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EVALUATION OF ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL SCREENING OF *MALUS DOMESTICA* BORKH (APPLE) AND *PHASEOLUS VULGARIS* L. (GREEN BEANS)

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ABSTRACT
The aim of this study was to determine the antioxidant activity as well as total phenol (TPC) and total flavonoid content (TFC) in two fruits, apple (*Malus domestica*) and green beans (*Phaseolus vulgaris*). The antioxidant activities were examined by two different methods namely DPPH free radical scavenging activity and reducing power scavenging activity. The results showed that considerable amount of TPC and TFC was present in these fruit extracts as well as these vegetables contain a vast array of different phytochemicals in their dry form. Apple showed higher antioxidant activity than green beans. Phytochemical screening revealed the presence of saponins, steroids, flavonoids and carbohydrates & glycosides in all the extracts. Overall, the present results provided basic data for choosing these fruits with high antioxidant capacity for consumption or for the development of antioxidant based medicines as value-added products.
Key words: Flavonoids, Phenols, DPPH assay, Apple, Green beans

INTRODUCTION

Oxidative damage in the human body plays an important causative role in disease initiation and progression. Damage from free radicals and reactive oxygen species has been linked to some neurodegenerative disorders and cancers and oxidation of low-density lipoprotein is a major factor in the promotion of coronary heart disease (CHD) and atherosclerosis[1]. Free radicals are reactive molecules with unpaired electrons that are able to exist independently. Endogenous metabolic processes, especially in chronic inflammations, are important sources of free radicals, which can react with and damage all types of biomolecules lipids, proteins, carbohydrates, and DNA. If damaged DNA is left unrepaired, and the mutated cell gains the ability to survive and divide aberrantly, it may become cancerous[2]. Free radicals due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress, cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins[3].

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders. Currently available synthetic antioxidants like Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toluene (BHT), Tertiary Butylated Hydroquinon (TBH) and gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity. In recent times, natural antioxidants have attracted considerable interest among nutritionists, food manufactures and consumers, due to their presumed safety and potential therapeutic value.

Interest in the role of antioxidants in human health has prompted research in the fields of food science and horticulture to assess fruit and vegetable antioxidants. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. Fruits and vegetables contain many different antioxidant components. The defensive effects of natural antioxidants in fruits and vegetables are related to three major groups: vitamins, phenolics, and carotenoids. Ascorbic acid and phenolics are known as hydrophilic antioxidants, while carotenoids are known as lipophilic antioxidants [4]. The consumption of fruits and vegetables has been associated with low incidences and mortality rates of cancer and heart disease. Eating fruits and vegetables also reduces blood pressure, boosts the immune system, detoxifies contaminants and pollutants, and reduces inflammation.

MATERIALS AND METHODS

Collection of the Fruit and Vegetable

The samples were collected from local markets of Coimbatore, Tamilnadu dust free samples. Soon after collection, the fruit peels were dried in shade for 20 days and then powdered to get a coarse powder. This powder was stored in air tight container and used for further successive extraction.

Preparation of Extracts

Aqueous extraction

25 g of air-dried powder was taken in 100 ml of water in a conical flask, plugged with cotton wool and they were shaken at room temperature for 2 days. After 2 days hours the supernatant was collected and the solvent was evaporated to make the final volume one fourth of the original volume (12) and stored at 4^0 C in airtight bottles.

Solvent extraction

25 g of air-dried powder was taken in 100 ml of methanol in a conical flask, plugged with cotton wool and they were shaken at room temperature for 2 days. After 2 days the supernatant was collected and the solvent was evaporated to make the final volume one fourth of the original volume (12) and stored at 4^0 C in airtight bottles. In the same conditions was obtained Ethyl acetate extract. The percentage yield of the extracts from each solvent extraction was calculated. The

crude extract was crushed into powder and then kept in desiccators.

Phytochemical screening

This was carried out according to the methods described by Trease and Evans[5]. Qualification phytochemicals analysis of the crude powder of the two plants for the identification of phytochemicals like as a tannins, alkaloid, steroid, phenols and terpenoid, flavonoid etc.

DPPH Radical scavenging activity:

DPPH scavenging activity was carried out by the method of Blois[6]. Different concentrations (1000, 500, 250, 125, 62.5 and 31.2 mg/ml) of dried powder of fruit and vegetable were dissolved in DMSO (dimethyl sulfoxide) and taken in test tubes in triplicates. Then 5 ml of 0.1mM ethanol solution of DPPH (1, 1, Diphenyl-2- Picrylhydrazl) was added to each of the test tubes and were shaken vigorously. They were then allowed to stand at 37^0 C for 20 minutes. The control was prepared without any extracts. Methanol was used for base line corrections in absorbance (OD) of sample measured at 517nm. A radical scavenging activity was expressed as 1% scavenging activity and was calculated by the following formula.

