1.0 and pH 4.5 was measured and calculated total anthocyanin pigments in the extract. For the pH adjustment dilution with the potassium chloride buffer (0.025M), pH 1.0 and buffer, pH 4.5 (sodium acetate, 0.4M) was done. The anthocyanin content was calculated as anthocyanins pigment (cyanidin-3-glucoside equivalents, mg/L) = (A * MW*DF*10^3) /ε*1; Where A = (A520nm – A 700nm) pH 1.0 – (A520nm – A700nm) pH 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside; DF = dilution factor1 = path length in cm; ε = 26 900 molar extinction coefficient, in L *mol^{-1} * cm^{-1}, for cyd-3-glu;10^3 = factor for conversion from g to mg.

Estimation of total flavonoid content

Total flavonoids contents were determined using the method of Ordon *et al* (2006). 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of sample solution. Then incubated for 1 hour at room temperature and absorbance was taken at 420 nm using double beam spectrophotometer (SPECORD-200, Analytical zena). The results are expressed as quercetin equivalents per ml of extract.

The total phenolic content of the extract

The total polyphenolic content was measured as per the procedure described by Zhang et al., 2006. A standard curve of gallic acid was prepared ranged from 0-120 µg/ml and results were expressed as µg gallic acid equivalent (GAE) ml^{-1}.

Measurement of bio functional attributes of strawberry polyphenolic extract

Antioxidant activity by ORAC and DPPH assay

ORAC (Oxygen Radical Absorbance Capacity) assay

Antioxidant activity by ORAC-FL assay was determined according to the methods developed by Ou *et al.,* 2001 and modified by Zulueta *et al*., 2009. Experiment was performed using Elisa microplate reader. In each well Fluoresein 150 µL, standard (trolox), sample and blank each 25 µL were pipette in triplicate. Then the microplate was sealed and incubated for 30 min. at 37°C in a Microplate reader (Model infinite 200, Austria) incubator without shaking. Fluorescence was estimated at Ex. 485nm, Em. 520nm after every 90 sec to measure the background signal. 2525 µL (240 mM) of AAPH was added manually after every 3 seconds. Again test was resumed and fluorescence measurement was taken upto 90 minutes. Area under the fluorescence decay curve (AUC) was calculated as

$$AUC = 1 + \sum_{i=1}^{i=90} fi/f0$$

Where, f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time i. The net AUC corresponding to a sample was calculated as Net AUC = $AUC_{antioxidant}$ - AUC_{blank}

The regression equation between net AUC and antioxidant concentration was calculated. Final ORAC-FL values were expressed as µM of Trolox equivalent.

DPPH radical scavenging activity

Antioxidant potential based on the DPPH (2, 2 diphenyl-1-picryl hydrazyl) assay was measured as per the method given by Brand Williams *et al*., 1995. 100 µL of sample/ trolox solution of appropriate dilutions were added to 4.0 ml of freshly prepared DPPH (0.028 mM) working solution in a test tube. The content were vertexed and kept in dark for 30 min. at 37^{0}C The absorbance of the solution was taken at 515 nm against methanol using SPECORD-200 double beam spectrophotometer (Analytical zena). The standard curve using trolox was prepared ranges from100-1000 µM concentration. The results were calculated as % DPPH scavenging activity= [(A515nm blank - A515nmsample) x 100/ A515nm blank]. The results were expressed in terms of trolox equivalent antioxidant capacity (TEAC) values i.e. µM of trolox equivalent / g of sample.

Analysis of carbohydrates hydrolyzing enzymes inhibitory activity

α-Amylase Inhibition Disk Assay

α-amylase inhibitory activity was measured as per the method described by Apostilidis et al. 2006. 800 µL polypehnol extract sample was added to the 200 µL of porcine pancreatic α-amylase (PPA) solution equivalent to 1000 U in 20-mM sodium phosphate buffer, pH 6.9. 100 µL of this solution was poured to the sterile 3.0 cm sterile paper disk (Whatman, Grade 1), placed in the periplates containing starch agar (5g agar+5g starch dissolved in 500mL distilled water). The plates were kept at room temperature for 3 days sealed with parafilm. Then, after 3 days incubation, 5 mL of iodine solution (5-mM iodine in 3% potassium iodide) was added to each plate and wait for 15 min. Excess iodine stain was removed, and the diameter was measured.

α-Glucosidase enzyme inhibition activity

α- glucosidase inhibition assay was performed essentially as described by Apostolidis *et al.*2006 with some modifications. 500 µl of sample extract was added to 11 ml of 0.1 M potassium phosphate buffer pH 6.90 containing α-glucosidase solution (1.0 U/ml). Then incubated at 25 ^{0}C in a water bath for 10 minutes. After 10 minutes 500µl of 5 Mm p-nitrophenyl-D-glucopyranoside solution in 0.1 M potassium phosphate buffer pH 6.90 was pipetted to each tube. Further incubation for 5 min at 25 ^{0}C for done. Absorbance was taken at 405 nm, before and after incubation.

