Antioxidant properties of aromatic plant alcoholic extracts

Received for publication, November 25,2007
Accepted, December 1, 2007

CAMELIA PAPUC, CRISTIANA DIACONESCU, NICORESCU V., CARMEN CRIVINEANU
University of Agronomical Sciences and Veterinary Medicine, Marasti, 59, 001464, Bucharest, Romania
E-mail cami_papuc@yahoo.com.

Abstract

In this study, the antioxidant properties of horseradish roots (Armoracia rusticana), coriander fruit (Coriandri fructus) and mustard seeds (Brassica alba) alcoholic extracts are investigated.

The scavenging activity of the extracts DPPH radical, superoxide anion and hydroxyl radical were evaluated in vitro. Superoxide anion was generated by phenazine methosulphate/NADH system; hydroxyl radical was generated by $FeCl_3$/ascorbic acid system and assayed by evaluating deoxyribose degradation using thiobarbituric acid method. The effect of alcoholic plant extracts on Fe^{2+} mediated lipid peroxidation was evaluated in rat brain homogenate in terms of thiobarbituric acid reactive substances. Mustard seeds and coriander fruit alcoholic extracts acted as scavenger DPPH radical, superoxide anion and hydroxyl radical and have antioxidant activity on rat brain homogenate. The antioxidant activity of horseradish roots alcoholic extract was 15 to 20 times lower than mustard seeds and coriander fruits alcoholic extracts.

Keywords: mustard, coriander, horseradish, DPPH radical, superoxide anion, lipid peroxidation

Under the usual conditions of food storage, unsaturated acyl-lipids can not be considered as stabile food constituents because thus are readily oxidized to hydroperoxides. Oxidation of unsaturated acyl-lipids is a complex process and involves a great number of interrelated reactions of intermediates. The latter, after subsequent degradation reaction, yield a great number of other volatile and nonvolatile compounds, responsible for deterioration of food quality.

Oxidation of unsaturated acyl-lipids can be retarded by addition of antioxidants in foods. Synthetic antioxidants such propyl, octyl and dodecyl gallate, 2,6-di-tert-butyl-p-hydroxytoluene (BHT) and tert-butil-hydroxyanisole (BHA) are suspected like possibly carcinogenic to humans.

Natural antioxidants such tocopherols and vitamin C are used as additives to increase food stability.

Plants containing phenolic compounds with multiple biological effects, including antioxidant activity. Crude extracts of fruits, herbs, vegetables, cereals, and other plants 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 [1]Kähkönen]. Plant polyphenols comprise a great diversity of compounds, among which flavonoids (anthocyanins, flavanols, flavonols, isoflavones, flavan-3-ols, proanthocyanidins) and several classes of nonflavonoids (hydroxybenzoic acids, hidroxycinamic acids, stilbenes, gallotannins) are usually distinguished. The antioxidant activity of plants polyphenols is based on the redox properties of their hydroxyl groups and the structural relationships between different parts of their chemical structure [Rice]. In recent years, phenolic compounds have attracted the interest of researches because they show powerful antioxidant activity that can protect foods lipid peroxidation. In addition, a lot off study have indicated beneficial effects of plant antioxidant compounds in the prevention of multitude of disease states (neurodegenerative disorders, cancer, and cardiovascular diseases).

The present study aims to assess the antioxidant activity of horseradish roots *(Armoracia rusticana),* coriander fruit *(Coriandri fructus)* and mustard seeds *(Brassica alba)* alcoholic extracts.

Materials and Methods

Obtaining vegetal extracts. In order to obtain vegetal extracts, 5g of each dry vegetable were minced, mixed well and then subdued to a solid-liquid extraction in a Soxhlet installation with ethanol 60%. Alcoholic extracts were evaporated to a volume of about 20 mL. Extractions and all analysis were performed in 2 replicates.

