Free Radical Scavenging Activities of Some Edible Fruit Seeds

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Abstract

Free radical scavenging activity of the seeds of some edible fruits from Turkey, namely *Citrus limon* (L.) Burm. (lemon), *Prunus armeniaca* L. (wild apricot), *P. laurocerasus* L. (cherry laurel), *P. cerasus* L. (sour cherry), *Citrullus vulgaris* Schrad. (watermelon), and *Cucumis melo* L. (melon), has been investigated by using stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide (O₂⁻) generated by xanthine/xanthine oxidase (XOD) systems. DPPH scavenger capacity of the extracts of the aforementioned edible fruit seeds was compared with the known antioxidative substances such as tert-butyl-4-hydroxytoluene (BHT), vitamin C, quercetin and gallic acid. Radical scavenging capacity of the extracts was compared with that of gallic acid.

Keywords: Free radical, seed, DPPH, XOD, antioxidant.

Introduction

Free radicals, oxidative stress and lipid peroxidation have been suggested as potentially important causative agents that lead to uncontrolled reactions resulting in the cross-linking of DNA, proteins, and lipids, or oxidative damage to functional groups of these important biological macromolecules. Damage to these functional groups causes molecular damage and cell injury (Halliwell & Gutteridge, 1985). Oxygen radicals and lipid peroxides are implicated in the etiology of cancer, multiple sclerosis, Parkinson’s disease, senile dementia, autoimmune disease, diabetes mellitus, Alzheimer’s disease and asbestosis (Loliger, 1991; Hudson, 1990).

A large amount of research has been carried out to find antioxidative drugs, which not only prolong the shelf life of food products but also participate as radical scavengers in living organisms. Currently, there is an increasing interest in the antioxidative activity of natural compounds (Da Silva, 1991). They can be an alternative to the use of synthetic compounds in food and pharmaceutical technology or serve as lead compounds for the development of new drugs with the prospect of improving the treatment of various disorders.

The aim of this study was to determine free radical scavenging activities of polyphenolic fraction of the seeds of some edible fruits using superoxide and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals.

Materials and methods

Chemicals

Xanthine sodium, xanthine oxidase (XOD), nitroblue tetrazolium (NBT), tert-butyl-4-hydroxytoluene (BHT), superoxide dismutase (SOD), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ethylene diamine tetraacetic acid disodium (EDTA), (CAPS), quercetin (Sigma Chemical Co, St. Louis, MO, USA), potassium dihydrogen phosphate, sodium hydroxide, sodium bisulfite, gallic acid, vitamin C, ethanol (Merck Co., Germany), methanol, ethyl acetate and chloroform (Carlo Erba, Italy) were used in the determinations.

Plant materials

*Citrus limon* (L.) Burm. (lemon, Rutaceae), *Prunus armeniaca* L. (wild apricot, Rosaceae), *P. laurocerasus* L. (cherry laurel, Rosaceae), *P. cerasus* L. (sour cherry, Rosaceae), *Citrullus vulgaris* Schrad. (watermelon, Cucurbitaceae), and *Cucumis melo* L. (melon, Cucurbitaceae) were purchased from Ankara. The seeds and the fruits of the plants used in this study were dried at room temperature and powdered in a grinder.

Preparation of polyphenolic fractions of the seeds

Seeds (5 g) of *Citrus limon*, *Prunus armeniaca*, *P. laurocerasus*, *P. cerasus*, *Citrullus vulgaris*, and *Cucumis melo*, and the fruits of *P. laurocerasus* were weighed. Ten mL of
NaHSO₃ solution (1 g/L) and 10 mL of ethanol were added to the seeds. They were mixed on a magnetic stirrer for 2 min. After adding 20 mL of chloroform to each seed and fruit fraction, they were stirred again for a minute. The mixtures were transferred to centrifuge tubes and centrifuged at 3000 rpm for 10 min. Each mixture was divided into three portions. Hydroalcoholic solution at the top phase was separated by a pasteur pipette. Seed leftovers in the middle and chloroform phase at bottom were also separated from each other by filtration. Chloroform phase was discharged. This extraction procedure was repeated six times for each extract. After combining the hydroalcoholic phases, they were evaporated at 30°C until only aqueous solution remained. The aqueous solution was extracted with 20 mL of ethyl acetate for six times. Combined ethyl acetate extracts were evaporated at 30°C until to dryness and the extracts obtained were stored at deep-freezer until experimental work.

