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ANTIOXIDANT ACTIVITY OF POLYPHENOLS EXTRACTED FROM HAWTHORN AND DOG-ROSE FRUITS ON LINOLEIC ACID EMULSION MODEL SYSTEM COMPARED TO BHA SYNTHETIC ANTIOXIDANT

Nicoleta Corina PREDESCU, Valentin NICORESCU, Carmen PETCU, Georgeta Stefan, Camelia PAPUC

University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Splaiul Independentei, District 5, 050097, Bucharest, Romania

Corresponding author email: durduncorina@yahoo.com

Abstract

The ethanolic extracts of hawthorn (Crataegus monogyna) and dog-rose (Rosa canina) were found to contain polyphenols with antioxidant activity. The aim of this study was to assess the antioxidant properties of hawthorn and dog-rose polyphenols in linoleic acid emulsion model system, comparatively with synthetic antioxidant BHA. Polyphenols and BHA were incorporated in a linoleic acid emulsion at the final concentration of 100 ppm and then incubated at 80°C for 7 days. For determination of the progress of oxidation processes, primary and secondary peroxidation products levels were evaluated at every 24 hours. Hawthorn and dog-rose polyphenols inhibited the formation of lipid hydroperoxides, conjugated dienes and thiobarbituric acid reactive substances. The protective effect of hawthorn and dog-rose polyphenols was superior to the one of BHA synthetic antioxidant.

Key words*: antioxidant activity, dog-rose, hawthorn, linoleic acid, polyphenols.*

INTRODUCTION

Polyphenols are a wide range of biological molecules present in various plant species and play an important role for normal growth, development and defence against infections and injuries (Scalbert and Williamson, 2000). Polyphenols can be classified into different groups depending on the number of phenol rings contained and on the basis of structural elements that bind these rings to one another. The main classes include flavonoids, phenolic acids, stilbenes and lignans (Spencer et al., 2008). Flavonoids are present in leaves, flowers and fruits and partially provide plant colours. They generally occur as glycosylated derivates in plants, although conjugations with inorganic sulphate or organic acids as well as malonylation are also known (Heldt, 1997). Phenolic acids are hydroxylated derivates of benzoic acid and cinnamic acid (Macheix et al., 1990). Stilbenes contain two phenyl moieties connected by a two-carbon methylene bridge. One of the best studied, naturally occurring polyphenol stilbene is resveratrol (3.4′,5-trihydroxystilbene) (Lőliger, 1991). Lignans are diphenolic compounds that contain a 2.3-dibenzylbutane structure that is formed by the dimerization of two cinnamic acid residues. Several lignans are considered to be phytoestrogens (Adlercreutz and Mazur, 1997). Plant polyphenols are most commonly known for their antioxidant activity. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have metal chelating properties (Papuc et al., 2007). Plant polyphenols are increasingly of interest in the food industry because of their capacity to retard oxidative degradation of lipids and thereby improve the quality and nutritional value of foods. The importance of antioxidant constituents of plant materials in maintenance of health and protection against coronary heart diseases and cancer is also raising interest among scientists, food manufactures, and consumers as the trend of the future is moving toward functional food with specific health effects (Roginsky and Lissi, 2005).

Hawthorn is a large genus of shrubs and trees widely spread in temperate zones, including Romania. Hawthorn (*Crataegus monogyna*) has been used in folk medicine and is widely

utilized in pharmaceutical preparations mainly because of its neuro- and cardiosedative actions and its low toxicity (Kirakosyan et al., 2003). The pharmacological effects of *Crataegus monogyna* have been attributed mainly to the polyphenolic contents such as flavonoids, proanthocyanidins, catechins, phenolic acids, essential oils and terpenoids (Bahorum et al., 1994; Chang et al., 2002). Hawthorn fruits contain a wide range of flavonoid compounds (eg. hyperoside, luteolin-7-glucoside, rutin, quercetin, vitexin, etc.) and phenolic acids (eg. chlorogenic acid, caffeic acid, etc.) (Rice-Evans et al., 1995; Durdun et al., 2010).

Rosa canina, commonly known as the dog-rose, is a variable climbing wild rose species native to Europe, North-western Africa and Western Asia. Dog-rose is mostly used for the prevention and treatment of the common cold, gastrointestinal disorders, diabetes, kidney disorders, and other infections. The dog-rose hips comprise several biologically active compounds, such as: sugars, organic acids, pectins, flavonoids, tannins, carotenoids, fatty acids, vitamins (particularly vitamin C and also vitamins B_1, B_2, K, PP, E), macro- and microelements (Demir and Özcan, 2001).

