Antioxidant activity of sea buckthorn (*Hippophae Rhamnoides)* extracts compared with common food additives

CAMELIA PAPUC, CRISTIANA DIACONESCU, V. NICORESCU
University of Agronomical Sciences and Veterinary Medicine Bucharest, 59 Marasti Blvd., 1st sector, Bucharest, Romania,

Abstract

Sea buckthorn (Hippophae rhamnoides) is a plant distributed all over the world, with diverse uses, such as controlling soil erosion, nutritious foods, drugs and skin-care products.

This study was undertaken in order to examine the antioxidant activity of water-acetone and alcoholic extracts obtained from sea buckthorn, using different tests including scavenging of 1,1-diphenyl-2-picryl-hydrazil radical (DPPH˙), superoxide anion radical ($O_2^{\cdot-}$) and total antioxidant activity.

The scavenging activity against DPPH radical was determined by the decrease of its absorbance at 517 nm induced by antioxidants. The scavenging activity against superoxide anions – generated by the phenazin methosulfate (PMN) / nicotinamid-adenin-dinucleotidphosphate, reduced form (NADPH) system – was detected within by the reaction with chloride of 2,2'-di-p-nitrophenyl)-5,5'-diphenyl-(3,3'-dimethoxy-4,4'-difphenylene) ditetrazolium chloride (nitro blue tetrazolium – NBT). Total antioxidant activity of sea buckthorn extracts was determined by thiocyanate method. The antioxidant activities of sea buckthorn extracts were compared with standard antioxidants 2,6-di-tert-butyl-p-hydroxytoluene (BHT) and tert-butyl-hydroxyanisole (BHA).

The results indicate that water-acetone extract and alcoholic extract prevent lipid peroxidation due to the ability to annihilate free radicals. Sea buckthorn extracts had a more antioxidant capacity than BHT and BHA.

Keywords: sea buckthorn, antioxidant activity, superoxide anion, DPPH radical, alcoholic extract.

Introduction

Sea buckthorn *(Hippophae rhamnoides)* is a bush species widely distributed throughout the temperate zone of Asia, Europe and all over subtropical zones, being found especially at high altitudes. Sea buckthorn fruits have been used as a drug in traditional medicine since ancient times. Their pharmacological effects are due to vitamins, trace elements, amino acids and other bioactive substances, such as β-carotene, zeaxanthin, lycopene, flavonoids, folic acid, triterpene, fatty acids, tannic acid, etc. The polyphenols contained in sea buckthorn fruits have antioxidant properties and they can protect human body against the damaging effect of oxidized radicals (Gao & al.[1], Yao & Tigerstedt [2], Yeb [3], Kallio & al. [4], Yang & al. [5], Yang & Kallio [6], Papuc & al. [7]).

Lipid oxidation can occur in foods that contain substantial amounts of fats, like milk and meat products, oil, nuts and also in those that contain only minor amounts of lipids, such as vegetable products (Kanner & Rosenthal [8]). Lipid oxidation can occur by three different mechanisms: 1) autoxidation by free radical action, 2) photo-oxidation and 3) enzyme action. The autoxidation is a radical-chain process involving three sequences: initiation, propagation and termination. The initiation sequence refers to the attack of reactive oxygen species (ROS), with sufficient reactivity, to abstract a hydrogen atom from a methylene group (-CH_2-). This

attack generates free radicals from polyunsaturated fatty acids. The most efficient ROS that attacks methylene group is hydroxyl radical (·OH). Peroxide anion ($O_2^{\cdot -}$) is insufficiently reactive to attack methylene group; it cannot be directly responsible for the initiation of the lipid oxidation chain, but there is the possibility of $O_2^{\cdot -}$ conversion to some other oxidizing species, such as hydroxyl radical, as the result of an $O_2^{\cdot -}$ driven Fenton reaction (Kanner & Rosenthal [8]):

$2\ O_2^{\cdot -} + 2H_2O \rightarrow H_2O_2 + 2{\cdot}OH + O_2$

$M^{n+} + O_2^{\cdot -} \rightarrow M^{(n-1)+} + O_2$

$M^{(n-1)+} + H_2O_2 \rightarrow M^{n+} + {\cdot}OH + HO^-$

The transition ion M^{n+}, can well be provided by hem-proteins.

Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals and also by acting as reactive species scavenger. Antioxidant supplement can be used in order to reduce the oxidative damage induced from reactive species. The most commonly used antioxidants at the present time are 2,6-di-tert-butyl-p-hydroxytoluene (BHT), tert-butyl-hydroxyanisole (BHA), propyl galate and *tert*-butylhydroquinone (Gulcin & al. [9]). These antioxidants have been suspected of being responsible for liver damages and carcinogenesis in lab animals.

Polyphenolic compounds, like flavonoids and phenolic acids, commonly found in plants, have been reported to have multiple biological effects, including antioxidant activity (Papuc & al. [7], Valentao & al. [10]).

The goals of this study were to obtain alcoholic extracts and water-acetonic extracts from sea buckthorn fruits, to emphasize the scavenging activity of these extracts against reactive oxygen species superoxide anion and 1,1-diphenyl-2-picrylhydrazyl (DPPH·) free radical and to determine the total antioxidant activity, comparatively with synthetic antioxidants 2,6-di-tert-butyl-p-hydroxytoluene (BHT) and tert-butyl-hydroxyanisole (BHA).

Materials and Methods

Preparation of sea buckthorn fruits extracts. Dried fruits of *Hippophae rhamnoides* were powdered and successively extracted with ethanol and acetone in a Soxhlet extractor at a temperature of 55-60ºC, for a period of 7-8 h. The obtained extracts were filtered in order to obtain particle free extracts and evaporated using a rotary evaporator under vacuum at 45ºC. The obtained residues were dissolved in ethanol and in a mixture of acetone-water (3:2, v/v) respectively.

Synthetic antioxidants BHA and BHT (Sigma) were used in concentration of 100µg/g.

Superoxide anion scavenging activity. The superoxide anions generated by phenazin methosulfate (PMN)/nicotinamid-adenin-dinucleotidphosphat, reduced form (NADPH) system, were detected by the reaction with 2,2'-di-p-nitrophenyl)-5,5'-diphenyl-(3,3'-dimethoxy-4,4'-diphenylene) ditetrazolium chloride (nitro blue tetrazolium – NBT) (Wang & al. [11]). The mixture of reaction contained: 2.7 ml PBS 50 mM pH 7.8, 50 µl Na_2EDTA 0.1 M, 100µl NBT 0.6 M, 50 µl phenasin metosulphat 1mM, 50 µl NADPH 0.5 mM and alcoholic or acetonic plant extract.

The annihilation activity of free radicals was calculated in % inhibition, according to the following relation:

$$\%\ Inhibition = \frac{(\text{A control - A test})}{\text{A control}} x100$$

In order to achieve the control test, the same operations were made, with the single exception that instead of the extracts, the solvent (ethylic alcohol / water-acetone) was used.

DPPH free radical scavenging activity. The ability of alcoholic and acetonic extract to annihilate the DPPH radical (1,1-diphenil-2-picrylhydrazyl) was investigated by the method described by Lie-Fen Shyur (Shyur & al. [12]). Equal volumes of diluted extract were mixed with an equal volume of DPPH $6x10^{-3}$ M in absolute ethanol, and the obtained mixtures were kept at room temperature for 30 minutes. Then, the absorption of the mixtures at 520 nm was determined, in comparison with the control solution (maximum absorption).

The annihilation activity of free radicals was calculated in % inhibition according to the following relation:

$$\% \ Inhibition = \frac{(\text{A control} - \text{A test})}{\text{A control}} x100$$

In order to achieve the control test, the same operations were made, with the single exception that instead of the extracts, the solvents were used.