Antioxidative assay

Method for determination of DPPH radical scavenging activity

A methanol solution (1.95 mL) of 0.1 mM DPPH solution and 50 μL of different concentration of each extract (20–400 mg/mL) were added to a spectrophotometric cuvette. Decrease in absorbance at 515 nm was recorded every 15 min until the reaction was fixed using a Shimadzu UV-265 spectrophotometer. Remaining percentage of DPPH was calculated by applying the standard calibration curve, DPPH/MeOH solutions at 10.9, 11, 12.5, 14, 16, 20, 25, 33, 50 μM concentrations. Measurements were done in triplicate for each sample.

Method for determination of superoxide radical scavenging activity

In this method, O₂⁻ was produced by the xanthine/xanthine oxidase (X/XOD) system. A reaction mixture (1.7 mL) containing CAPS 50 mmol/L, EDTA 0.94 mmol/L, xanthine sodium 0.05 mmol/L, NBT 0.025 mmol/L, pH 10.2, and 50 μL of different concentrations of each extract (20–400 mg/L) were added to spectrophotometric cuvettes. After adding 250 μL of xanthine oxidase (1.67 U/mL), the absorbance was measured at the beginning (A₀) and at the 10th min (Aₓ) against air at 560 nm using a Shimadzu U-265 spectrophotometer. Absorbance difference was calculated (ΔA = A₀ - Aₓ) for the samples, and coded as Pₓ. Phosphate buffer (50 μL) was used in the same reaction medium in place of extract and P₀ was calculated. Phosphate buffer (50 μL) in place of extract and 250 μL of distilled water in place of xanthine oxidase were used in the same reaction medium and P₀ was calculated. Unscavenged O₂⁻ % was found according to the equality given below:

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\text{Unscavenged } O_2^- \% = \frac{(P_x - P_0)}{P_x} \times 100
\]

Results and discussion

Antioxidative nutrients may play a major role in the prevention of a number of diseases, including cardiovascular and cerebrovascular diseases, some forms of cancer and many age-related disorders. Among the major lipid antioxidants are vitamin E, ubiquinol (coenzyme Q₀) and various carotenoids derived from dietary sources.

DPPH scavenging capacity of the extracts was compared with tert-butyl-4-hydroxytoluene (BHT), vitamin C, quercetin and gallic acid. Of all the extracts, only the extract of P. laurocerasus fruits possessed a scavenger activity against DPPH free radical at 290 mg/mL, while BHT, vitamin C, quercetin and gallic acid scavenged 50% of DPPH radical at 195 mg/mL, 126 mg/mL, 85 mg/mL and 40 mg/mL, respectively (Fig. 1). The extract of the seeds of P. cerasus had also a neglectable activity at 4800 mg/mL.

![Figure 1: Extract concentration of seeds scavenged 50% of DPPH radical (EC₅₀).](image-url)
When the $O_2^-$ scavenger capacity was evaluated, the seed extracts of *Citrus limon* and *Citrullus vulgaris* were found to be the most active at a 113 mg/mL concentration. The seed extracts of *Prunus armeniaca*, *P. laurocerasus* (seed and fruit), *P. cerasus* and *Cucumis melo* showed activity at 156 mg/mL, 121 mg/mL, 128 mg/mL, 125 mg/mL and 151 mg/mL, respectively (Fig. 2).

In both tests, the fruit extract of *P. laurocerasus* exhibited remarkable antioxidant activity. *P. laurocerasus* L. (Rosaceae) (syn. *Laurocerasus officinalis* Roemer) has been found to contain some phenolic-type compounds which might be possibly considered to be responsible of the antioxidant activity in *P. laurocerasus* (Baytop, 1984). As to the other extracts that were moderately active in the superoxide radical scavenging activity assay, *P. cerasus* L. (syn. *Cerasus vulgaris* Miller) (Rosaceae), *P. armeniaca* L. (Rosaceae), *Citrus limon* (L.) Burm. ( Rutaceae), *Citrullus vulgaris* Schrader (syn. *C. lanatus* (Thunb.) Matsum et Nakai (Cucurbitaceae) and *Cucumis melo* L. (Rosaceae) are also known to be rich in phenolic compounds, heterosides and flavonoids.

These fruits have been a part of the human diet for centuries and their health benefits are mainly attributed to the phenolic compounds as well as vitamins, pectins, tannins and flavonoids which are biologically active compounds found in high levels within the fruits. They act as natural agents to protect organisms against free radicals, oxidative stress and lipid peroxidation (Manners & Hasegawa, 1999). On the basis of current evidence, it would be premature to designate these fruits and their phenolic components as significant antioxidative agents. However, they are readily available and have been safely consumed for many years, the preliminary results would provide a basis for starting bioactivity-guided isolation in order to prove if the active components are phenolic-type of compounds.

**References**


