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Antioxidant potentials of dry fruits from district: Astore, Gilgit, Baltistan

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Abstract

The walnut shell and apricot fruit were subjected to test under various methods i.e. Folin-ciocalteu methods, DPPH radical scavenging assay and metal chelating assay method. The findings exhibited that the radical scavenging activity of walnut fruit and shell was found to be 77.45% and 76% respectively which is quite significant. On the other hand apricot fruit showed a week activity i.e. 6.52%. A standard antioxidant ascorbic acid was run parallel to compare the results it showed 91%. Results regarding actual potency to scavenge radicals indicated that walnut fruit showed promising potential against DPPH radicals and its IC_{50} value was found to be 1.28 µg/mL, which indicates it greater potency than standard antioxidant ascorbic acid (4.6 µg/mL). The walnut shell also showed a significant activity and its IC_{50} was determined to be 6.62 µg/mL which is comparable activity with ascorbic acid.

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Introduction

Antioxidants play a crucial function in the body. They hunt radicals in the plants and as result radicals may be converted into less reactive species (Mateo *et al*., 2011). There are many types of antioxidants which can scavenge free radicals are present in various vegetables, fruits and tea (Hong *et al*., 1996). According to food and drug administration authority (FDA) antioxidants are those substances which decelerate rancidity, deterioration and discoloration caused by oxidation. The natural antioxidants are low in molecular weight (Kumar, 2011). The antioxidants which are found naturally such as Alpha tocopherol and L ascorbic acid are extensively used since they are harmless and their antagonistic reactions are less. In food additives the uses of antioxidants from edible plants have been recently boost up due to their non-toxic effects (Seong, 2004). The application of plants parts, fruits, vegetables, cereals and oil seeds as a antioxidants is significantly employed because they are natural (Peiyuan, 2011). Vitamin C, E and A, beta-carotene and lycopene as well as some synthetic antioxidants are consider as non-enzymatic antioxidants (Merinal and Viji, 2012; Hamid *et al*., 2010).

In the food and food supplements vitamin A, E, D and Beta carotene while in the body glutathione and uric acid are antioxidants which can directly remove free radicals (Hamid *et al*., 2010). In addition to this some macromolecules for instance caeruloplasmin and melatonin which exhibits antioxidant properties. These are natural source of antioxidants (Nabila *et al*., 2012). The antioxidants are produced in the body however some of them can also be extracted from human food i.e. vegetables, fruits, meat, oil and seeds etc (Merinal and Viji, 2012).

Several diseases such as cardiovascular disease, tumor growth, alzheimer's disease, wrinkled skin and cancer can be caused by oxidation when the cells are accelerated for oxidation even decrease in energy (Freidovich, 1999; Yun-Zhong *et al*., 2002). The epidemiological research proposed that the antioxidant capacity of plasma increases and decrease the risk of due to utilization of food containing natural antioxidants however not cancer and heart diseases (Dongmei *et al*., 2007).

Free radicals play significant role in the basis of life as well as essential for development of living systems. Their effects on the organisms are significant. Oxygen is the basic necessity of humans and radicals of oxygen employed vital actions like gene transcription and signal transduction (Yun-Zhong, 2002). The structure of large number of phenolics have been clarified by chemists however the phenolic compounds which may be extracted have not yet been fully described. For the reason that several extracts of berry, essential oils and redolent plants are gaining interest because of their nontoxic effects and increasing demand by customers. Many studies revealed that fruits, berries, spices and essential oils bear antioxidant and radical scavenging features (Maestri *et al*., 2006).

Apricot (*Prunes armeniaca* L.) belongs to genus prunes and is grouped in the Rosaceae family. Cultivation of apricot started from wild apricot. Moreover it has been domesticated by cultivation. There are various uses of apricot because of its nutritional value. It can be used as fresh dried and with its treatment jam, jelly and pulp etc are obtained (Mirzaee *et al*., 2009). The current study was conducted keeping the aim to evaluate the biological potential of some domestic use plants fruits of district Astor.

Material and methods

Chemicals

The chemicals were demanded from different contractors to determine the free radicals scavenging features and to find out total phenolic contents. Various chemicals such as gallic acid, sodium carbonate (Na_2CO_3) and iron sulphate were ordered from scharlau (Spain) and methanol from TEDIA (Spain). While ethanol and dimethyl sulfoxide (DMSO) were bought from scharlau (Japan). The reagents containing folin ciocalteu was obtained from MP bio-medicals (France) and 1, 1 dimethyl (DPPH) was gpurchased from Alfa Aesar (Spain).

