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Antioxidant properties of various solvent extracts from purple basil

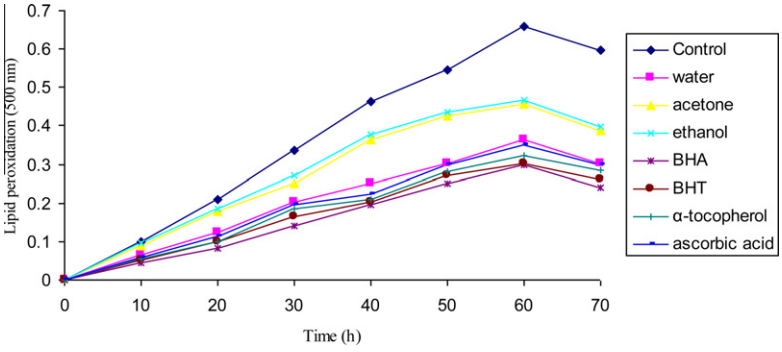
Yeşim Yeşiloğlu *, Latifşah Şit

Trakya University, Faculty of Science, Department of Chemistry, Edirne, Turkey

HIGHLIGHTS

- We investigated antioxidant and radical scavenging activities in different assays.
- In ethanol extract, 116 ± 0.2 mg gallic acid equivalent of phenols was detected.
- Extracts had effective radical scavenging and metal chelating activities.
- It can be considered as a source of natural antioxidants for food and nutraceutical products.

GRAPHICAL ABSTRACT



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ABSTRACT

Water, ethanol and acetone extracts from leaves and flowers of purple basil, one of the most popular spices consumed in the Thrace region of Turkey, were tested *in vitro* for their ability to inhibit peroxidation of lipids, to scavenge DPPH, hydrogen peroxide, superoxide anion, to reduce Fe(III) to Fe(II) and to chelate Fe(II) ions. The results showed that purple basil contained naturally occurring antioxidant components and possessed antioxidant activity which may be attributed to its lipid peroxidation inhibitory, radical scavenging and metal chelating activities. It was concluded that purple basil might be a potential source of antioxidants.

Introduction

Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxygen-centered free radicals and other reactive oxygen species (ROS), which are continuously, produced *in vivo*, result in cell death and tissue damage. The role of oxygen radicals has been implicated in several diseases, including cancer, diabetes and cardiovascular diseases, ageing, etc. [1]. Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress [2]. There is an increasing interest in natural antioxidants, e.g., polyphenols, present in medicinal and dietary plants, which might help in preventing oxidative damage [3]. Polyphenols possess ideal structural chemistry for free radical scavenging activity, and they have been shown to be more effective antioxidants *in vitro* than tocopherols and ascorbate. Antioxidant properties of polyphenols arise from their high reactivity as hydrogen or electron donors, and from the ability of the polyphenol

Abbreviations: NADH, nicotinamide adenine dinucleotide; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; NBT, nitroblue tetrazolium; PMS, phenazine methosulfate; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ferrozine, 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine; Tween-20, polyoxyethylenesorbitan monolaurate; TCA, trichloroacetic acid; ROS, reactive oxygen species.

* Corresponding author. Tel.: +90 284 235 95 92; fax: +90 284 235 87 54.
E-mail addresses: yesimyesiloglu@trakya.edu.tr, yesimyesiloglu@yahoo.com (Y. Yeşiloğlu).

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derived radical to stabilize and delocalize the unpaired electron (chain-breaking function), and from their ability to chelate transition metal ions [4].

Human body has multiple mechanisms especially enzymatic and non-enzymatic antioxidant systems to protect the cellular molecules against reactive oxygen species (ROS) induced damage [5]. However, the innate defense may not be enough for severe or continued oxidative stress. Hence, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the ROS in human body. Many synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are very effective and are used for industrial processing but they possess potential health risk and toxic properties to human health and should be replaced with natural antioxidants [6]. Hence, compounds especially from natural sources capable of protecting against ROS mediated damage may have potential application in prevention and/or curing of diseases.

The phenolic compounds in herbs act as antioxidants due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators [7].

Among the various medicinal and culinary herbs, some endemic species are of particular interest because they may be used for the production of raw materials or preparations containing phytochemicals with significant antioxidant capacities and health benefits [8]. Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [7].

Purple basil, locally known as "reyhan", is one of the most popular leafy vegetables consumed in Thrace region of Turkey. The objectives of this study were to evaluate the antioxidant activity of purple basil extracts obtained from three solvents. No reports are available on the antioxidant activity of purple basil.

Experimental

Chemicals

Linoleic acid, α-tocopherol, potassium persulfate, nicotinamide adenine dinucleotide (NADH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, catechin, and 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine (ferrozine) were obtained from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany).