$$\text{Radical scavenging activity (\%)} = \frac{\text{OD Control} - \text{OD Sample} \times 100}{\text{OD control}}$$

Reducing power

Reducing activity was carried out by using the method of Oyaizu[7]. Different concentrations (1000, 500, 250, 125, 62.5 and 31.2 mg/ml) of dried powder of fruit and vegetable were dissolved in DMSO (dimethyl sulfoxide) and taken in test tubes in triplicates. To the test tubes 2.5 ml of sodium phosphate buffer and 2.5 ml of 1% Potassium ferric cyanide solution was added. These contents were mixed well and were incubated at 50^0 C for 20 minutes. After incubation 2.5ml of 10% TCA was added and were kept for centrifugation at 3000rpm for 10 minutes. After centrifugation 5ml of supernatant were taken and to this 5ml of distilled water was added. To this about 1ml of 1% ferric chloride was added and was incubated at 35 ^{0} C for 20 minutes. The O.D (absorbance) was taken at 700nm and the blank was prepared by adding every other solution but without extract and ferric chloride (0.1%) and the control was prepared by adding all other solution but without extract. The reducing power of the extract is linearly proportional to the concentration of the sample

Total phenolic content

Total phenolic contents were determined by Folin Ciocalteu reagent[8]. A dilute extract of each plant extract (0.5 ml of 1:10g ml $^{-1}$) or garlic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5ml, 1:10 diluted with distilled water) and aqueous sodium carbonate (4ml, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/ml solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg g-l of dry mass), which is a common reference compound.

Determination of Total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination [9]. Each plant extracts (0.5ml of 1:10 g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). The calibration curve was prepared by preparing quercetin solution at concentrations 12.5 to 100g ml $^{-1}$ in methanol.

RESULTS AND DISCUSSION

Free radicals have been shown to be harmful as they react with important cellular components such as proteins, DNA and cell membrane[10]. The body on the other hand, requires free radicals for immune system responses. However, an overload of these molecules had been linked to certain chronic diseases of heart, liver and some form of cancers[11]. Vegetables are a good source of dietary antioxidants, such as vitamin C, vitamin E and beta carotene. The antioxidative phytochemicals in grains, vegetables, fruits and medicinal plants have received increasing attention for their potential role in preventing human diseases[12].

Methanol and aqueous extracts of the selected samples were subjected to qualitative organic analyses. Extracts of apple and beans answered positively for steroids. Extract of the fruit apple answered for alkaloids, saponin, phenolic compounds and flavonoids. (Table 1). The medicinal value of these secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body. The most important of these substances include, alkaloids, glucosides, steroids, flavonoids, fatty oils, resins, mucilages, tannins, gums, phosphorus and calcium for cell growth, replacement, and body building[13].

Two different tissues using water and methanol solvents were prepared to examine the antioxidant activity and concentrations of Phenols and flavonoids. The concentration of phenols in the examined fruit and vegetable extracts using the Folin-Ciocalteu reagent was expressed in terms of gallic acid equivalent (Table 2). The concentrations of phenols in the examined fruit extracts ranged from 124.61 to 216.14 mg/g. The high concentration of phenols was measured in methanol extract of apple. The extracts obtained using more polar solvents had higher concentrations of phenols while the extracts obtained using low polar solvents contained small concentrations[14] (Table 2). The concentration of flavonoids in two different extracts was determined using spectrophotometric method with aluminium chloride. The summary of quantities of flavonoids identified in the tested extracts is shown in Table 2. The concentrations of flavonoids in fruit extracts ranged from 49.13 to 92.20 mg/g. High concentrations of flavonoids were measured in methanol extracts of apple.

Phenolic and other phytochemical antioxidants found in fruits, vegetables and legumes are bioactive compounds capable of neutralizing free radicals and may play a role in the prevention of certain diseases. Also, dietary supplements and food fortification may be an alternative route to the consumption of minor plant components that may have health effects. The high phenol and flavonoids contents of apple may cause high antioxidant activity of this plant. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities. The correlation between total phenol contents and antioxidant activity has been widely studied in different foodstuffs such as fruit and vegetables [15].

The extracts of the tested fruit and vegetable tissues possessed free radical scavenging properties, but to varying degrees, ranging from 31.05 to 86.19% DPPH scavenging. Using the organic solvent extraction, methanol showed better DPPH scavenging activity. A maximum scavenging activity was offered by methanol extract of apple (86.19%), beans (75.86%) followed by aqueous extract of apple (65.54 %) and beans (65.52%) (Table 3 & 4). The methanol extracts of apple showed very good DPPH free radical scavenging activity, which were almost near to that of standard ascorbic acid. The free radical inhibition activity of crude methanolic extract of apple showed significant activity, which is in agreement with that of the previous reports on Banana (*Musa*, AAA cv. Cavendish [16] and *Hylocereus undatus* [17]. Among the fruit and vegetable samples, the fruit (apple) showed the highest free radical scavenging activity.