Several studies demonstrated the capacity of hawthorn and dog-rose polyphenols to annihilate reactive oxygen species and to inhibit different lipid peroxidation processes in rat brain homogenates, as well as to reduce thermal oxidation processes of vegetal oils (Gao et al., 2000; Wenzig et al., 2008, Papuc et al., 2013).

The aim of this study was to assess the antioxidant properties of hawthorn and dog-rose alcoholic extracts in linoleic acid model system, comparative with BHA synthetic antioxidant.

MATERIALS AND METHODS

Obtaining vegetal extracts

In order to obtain vegetal extracts, dried hawthorn and dog-rose fruits were grounded and then subdued to a solid-liquid extraction (1:10; w: v) with ethanol in a solvent extractor (VELP Scientifica).

Determination of total phenolic content

Total phenolic content (TPC) was determined by spectrophotometry with the Folin-Ciocalteu reagent, according to a procedure described by Singleton and Rossi (1965). Briefly, 0.5 mL of the diluted sample and 7.0 mL distilled water reacted with 0.5 mL of Folin-Ciocalteu reagent for 3 min, and then 2 mL saturated sodium carbonate solution (about 20 %) was added into the reaction mixture. The absorbance readings were taken at 765 nm after incubation at room temperature for 2 h. The concentration of polyphenols in samples was derived from a standard curve of gallic acid. The results were expressed as mg gallic acid equivalent/100 mL (mg GAE/100 mL).

Preparation of linoleic acid emulsion

Linoleic acid emulsion was prepared according to the procedure described by Yen et al. (2003). Briefly, 0.285 g linoleic acid were mixed with 0.289 g Tween 20 and 50 ml phosphate buffer 0.067M, pH 7.2 and then the mixture was homogenized for5 min.

Evaluation of the antioxidant activity

Hawthorn and dog-rose alcoholic extracts were added to linoleic acid mixture at the final concentration 100 ppm total polyphenols. In parallel, ethanolic solution of butylated hydroxyanisole (BHA) was used too as antioxidant for linoleic acid emulsion, at the final concentration 100 ppm. Linoleic acid emulsion without antioxidants was used as control. The mixtures and the control samples were incubated at 80°C for 7 days. The progression of oxidation processes was monitored at every 24 hours by measuring the hydroperoxides (HP), conjugated dienes (CD), and thiobarbituric acid reactive substances (TBARS) levels.

Inhibition of hydroperoxides formation

Hydroperoxides were determined using the method described by Romero et al. (2008). Briefly, 0.02 g linoleic acid emulsion – plant ethanolic extract / BHA solution mixture was dissolved in 9.8 ml methanol:chloroform mixture (70:30, v/v) and then 0.1 ml of 300 g/l ammonium thiocyanate was added and mixed. After 5 min., 0.1 ml ferrous chloride prepared in 3.5% HCl was added to the previous mixture and the absorbance was measured at 501 nm.

Inhibition of hydroperoxide formation was calculated using formula (1).

$$(1) \quad \% \text{ HP Inhibition} = [(A_{control} - A_{sample})x100]/A_{control}$$

Inhibition of conjugated dienes formation

20 µl linoleic acid emulsion – plant ethanolic extract / BHA solution mixture were mixed with 2 ml isooctane and absorbance was measured at 232 nm wave-length using a Jasco V 670 spectrophotometer. Inhibition of conjugated dienes formation was calculated using formula (2).

$$(2) \quad \%\ CD\ Inhibition = [(A_{control} - A_{sample})x100]/A_{control}$$

Inhibition of thiobarbituric acid reactive substances formation

100 µl linoleic acid emulsion – plant ethanolic extract / BHA solution mixture were mixed with 2 ml 20 mM thiobarbituric acid prepared in 15% trichloroacetic acid solution. The mixture was heated at 90°C for 15 min. and cooled at room temperature. After the addition of 2 ml of chloroform, the mixture was strongly agitated and then centrifuged at 1000 rpm for 15 min. The absorbance of organic layer was estimated at 532 nm. Inhibition of TBARS formation was calculated using formula (3).

$$(3) \quad \%\ TBARS\ Inhibition = [(A_{control} - A_{sample})x100]/A_{control}$$

RESULTS AND DISCUSSIONS

Inhibition of hydroperoxides formation

Hydroperoxides are the primary and main products of lipid peroxidation because they can also be a source of active free radicals due to their thermolysis or catalytic destructions (Lőliger, 1991). From Figure 1 it can be observed that hawthorn and dog-rose polyphenols had an inhibitory effect upon hydroperoxides formation process higher than the one of BHA. For hawthorn and dog-rose ethanolic extract and BHA, the maximum inhibitory effect was recorded after 4 hours of incubation at 80°.