Ammonium thiocyanate, ferrous chloride, polyoxyethylenesorbitan monolaurate (Tween-20), trichloroacetic acid (TCA), ethanol and acetone were purchased from Merck. All other chemicals used were in analytical grade and obtained from either Sigma–Aldrich or Merck.

Plant material and extraction procedures

The leaves and flowers of purple basil were purchased from a traditional market at Edirne City (Edirne, Turkey). Plant materials were washed with distilled water and dried at room temperature. For water extraction, 25 g sample was put into a fine powder in a mill and was mixed with 500 mL boiling water by magnetic stirrer for 15 min. Then, the extract was filtered over Whatman No. 1 paper. The filtrates were frozen and lyophilized in lyophilizator at 5 μm Hg pressure at −50 °C (Labconco, Freezone 1L). For solvent extraction, 25 g sample was put into a fine powder in a mill and was mixed with 500 mL solvent. The residue was re-extracted until extraction solvents became colorless. The obtained extracts were filtered over Whatman No. 1 paper and the filtrate was collected, then solvent was removed by a rotary evaporator (Buchi R-200, Switzerland) at 40 °C to obtain dry extract. All the extracts were kept at −20 °C and were dissolved in water or solvent before use.

Determination of total phenolic compounds

Total soluble phenolics in the extracts were determined with Folin–Ciocalteu reagent according to the method of Slinkard and Singleton [9] using gallic acid as a standard phenolic compound. Briefly, 1.0 mL of extract solution containing 1.0 mg extracts in a volumetric flask was diluted with distilled water (46 mL). 1.0 mL of Folin–Ciocalteu reagent was added and the content of the flask mixed thoroughly. Three minutes later, 3 mL of Na_2CO_3 (2%) was added, and the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm. The amount of total phenolic compounds was calculated as mg of gallic acid equivalents (GAE) from the calibration curve of gallic acid ($y = 0.001x + 0.001$; $r^2 = 0.9970$) and expressed as mg gallic acid/g dry weight (dw) of the plant material. Data were presented as the average of triplicate analyses.

Determination of total flavonoid content

Total flavonoid content was determined by a colorimetric method described by Wang et al. [10] with minor modification. An aliquot of 10 mL of appropriate dilution of each extract was added to volumetric flask containing 1 mL of 5% (w/v) sodium nitrite and placed for 6 min, followed by reaction with 1 mL of (10%) (w/v) aluminum nitrate to form a flavonoid-aluminum complex. After 6 min, 10 mL of 4.3% (w/v) NaOH was added and the total was made up to 25 mL with distilled water. After 15 min at room temperature, the final solution was mixed well again and the absorbance was measured against a blank at 510 nm with a UV-1601 UV/VIS Recording Spectrophotometer (Shimadzu UV-1601, Japan). (+)-Catechin was utilized for constructing the standard curve ($y = 0.012x + 0.036$; $r^2 = 0.9900$; y is the absorbance; x is the solution concentration). The results were expressed as mg catechin equivalents (CE)/g extract (dw).

The lipid peroxidation assay

Inhibition of lipid peroxidation of purple basil extracts was determined according to the thiocyanate method [11]. For stock solutions, 10 mg of each purple basil extracts was dissolved in 10 mL water (or solvent). Then, the solution, which contains the same concentration of purple basil extracts or standard samples (60 μg/mL) in 2.5 mL of potassium phosphate buffer (0.04 M, pH 7.0) was added to 2.5 mL of linoleic acid emulsion in potassium phosphate buffer (0.04 M, pH 7.0). Fifty milliliters of linoleic acid emulsion contained 175 μg Tween-20, 155 μL linoleic acid, and 0.04 M potassium phosphate buffer (pH 7.0). On the other hand, 5.0 mL control was composed of 2.5 mL linoleic acid emulsion and 2.5 mL, 0.04 M potassium phosphate buffer (pH 7.0). The mixed solution (5 mL) was incubated at 37 °C in a glass flask. At regular intervals during incubation, a 0.1 mL aliquot of the mixture was diluted with 3.7 mL of solvent (ethanol or methanol), followed by the addition of 0.1 mL of 30% ammonium thiocyanate and 0.1 mL of 20 mM ferrous chloride in 3.5% hydrochloric acid. The peroxide level was determined by reading the absorbance at 500 nm in a spectrophotometer (Shimadzu UV-1601, Japan). This step was repeated every 10 h until the control reached its maximum absorbance value. Therefore, high absorbance indicates high linoleic acid emulsion oxidation. All data on total antioxidant activities are the average of triplicate experiments. The percent