Determination of total antioxidant activity. The total antioxidant activity of sea buckthorn fruits extracts was determined according to the ferric thiocyanate method (Gulcin & al. [9]). Linoleic acid emulsion in potassium phosphate buffer (0.04 M, pH 7.0) was used. Triton X was used as emulgator. The mixted solution sea buckthorn fruits extract - linoleic acid emulsion was incubated at 37ºC in a flask glass for 3 min. The peroxide value was determined at a Karl Zeiss Jena spectrophotometer by reading the absorbance at 500 nm, after the reaction with $FeCl_2$ and thyocianate.

The inhibition of lipid peroxidation was calculated by the following equation:

$$\% \ Inhibition = \frac{(\text{A control} - \text{A test})}{\text{A control}} x100$$

As control samples were used solutions without the adding of sea buckthorn extracts or standard antioxidant.

Results

Superoxide anions scavenging activity. The obtained results have emphasized the capacity of sea buckthorn fruit extracts to annihilate the superoxide anions generated in PMS-NADPH-NBT system (Fig. 1). The capacity to annihilate superoxide anions depended on the extraction medium. Water–acetonic extract showed lower scavenging activity against superoxide anion than alcoholic extract. Superoxide anion scavenging activity was 33.3 ± 1.7% in case of the alcoholic extract and 25.5 ± 2.4% in case of the water-acetonic extract. Superoxide anion scavenging activity of synthetic antioxidants BHT and BHA was 77.3 ± 3.2% and 65.7 ± 2.8%, respectively.

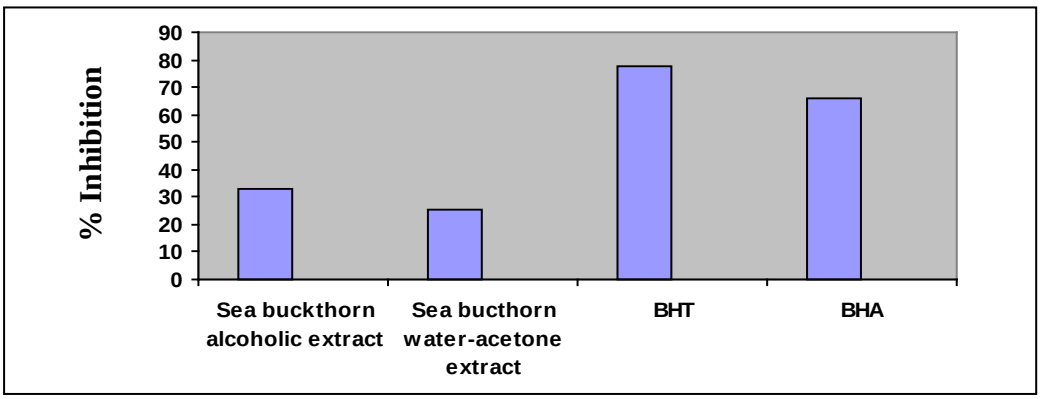

Figure 1. Superoxide anion scavenging activity of sea buckthorn extracts compared with synthetic antioxidants BHT and BHA

DPPH radical scavenging activity. DPPH radical reacts with suitable reducing agents losing color stoichometrically with the number of electrons consumed, witch is measured spectrophotometrically at 517 nm. The obtained results for sea bucthorn fruits extracts and synthetic antioxidants BHT and BHA are shown in figure 2. The annihilation of DPPH radical, expressed in % inhibition, was 94.7 ±3.2 % for the alcoholic extract and 74.7 ± 2.6% for the water-acetonic extract. The scavenging effect of synthetic antioxidants was 50.2 ± 3.3% for BHT and 54.8 ± 2.4% for BHA (Fig. 2).

