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Research Article

Antioxidant Profile Determination of off-Seasonal Guava, Allahabad Safeda (*Psidium guajava* L.) Fruits

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Abstract

Antioxidants received from plant sources are found to be one of the best remedies for a number of human diseases. Guava with high amount of dietary fiber is recommended as natural and secured source of antioxidants. In this study, antioxidant capacity in guava fruits is determined by the assessment of total phenol content and DPPH scavenging activity. Results obtained emphasize on the usage guava fruits to eradicate destructive free radicals from the human body. Daily intake of guava fruits can help fighting noxious diseases like cancer and arthritis.

Keywords antioxidants, chlorophyll a, chlorophyll b, carotenoid, protein

Introduction

Natural antioxidants from plant materials have recently drawn substantial interest of researchers. Flavonoids and other polyphenols that classifies major antioxidant phytochemicals from plants has been reported to inhibit the propagation of free radical reactions, to protect the human body from diseases (Kinsella et al, 1993). The use of fruit and vegetable juice has been increasing day by day due to their health benefit to human beings. Song et al. 2010 stated antioxidants as the substances capable of averting oxidative injury of nucleic acids, lipids and proteins by reactive free radicals containing reactive oxygen species. High ascorbic acid (50-300mg/100g fresh weight) content of guava which is three to six times more than oranges compels it to be used as health food. The intake of guava is rational strategy for the augmentation of nutritional antioxidant level. Phytochemical features like antioxidant and asiaticoside are performing a major function in activating the pharmacological activity of guava fruits. Due to these characteristics, people want to consume natural and secured antioxidants and this has given a new direction to the exploration of natural resources of antioxidants (Gulcin, 2006, 2007, 2010).

Guava is known to play a significant role in fighting cancer and developing immunity due to presence of numerous antioxidant polyphenols including amritoside, leucocyanidin, guaijaverin, (+)-gallocatechin and flavonoid compounds with less fats and carbohydrates. The fruit is an excellent laxative supplying a good amount of soluble dietary fiber (about 14% of DRA). It is a good source of vitamin A and C fulfilling the daily recommended intake. The objectives of this study were (a) to estimate the carotenoid, chlorophyll and protein content in guava fruits (b) To analyze the antioxidant capacity in guava fruits by determination of total phenol content, DPPH assay.

Methodology

Procurement of samples

The guava used as samples were off-seasonal guava, Allahabad Safeda (*Psidium guajava* L.) obtained from local fruit market of Allahabad, U.P., India.

Chemical Analysis

Chlorophyll and Carotenoid Content Measurement

Schopfer (1989) method was used to estimate the total chlorophyll and carotenoid content. Ten ml of

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80% acetone was used to crush the fruit samples and kept overnight at 4°C. Then samples were homogenized and centrifuged at 5000g for 7 minutes. Final volume of supernatant was measured in a UV-spectrophotometer at 480 nm and 510 nm for carotenoid and at 644nm and 662nm for chlorophyll using following formulas:

Chlorophyll a = $9.784\ OD_{662} - 0.99\ OD_{644}$

Chlorophyll b = $21.426\ OD_{644} - 4.65\ OD_{622}$

Carotenoid = $7.9\ OD_{480} - 3.6\ OD_{510}$

(Concentration in mg/g of fresh weight).

Estimation of Protein by Lowry's Method

Protein estimation was performed in accordance with method of Lowry et al. (1951). Two grams of the sample was weighed and ground with 20 ml of distilled water, centrifuged and the supernatant was used for protein estimation. Different volumes of working standard and sample extract were prepared in separate test tubes. Five ml of alkaline copper sulphate was added in to the tubes and after 10 minutes, subsequent addition of 0.5 ml of Folin-Ciocalteau Reagent (FCR) was done. After mixing, samples were incubated at room temperature in the dark for 30 minutes. Subsequently absorbance was measured at 660 nm spectrophotometrically and standard graph was drawn to calculate the amount of protein in guava sample. Results were expressed in μg ml^{-1} using the calibration curve.