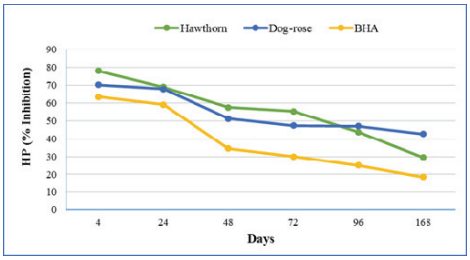

Figure 1. Inhibitory effect of hawthorn and dog-rose fruits polyphenols on hydroperoxides formation in linoleic acid emulsion model system, compared to BHA

After this interval, for the natural and synthetic tested antioxidants it was recorded a decrease of the protective effect against lipid peroxidation process.

Inhibition of conjugated dienes formation

Conjugated dienes are considered primary peroxidation products, resulted from fatty acids with two double bonds oxidation. The inhibitory effect of the hawthorn and dog-rose extracts and BHA synthetic antioxidant upon conjugated dienes formation process is presented in Figure 2. The obtained results demonstrate that hawthorn and dog-rose polyphenols inhibited the formation of conjugated dienes during incubation at 80°C for 168 hours strongly than BHA synthetic antioxidant. The inhibitory action of hawthorn and dog-rose polyphenols upon conjugated dienes formation was more accentuated in the first 96 hours of exposure to 80°C, after that being recorded a slight decrease of this effect.

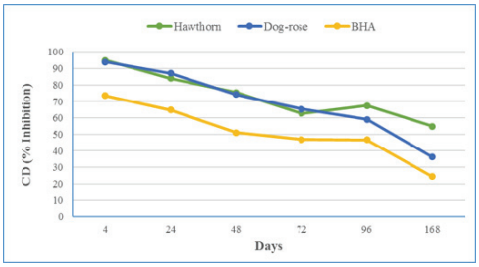

Figure 2. Inhibitory effect of hawthorn and dog-rose fruits polyphenols on conjugated dienes formation in linoleic acid emulsion model system, compared to BHA

Inhibition of thiobarbituric acid reactive substances formation

Linoleic acid peroxides generate a high number of carbonyl compounds upon decomposition. These compounds are secondary products of lipid peroxidation and, in reaction with 2-thiobarbituric acid, they are widely used as a measure of rancidity development.

The inhibitory effect of hawthorn and dog-rose polyphenols upon linoleic acid emulsion, comparative to BHA, is presented in Figure 3. Hawthorn polyphenols protected linoleic acid against peroxidation process in a manner superior to BHA synthetic antioxidant. The most pronounced inhibitory effect was recorded after 48 hours of incubation at 80°C, after that interval being observed an accentuated

decrease especially for dog-rose alcoholic extracts. After 168 hours of incubation, the inhibitory effect upon linoleic acid peroxidation process slightly increased for both hawthorn polyphenols and synthetic antioxidant BHA compared to dog-rose polyphenols.

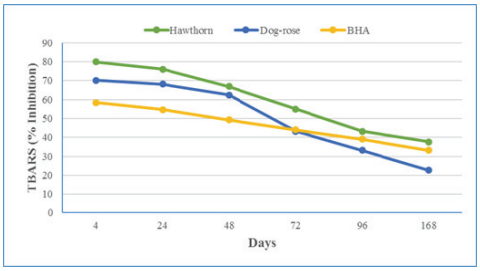

Figure 3. Inhibitory effect of hawthorn and dog-rose fruits polyphenols on thiobarbituric acid reactive substances formation in linoleic acid emulsion model system, comparatively with BHA

CONCLUSIONS

Hawthorn and dog-rose fruits polyphenols are able to protect linoleic acid against lipid peroxidation process.

Hawthorn and dog-rose fruits polyphenols inhibited the formation of conjugated dienes, lipid hydroperoxides and thiobarbituric acid reactive substances.

The protective effect of hawthorn and dog-rose fruits polyphenols was superior to the one of BHA synthetic antioxidant.

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