Collection of Plant Species and Extraction

Walnut (*Juglans regia* L.) and its shell and Apple (*Prunes armeniaca* L.) were obtained from District Astore of Gilgt-Baltistan. The fruits and plants parts were kept in the laboratory for one month till it has been dried completely. Then the dried plants were press into powdered by cutter and mortar. Then the powdered plants were weighted and after taken weight it was soaked into 80 % methanol for one month. After that extract was taken and through rotatory evaporator the extract was dried into semi solid. The obtained extracts were further treated for biological processes.

Radical Scavenging Method

In the present study the antioxidant activity was determined by using UV-VIS spectrophotometer. The technique of chromatography (HPLC) was also used to find out antioxidant activity. The essential members of hydrazine derivatives are 1, 1 diphenyl and 2-picrylhydrazyl. They both contain nitrogen which act as a free radical center. Such a nitrogen center oriented molecules are highly stable because the structure of these molecules are conjugated. Therefore DPPH was used which emits deep violet color due to unpaired electrons. By adding the antioxidants compounds the color of DPPH was reduced this showed that the compound has the capacity of removing hydrogen atom. DPPH electron becomes paired as a result the color changed such as light green which is property of hydrazine.

Procedure

In order to prepare stock solution 13 mg DDPH (Diphenyl 2-picrylhydrazyl) was added in 104.5 ml ethanol. In this reaction mixture 4750 µL of 300 µM DPPH solution whereas 250 µL of 500 µg/mL sample solution was taken. To prepared DPPH ethanol was used while to prepared samples DMSO was used. In a test tube 4750 µL DPPH solution from the stock solution was pour into 250 µL DMSO. After that the solution was kept for incubation for 30 minutes at 37 ^{o}C and the test tube was covered with aluminum foil. The absorbance was set at 515 nm for control reading.

After that in three test tubes 250 µL of walnut, walnut shell, apricot and standard compound (ascorbic acid) sample solution was taken and DPPH solution of 4750 µL was put into the tube. Aluminium foil was used to cover the reaction mixture kept for incubation for 30 minutes at physiological temperature of 37 ^{o}C. Then the samples were shaking manually after every 5 minutes. The absorbance was adjusted to 515 nm after 30 minutes. The DMSO as a blank was used on UV visible spectrophotometer. The radical scavenging activity was calculated by following formula:

$$\% \text{ RSA} = 100 - \{(\text{OD test compound} / \text{OD control}) \times 100\}$$

Determination of IC_{50}

The 50 % free radicals were inhibited at concentration of IC_{50}. The value of IC_{50} showed that 50 % of DPPH free radicals may be scavenge.

Procedure

In order to determine IC_{50}, 1 mL solution was taken from each sample solution and from stock solution (1000 mg/mL) ascorbic acid solution was taken. The solution was diluted by addition of 1 mL DMSO in order to get 500 mg/mL concentration of each then from this solution 1 mL concentration was taken to further diluted the solution through adding 1 mL more DMSO to every subsequent concentration. The different concentrations of 15.625 mg/mL, 31.125 mg/mL, 62.5 mg/mL, 125 mg/mL, 250 mg/mL and 500 mg/mL were obtained in the test tube. Then from all these concentrations 250 µL solution was taken in which 4750 µL DPPH standard was added through micro pippet. Then all the test tubes were enclosed in aluminum foil and left for incubation to 30 minutes at 37 ^{o}C and shaked after every 5 minutes. Absorbance was set to 515 nm and reading was taken after 30 minutes by DMSO as blank proceeding with UV visible spectrophotometer.

Results and discussion

Antioxidants are those substances which prevent oxidation process by removing free radicals in spite of having at relatively small concentration (Gilgun-Sherki *et al.*, 2001).

It is obvious that antioxidant compounds play significant role regarding health protection. Scientific studies proved that antioxidants help to minimize the chronic liver diseases such as heart and cancer disease (Sun *et al.*, 2002). The functions of antioxidants in the body are crucial to remove free radicals. They react as radical inhibitors in plants and change radicals into less reactive species (Poulson *et al.*, 1998; Yun-Zhong *et al.*, 2002). The food and drug administration defined antioxidants as that those species which hinders deterioration, rancidity or loss of colors caused by oxidations. Moreover antioxidants are present in many fruits, vegetables and tea (Kumar, 2011). In the present study two antioxidant analyzes were used namely; iron chelating assay and DPPH free radical scavenging assay. The folinciocalteu procedure was also used to find out the total phenolic contents from the samples.