Estimation of Total Phenol Content

Folin-Ciocalteu method was used in the estimation of total phenol content. One gram of guava sample was weighed and ground with mortar and pestle in 10 times volume of 80% ethanol. Homogenate was centrifuged at 10,000 rpm for 20 minutes and supernatant was collected in another test tube. The left residue was re-extracted with five times volume of 80% ethanol, centrifuged and supernatant was pooled out. **S**upernatant was evaporated to dryness and dissolved in 5 ml of distilled water. Different aliquots (0.2-2 ml) were pipetted into test tubes. Volume was made up to 3 ml in each test tube with distilled water and 0.5 ml of FCR was added into each test tube. After 3 minutes 2 ml of 20% sodium bicarbonate was added in each test tube. The absorbance was measured at 650 nm against a reagent blank. Standard curve was prepared using different concentration of catechol. Total phenol value was expressed in terms of Gallic acid equivalent (mg ml^{-1} of extracted compound).

DPPH free radical scavenging activity

The DPPH free radical scavenging activity was determined by the method given by Hatano et al. (1988) as modified by Gow-Chin Yen and Hui-Yin Chen (1995).

One millimolar DPPH solution was prepared by dissolving DPPH in 95% v/v methanol and made up to 50 ml with same. Fruit extract (200-1000 μg) corresponding to 0.2, 0.4, 0.6, 0.8, 1.0 ml were made up to 4 ml with distilled water in different test tubes and 1ml, 1mM DPPH was added and was left at room temperature for 30 minutes. Absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The effect of BHA on DPPH was also assessed for comparison with that of fruit extract. Methanolic dilutions (0.2, 0.4, 0.6, 0.8, 1.0 ml) of 1mg/ml BHA was made to 4 ml with distilled water. One ml DPPH (1mM) solution was added to each tube, and same procedure as in DPPH scavenging experiment was followed.

Calculation: Percentage of Scavenging Activity was calculated using the following formula

$$\%SA = (AC - AE) / AC \times 100$$

where % SA is percentage scavenging activity, A_C is the absorbance of control and A_E is the absorbance of extract

Table 1: Carotenoid, chlorophyll and protein content in off seasonal guava (P. guajava L.), Allahabad Safeda fruits

S.No.	Experimental Material	Guava fruits (*Psidium guajava* L. cv. Allahabad Safeda)
1.	Chl. a content (mg/g)	2.738
2.	Chl. b content (mg/g)	5.139
3.	Carotenoid content (mg/g)	0.642
4.	Protein content (μg/ml)	224

Results and Discussion

Chlorophyll and Carotenoid Content

Chlorophyll a, chlorophyll b and carotenoid content were found to be 2.738 mg/g, 5.139 mg/g, 0.642 mg/g respectively in the off seasonal guava fruits (Table 1). Gill et al. (2002) reported that chlorophyll a and chlorophyll b content was in range of 1.965 mg/g and 4.396 mg/g respectively and carotenoid content to be nil in off seasonal 'Allahabad Safeda' Guava fruits. The slight difference in result may be due to different assay method adopted and also due to fruit types which were used as sample.

Protein Content

Protein content was found to be 224 µg/ml in off-seasonal guava fruits (Table 1). Protein content was found to be much higher than certain other fruits like cherry, carrot and grapes (source: Fruit nutrition facts, www.thefruitpages.com).

Total phenol content

The total phenol content was found to be 460 mg GAE / 100 g in off seasonal guava fruits. Thaipong et al. (2006) reported total phenol content in range of 430-480 mg GAE/100g in off-seasonal 'Allahabad Safeda' Guava fruits. They also suggested that phenol compound is a class of antioxidants agents acting as free radical terminators.

Table 2: DPHH Scavenging Activity of Guava Fruit Extract

Concentration of extract (mg/ml)	*Psidium guajava* (absorbance at 517 nm)	BHA (absorbance at 517 nm)
0.04	0.467	0.316
0.08	0.406	0.302
0.12	0.313	0.158
0.16	0.282	0.126

Absorbance of Control at 517 nm -0.725

DPPH Scavenging Activity

At minimum concentration of 0.04 mg/ml, fruit extract and BHA scavenged 35% and 56% DPPH respectively. DPPH scavenging activity of 61% and 83% at a maximum concentration of 0.16 mg/ml was observed respectively for fruit extract and BHA. Though both fruit extract and BHA scavenged DPPH in concentration-dependent manner, BHA displayed better DPPH scavenging efficiency over fruit extract. This may be attributed to the presence of methoxy group in BHA which enhances the accessibility of the radical centre of DPPH to BHA. The percentage scavenging activity for all the concentration can be calculated from Table 2 and Figure 1. The results obtained were in accordance with the previous research work conducted by Ogunlana and Ogunlana (2008).