Angiotensin converting enzyme (ACE) Inhibition assay

ACE inhibition activity was measured as per the method developed by Cushman and Cheung in 1971 with some modification. The Hip-His-Leu was dissolved in 0.1 M sodium borate buffer (pH 8.3) containing 0.3 M NaCl. Then, 110 µl of 5 mM Hip-His-Leu solution was mixed with 100 µl 0.1M sodium borate buffer (pH 8.3),20µl of test sample was added. The reaction was initiated by the addition of 20 µl (4 mU in 250 µl of reaction mixture) of ACE enzyme and the mixture was incubated for 30 min at 37^{0}C. 250 µl of 1N HCl was added to stop the reaction. Then added 1.5 ml ethyl acetate and centrifuged at 3000g/10 min, evaporated at 95^{0}C for 10min, redissolved in 1 ml distilled water and measured the absorbance at 228 nm. The extent of inhibition was calculated as; (B-A)/(B-C) x 100 Where A = absorbance in the presence of ACE and ACE inhibitory component; B = absorbance without ACE inhibitory component, and C = absorbance without ACE.

RESULTS AND DISCUSSION

Polyphenol extract preparation and analysis

The water soluble extract of strawberry polyphenols was prepared and concentrated. The water soluble strawberry polyphenol extract was also assessed for the total phenolics, total monomeric anthocyanins, and total flavonoids content (as presented in table no.1). The phenolic content was observed in the 10 mg/ml of extract, which corresponds to 2.5mg/g of strawberry pulp. Total monomeric anthocyanin content was 164±1.15 µg cyanidin-3-glucoside equivalents/mL of extract, which corresponds to 41µg/g of strawberry puree, and 1.64% of total phenol. The flavonoids content was 526±0.88µg quercetin equivalents/mL of extract which corresponds to 131.5µg/g of strawberry puree, and 5.26% of total phenol. The pH of 0.05% (w/v) aqueous solution of strawberry hydrophilic extract was observed to be 3.57.

The phenolic content in the plants observed to be linked with antioxidant potential (Viuda-Martos et al., 2010). The phenolic content measured in the present investigation by Folin-Ciocalteu assay, is based on the oxidation reduction reaction. This method measures the other chemical components such as carotenoids, amino acids, sugars and vitamin C, part from phenolic content by Folin-Ciocalteu assay (Vinson et al., 2001). Besides this, this method is routinely used for the analysis of polyphenols. The phenolic content in the strawberry is related to the total phenol level in addition to the anthocyanin, which is the major phenolic acid component (Skrede and Wrolstad, 2002; Kahkonen *et al.,* 2003). Strawberries also considered to be enriched with phenolic compounds with antioxidant and anti-proliferative activities (Halvorsen *et al.*, 2002, Wang *et al.*, 1996, Guo *et al.*, 2003, Sun *et al.*, 2002, Meyers *et al.*, 2003).

Heo and Lee (2005) reported the total anthocyanin content in strawberry was 19.430 ± 1.11 mg of cyanidin- 3-glucoside/100g of fruit. However, Wang *et al* (2000a) observed the same as 38.9 mg/100g of fresh matter and Clifford and Scalbert (2000) measured values of anthocyanin 15-35 mg/100g of fresh matter. This indicated that the anthocyanin content might vary with the type of fruit. Flavonoids are the major group of the polyphenolic compounds in the fruits. These amy have several health beneficial properties such as antioxidant, antiviral, and antimutagenic properties. Quercetin observed to be a well-known plant-derived flavonoid that might have antioxidant and anti-inflammatory properties (Davis *et al.*, 2009). Flavonoids are capable of chelating $Fe^{3+,}$ Fe^{2+} and Cu^{2+} cations. Major flavonoids estimated in strawberries are the glucosides and glucuronides of quercetin and kaempferol aglycons. The flavonols present in strawberries are quercetin-rutinoside, quercetin-glucoside, quercetin glucuronide and kaempferol-glucuronide (Seeram *et al.*, 2006c). Meyers *et al* (2003) reported total flavonoid content which ranged between 46.2 and 72.0 mg catechin equivalents/100 g fresh weight.

Antioxidant activity of strawberry hydrophilic extract

The total antioxidant activity of hydrophilic extract was determined using DPPH radical scavenging assay and ORAC-

FL assay. Based on DPPH assay the TEAC value were 2.76±0.01mM , 7.76±0.08 mM and 9.06±0.01 mM respectively at 0.5 mg/ml, 2.5mg/ml and

Table 1: Total phenolic substances of the hydrophilic extract

Total Polyphenol Content	10 ± 0.47 mg Gallic acid equivalents per ml of extract
Total Anthocyanin Content	164±1.15 µg cyanidin-3-glucoside equivalents per ml of extract
Total Flavonoids Content	526± 0.88 µg quercetin equivalent per ml of extract

Results are expressed as mean ± standard deviation of mean (n-3)