DPPH Radical Scavenging Activity

In order to evaluate antioxidant activity obtained from fruit extracts to check the free radical scavenging activity the most well recognized method is DPPH free radical scavenging. The DPPH analysis is very simple, fast and sensitive. The method does not require any special tool and treatment of sample. It requires common reagents to function. To find out antioxidant activity the DPPH free radical scavenging technique was applied in the dry fruits collected from district astore of Gilgit-Baltistan. DPPH has the potential to abstract hydrogen and it is a stable radical. The hydrogen is abstract from other compounds and it converts the DPPH into its reduced form by pairing up odd electron of DPPH. By this method the antioxidant activity of the sample extract was find out by check out their reducing ability.

Table 1. Showing antioxidant activity of dry apricots.

Sr. No.	Samples	Concentration µg/mL	% RSA±SEM
1	Walnut fruit	250	77.45±1.78
2	Walnut shell	250	76.93±2.14
3	Apricot fruit	250	6.52±1.43
4	Ascorbic Acid	250	91.34±0.54

RSA = Radical scavenging activity; SEM = Standard error of mean.

The extracts taken from walnut fruit, walnut shell and apricot fruit were used in contrary to DPPH free radicals and they exhibited stimulating radical scavenging activity. The radical scavenging activity for walnut fruit was 77.45 % and walnut shell was 76 % which found to be significant. On the other hand apricot fruit showed a week activity i.e. 6.52 % (Table 1). A standard antioxidant ascorbic acid was run parallel to compare the results it showed 91 %. The significant activity of walnut fruit and shell indicates that walnut can serve as important source of antioxidants and can be used to maintain antioxidant level and further investigations might be productive for the discovery of an important drug. However the walnut fruit showed highest antioxidant activity than walnut shell which was very close to the standard Ascorbic acid. Walnut fruit and shell both as well as Apricot serve as a good source of Antioxidant Activity.

Kinetic Studies on Different Fractions

The determination of inhibition concentration is quite important to identify a sample for its efficacy since the active samples were further investigated to determine their inhibition concentration by using kinetic program. The obtained results are presented in the Table 2.

Table 2. Inhibition concentration (IC_{50}).

Sr. No.	Sample	IC_{50} (µg/mL)±SEM
1	Walnut fruit	1.28±1.04
2	Walnut shell	6.62±3.20
3	Apricot fruit	ND
4	Ascorbic acid	4.6±0.27

SEM = Standard error of mean; ND = Not detected.

The samples which showed significant activity were again evaluated against DPPH radicals at different concentration to find their actual potency to scavenge radicals. Walnut fruit showed promising potential against DPPH radicals and its IC_{50} value was found to be 1.28 µg/mL, which indicates it greater potency than standard antioxidant ascorbic acid (4.6 µg/mL). The walnut shell also showed a significant activity and its IC_{50} was determined to be 6.62 µg/mL which is comparable activity with ascorbic acid (Table 2). The comparison of samples with standard are shown in the Fig. 1.

These results showed the therapeutic value of walnuts and they can be used for the betterment health especially against oxidative stress related metabolic disorders.

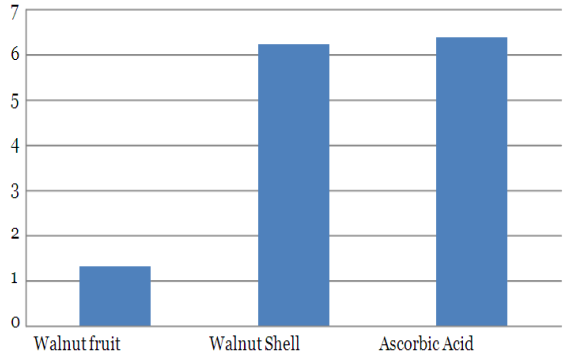

Fig. 1. IC_{50} values comparison of samples and standard.

Conclusion

The antioxidants can inhibit, remove and or scavenge the oxidation other substances or molecules by eliminating reactive species. It was concluded from the study that walnut fruit and shell showed extremely significant activity as compared to world standard antioxidant ascorbic acid. The inhibition concentration values of walnut fruit and shell against DPPH radicals were found to be 1.28 and 6.62µg/mL respectively. While the IC_{50} value of ascorbic acid was 4.6 µg/mL. Although this is very basic and rough information but quite valuable to divert attention of the scientific community towards the molecular research on walnut.

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