The reducing power assay of two different tissues of fruit and vegetable was given in table 5 and 6, which was found to be ranged from 0.100 ± 0.9 - 0.850 ± 2.4% of the dried powder. The reducing power of the fruit tissues increased in a concentration dependent manner. Among the two different sp. the apple *C. sinensis* (0.850 ± 2.4) have exhibited the highest rate of reduction of ferric ions. The reducing power of a compound is related to its electron transfer ability and may serve as a significant indicator of its potential antioxidant activity.

This is in agreement with an earlier report on *Hylocereus undatus* [17]. The results of the present study indicated that the apple have good antioxidant activity with high levels flavonoids comparable with that of vegetable. Among the two plant tissues, apple was found to possess a rich source of poly phenols and flavonoids. The abundance of these compounds, as well as of the other fractionated phenols, presents opportunities for the use of fruit and vegetable peel and peel extracts as future nutraceuticals and health-related ingredients for the food industry. Concluding, it should be stressed that the fruits can be considered as a potential source of different antioxidant components, which are not exploited at the moment, but could find practical application in many industrial branches.

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Table 1. Phytochemical screening of Apple (*Malus domestica* Borkh.) and Green Beans (*Phaseolus vulgaris* L.)

S.No.	Phytoconstituents	*Malus domestica*		*Phaseolus vulgaris*	
		Methanol	Aqueous	Methanol	Aqueous
1	Alkaloids	+	-	+	-
2	Saponins	+	+	-	+
3	Steroids	+	+	+	-
4	Phenolic compounds	+	-	-	-
5	Tannins	-	-	-	-
6	Flavonoids	+	-	-	-
7	Carbohydrate & Glycosides	+	-	+	-
8	Protein & Amino acids	+	-	+	-

+ indicates present - indicates absent

Table 2. Total phenol and Flavonoid contents in the fruit and vegetable extracts (mg/100mg of fruit/vegetable powder)*

Plant Name	Total Phenol		Flavonoids	
	Methanol	Aqueous	Methanol	Aqueous
M.domestica	216.14 ± 0.01	128.01 ± 0.29	92.20 ± 0.01	58.01 ± 0.12
P. vulgaris	198.4 ± 0.12	124.61 ± 0.21	78.01 ± 0.11	49.13 ± 0.05

*Each value in the table was obtained by calculating the average of three analyses ± Standard deviation

Table 3. DPPH Assay of Apple (*Malus domestica* Borkh.)*

S.No.	Concentrations (mg/ml)	Plant Extracts		Standard (Ascorbic acid)
		Methanol	Aqueous	
1	1000	86.19 ± 0.01	65.54 ± 0.03	
2	500	68.97 ± 0.015	55.19 ± 0.020	
3	250	51.73 ± 0.011	44.82 ± 0.020	71.87 ± 2.21
4	125	44.83 ± 0.020	37.96 ± 0.035	
5	62.5	41.39 ± 0.02	31.05 ± 0.032	

*The data are means of triplicate determinations; ± standard errors

Table 4. DPPH Assay of *Phaseolus vulgaris* L.*

S.No.	Concentrations (mg/ml)	Plant Extracts		Standard (Ascorbic acid)
		Methanol	Aqueous	
1	1000	75.86 ± 0.015	65.52 ± 0.011	
2	500	62.08 ± 0.02	55.19 ± 0.020	
3	250	51.74 ± 0.025	48.27 ± 0.011	71.87 ± 2.21
4	125	41.38 ± 0.015	41.35 ± 0.011	
5	62.5	31.03 ± 0.020	34.49 ± 0.011	

*The data are means of triplicate determinations; ± standard errors

Table 5. Reducing Power Assay of Apple (*Malus domestica* Borkh.)*

S.No.	Concentrations (mg/ml)	Plant Extracts	
		Methanol	Aqueous
1	1000	0.851 ± 0.01	0.566 ± 0.05
2	500	0.706 ± 0.05	0.426 ± 0.15
3	250	0.636 ± 0.152	0.306 ± 0.11
4	125	0.546 ± 0.05	0.136 ± 0.15
5	62.5	0.423 ± 0.011	0.113 ± 0.11

*The data are means of triplicate determinations; ± standard errors

Table 6 . Reducing Power Assay of *Phaseolus vulgaris* L.*

S.No.	Concentrations (mg/ml)	Plant Extracts	
		Methanol	Aqueous
1	1000	0.843 ± 0.05	0.676 ± 0.15
2	500	0.716 ± 0.11	0.54 ± 0.01
3	250	0.64 ± 0.02	0.37 ± 0.05
4	125	0.513 ± 0.11	0.186 ± 0.15
5	62.5	0.333 ± 0.015	0.126 ± 0.11

*The data are means of triplicate determinations; ± standard errors