Table 2: Functional properties of the strawberry hydrophilic extract

Parameters	Polyphenol concentration		
	0.5 mg/ml	2.5 mg/ml	5 mg/ml
Antioxidant activity (mM TE)	2.76±0.01	7.76±0.08	9.06±0.01
DPPH ORAC	8.3±0.15	35±0.88	64.33±1.45
α-amylase inhibitory activity (%)	26.33±1.45	45±1.15	90.33±1.45
α-glucosidase inhibitory activity(%)	25.33±0.88	50.05±0.02	81.66±0.88
ACE inhibition Activity (%)	24.16±0.44	71.16±0.44	86±0.57

Results are expressed as mean ± standard deviation of mean (n-3)

5mg/ml of extract while by ORAC-FL the corresponding values were 8.3±0.15 mM, 35±0.88 mM, 64.33±1.45 mM respectively as presented in table no. 2. Similarly, Skrede *et al* (2004) reported the antiradical power of strawberry to be 9.5 µmol TE/g, using DPPH radical assay. Wang *et al* also (2000) analysed the fruits and leaves from different cultivars of blackberry, red raspberry, black raspberry and strawberry plants for total antioxidant capacity(ORAC) and reported in fruits the ORAC values ranging from 7.8 to 33.7 µmol of TE/g of fresh berries (35.0-162.1 µmol of TE/g of dry matter). Whereas in leaves, ORAC values ranged from 69.7 to 182.2 µmol of TE/g of fresh leaves (205-728.8 µmol of TE/g of dry matter).

Similarly, Richa et al., 2011; evaluated the total antioxidant activity of hydrophilic strawberry polyphenol extract using DPPH radical scavenging assay and ORAC assay. Based on the DPPH assay, the trolox equivalent antioxidant activity was 5.19 ±0.81µM, while in case of ORAC it was 11.055±0.49 µM trolox equivalent/ml of extract. The results in the present investigation are also in the similar lines with respect to the increase in the concentration.

α - amylase inhibitory activity of strawberry hydrophilic extract:

As shown in the Table no. 1, the percentage α-amylase inhibitory activity of water soluble strawberry extract was analysed by disc assay was observed to be 26.33±1.45, 45±1.15% and 90.33±1.45% respectively at 0.5 mg/ml, 2.5 mg/ml and 5mg/ml of strawberry extract. Cheplick *et al* (2010) evaluated the α-amylase inhibition of water and ethanol soluble extract of different cultivars of strawberry and found that the water soluble extract had the higher inhibitory activity than that of the ethanol soluble extract, which showed the inhibition around 12%, 23% and 50% at the corresponding concentrations 10, 50 and 100 µg/ml in a dose dependence manner in the Honeoye cultivar, while in the ethanol soluble extract the % inhibition was not found to increase with respect to the increase in the corresponding concentrations.

The results obtained in the present investigation also showed the concentration dependency, but up to a certain level of polyphenol concentration (as presented in table no.2). After a particular saturation level, the observations in the present investigation indicated the increase in the % inhibtition activity, but not linearly with the increase in the concentration.

α-Glucosidase inhibitory activity of hydrophilic strawberry extract:

The percentage inhibitory activity of α- glucosidase for strawberry water soluble extract was determined as 25.33±0.88 at 0.5 mg/ml of the extract and the inhibition increased to 50.05±0.02% and 81.66±0.88% at the concentrations 2.5 mg/ml and 5mg/ml of the extract as presented in table no.2.

The results obtained in the present study corroborating with the study conducted by Cheplick et al., 2010; who reported the α-glucosidase inhibition activity in the water and ethanol soluble extracts of different cultivars of strawberry and observed that the % inhibition was increased with the increase in the polyphenolic concentration upto 50 µg/ml, thereafter it remains almost constant. In the present investigation also, the %inhibition was observed to be increased linearly with the increase in the polyphenol concentration upto 2.5 mg/ml, but after 2.5 mg/ml, increased was observed but not as proportional to the concentration.

Antihypertensive activity of strawberry polyphenol extract:

The % ACE-I inhibitory activity of strawberry water soluble extract was found to be 24.16±0.44, 71.16±0.44 and 86±0.57 at the polyphenol concentrations 0.5 mg/ml, 2.5 mg/ml and 5mg/ml respectively (table no.2). In another study conducted by Balasuriya *et al.* (2011) on the apple skin extract (ASE), showed that the enzymes inhibition is related to the concentration of phenolic compounds in the extract. Increase in the concentration of polyphenols in the apple skin extract from

0.01 ppm to 100 ppm showed an increase in the % inhibition activity from 29% to 64% ACE inhibition activity. Hence the % inhibition in the present study also observed to be increased from 0.5 to 2.5 mg/ml polyphenol concentration. Beyond the 2.5 mg/ml polyhenol concentration the increase in the % inhibition was slow.

CONCLUSION

The prepared strawberry polyphenol extract was observed to be possessed a good antioxidant, ACE inhibitory and antidiabetic property. Hence due to the various health promoting properties of strawberry extract, can be included in the diet of human being. Hence by incorporation in various food products, this concentrated form of polyphenols can be proved a better alternative to the synthetic drugs.

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