Discussion and Conclusion

Common guava popularly described as poor man's apple is an abundant source of antioxidants. The antioxidant derived from fruits and vegetables are very important due to their health benefit to human being. Present study has been done to find out the chlorophyll, carotenoid and protein content in guava fruit along with the antioxidant capacity by DPPH scavenging assay. The chlorophyll and carotenoid content in Guava have a positive function in the epithelization process and affect the cell cycle development of the fibroblasts. Different carotenoid pigments protect against light and diminishes the harm of sunburns and skin allergies. Results showed that *P. guajava* is a good source of carotenoid and it can be promising fruit for use in pharmacological products designed for antioxidant activity. As shown in the results, guava has also rich protein content. As phenolics are the type of chemical components functioning as chief antioxidants or free radical terminators, we found it logical to estimate their total content in fruit samples. Redox characteristics develop the radical scavenging effect in hydroxyl groups of phenols. In the results obtained it was quite evident that guava is richly benefitted with phenol as one its constituent and hence posses antioxidant capacity. DPPH assay was performed to determine the scavenging capacity and thereby determining the antioxidant activity. From the results obtained it was evident that guava has high antioxidant activity. To conclude, the result of this work is in support of the fact that the fruits of *Psidium guajava* are a promising source of natural antioxidants. Present study favors the positive assumptions regarding the antioxidant activity of *P. guajava* fruits. The chlorophyll content is much high in off-seasonal guava fruit whereas as the carotenoid content is much lesser due to the more ripened guava fruit extract.

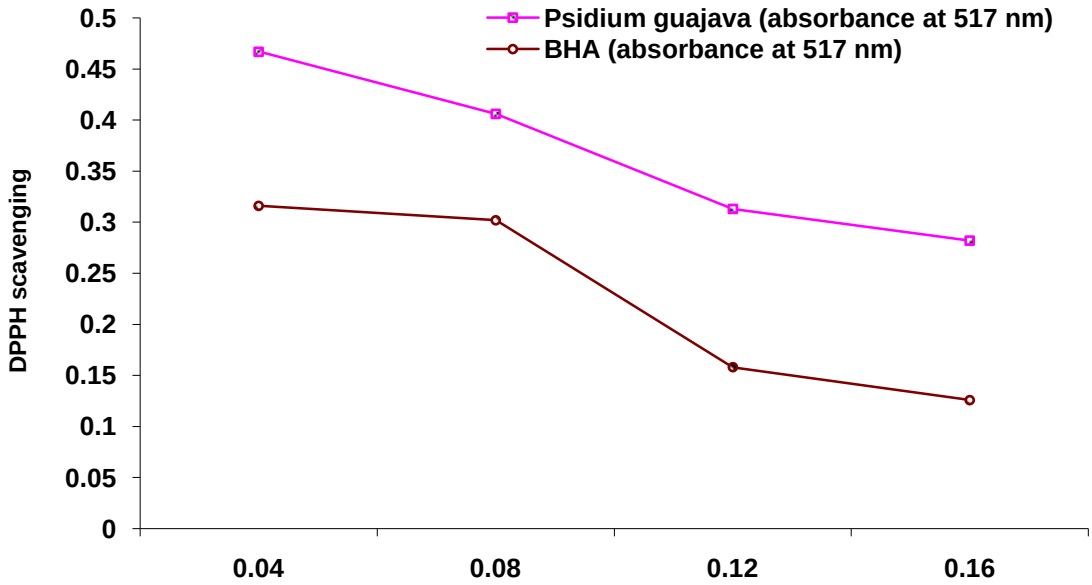

Figure 1 Percentage DPPH scavenging activity of fruit extract of guava

Protein content in guava fruits is much more in amount as compared to cherry, grapes and carrot. Scavenging activity is determined by DPPH assay and BHA was used as control. At maximum concentration, the percentage scavenging activity of DPPH is one of the oldest indirect methods of determining antioxidant activity. Antioxidant profile determined for *P. guajava* species are of immense benefit in developing immunity in opposition to infectious causes and hunt cancer removing harmful free radicals from the body. It will be promising in the reduction of occurrence of degenerative disease such as cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction and will slow down the ageing process if utilized properly.

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