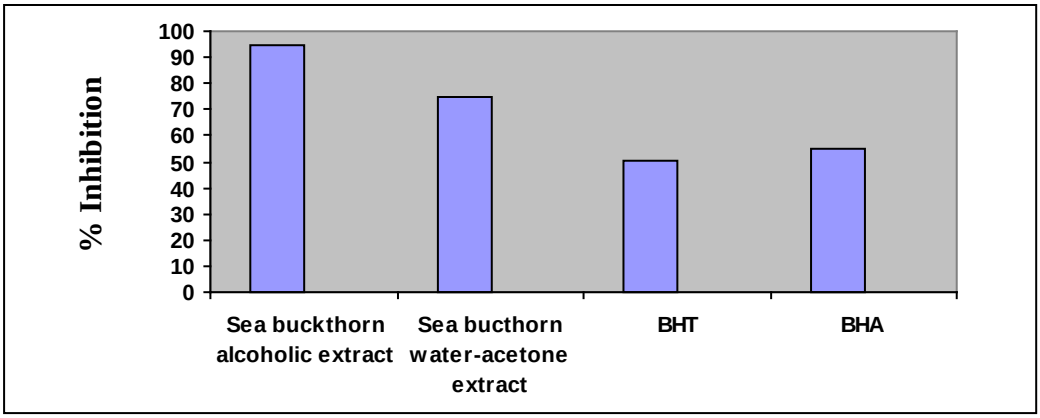

Figure 2. DPPH radical scavenging activity of sea buckthorn extracts compared with synthetic antioxidants BHT and BHA

Total antioxidant activity. During oxidation of linoleic acid, the formed peroxides oxidize Fe^{2+} to Fe^{3+}. The latter Fe^{3+} ions form complex with SCN^- and this complex has maximum absorbance at 500 nm. The higher absorbance indicates high linoleic acid oxidation. The effect of sea buckthorn extracts and synthetic antioxidants on peroxidation of linoleic acid emulsion is shown in Fig. 3. The antioxidant activity of sea buckthorn alcoholic extract was the strongest. The percentage inhibition of 100µg/g BHT and BHA was 91.1 ± 2.8% and 87.9 ± 3.5%, respectively.

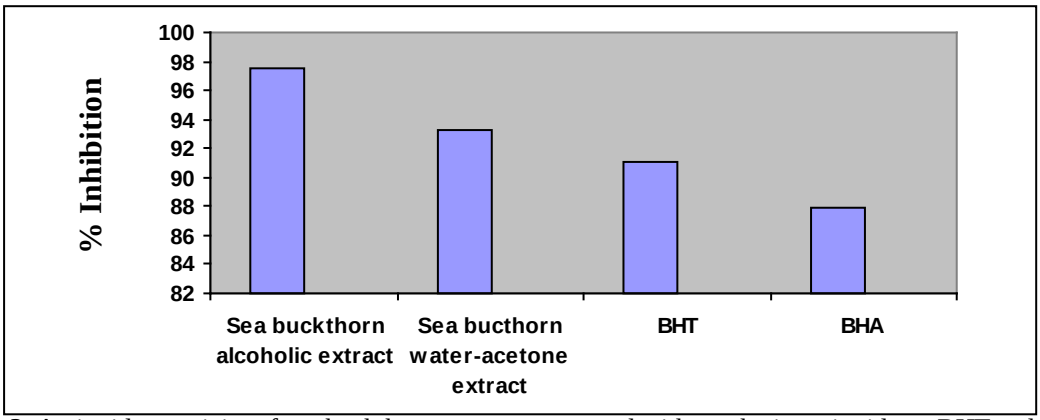

Figure 3. Antioxidant activity of sea buckthorn extracts compared with synthetic antioxidants BHT and BHA

Discussion

Sea buckthorn alcoholic and water-acetone extracts showed scavenging activity against superoxide anion and DPPH radical.

Sea buckthorn alcoholic extract exhibited the strongest scavenging activity against

superoxide anion and DPPH radical, while water-acetone extract exhibited a lower activity. The results also showed that sea buckthorn extracts scavenge superoxide anion and DPPH free radical better than BHA and BHT.

Sea buckthorn extracts exhibited antioxidant activity. Alcoholic extract was the most efficient antioxidant, while BHT and BHA showed a lower antioxidant activity. Antioxidant activity of sea buckthorn fruits extracts can be attributed to the property of capturing free radicals.

These properties make sea buckthorn extracts applicable to be used as natural antioxidant in medical, pharmaceutical and food industry.

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