The annihilation of DPPH radicals. The ability of the alcoholic extract to annihilate the DPPH radicals (1,1-diphenil-2-picrylhydrazyl) was investigated by the method described by Sihamada and col [2]. Briefly, 0,1 mM solution $DPPH^{\cdot}$ in absolute ethanol was prepared. 1,5 ml solution was added to 50µl alcoholic extract, and the obtained mixtures were kept at room temperature for 30 minutes. Then, the absorption of the mixtures at 517 nm was determined, in comparison with the control solution (maxim absorption). The annihilation activity of free radicals was calculated in % inhibition according to the following relation:

$$\% \text{ Inhibition} = (A \text{ control} - A \text{ test}) \times 100 / A \text{ control}$$

The annihilation of superoxide anions. The superoxide anions generated by the phenazin methosulfate (PMN)/nicotinamid-adenin-dinucleotidphosphat, reduced form (NADPH) system, were 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) [3]. 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 (PMN) 1mM, 50 µl NADPH 0,5 mM and 50 µl alcoholic plant extract.

At the same time, control tests were prepared. The results were expressed in % inhibition.

The annihilation of hydroxyl radicals. For the investigation of the annihilation process of hydroxyl radicals, the modified method described by Halliwell and col. [4] was used. The mixture of reaction consisted of $FeCl_3$ (300 µmols) premixed with EDTA, 2-deoxyribose (2.8 mmols), ascorbic acid (300 µmols) and hydrogen peroxide prepared in buffer phosphates 50 mM pH 7,4. After adding alcoholic plant extract, the mixture was incubated for 1 hour at 37º C. After incubation, trichloroacetic acid 2.8 % and thiobarbituric acid 1% were added. Then, the tests were maintained on water bath at 100 ºC for 20 minutes and then the extinction was read at 532 nm. The obtained results were expressed in % inhibition.

The inhibition of the lipid peroxidation process. The effect of different concentrations of alcoholic extracts on lipid peroxidation process inducted by the Fe^{3+}/ascorbic acid system on the phospholipids from homogenate in rat brain was determined through the thiobarbituric-acid reactive substances (TBARS) method [6, 7]. This method is based on the reaction of the TBA with the malondialdehyde (MDA), the secondary product of the lipid peroxidation process. Rat brain homogenate 25% (w/v) was used. At 200 µl of suspension there were added 500 µl phosphates buffer pH 7,4, 50 µl alcoholic extracts, 100 µl solution $FeCl_3$ 1mM and 100 µl ascorbate 1mM. After incubating the mixtures at 37º C, for 60 minutes, the following were added: 50 µl butylated hydroxytoluen (BHT) 2%, 1 ml trichloracetic acid (ATA) 2,8% and 1 ml thiobarbituric acid (TBA) 1%. The mixtures that resulted from this process were maintained for 20 minutes at 80º C. The resulting colored complex was extracted in buthanol and then, the extinction was read at 535 nm.

The inhibition of peroxides formation was expressed in % inhibition.

Results and Discussions

The annihilation of DPPH radicals.

DPPH radicals react with suitable reducing agents losing color stoichometrically with the number of electrons consumed witch is measured spectrophotometrically at 517 nm. As shown in Fig. 1, alcoholic extract obtained from coriander fruits strongly scavenged DPPH radical, followed by mustard fruits and horseradish.

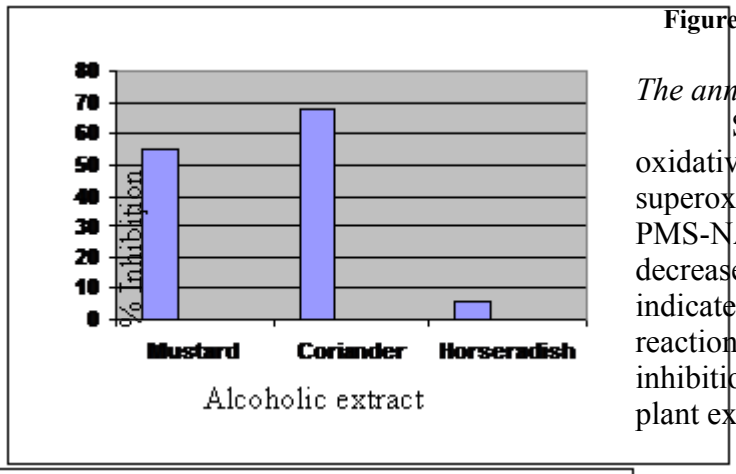

Figure 1. Effect of alcoholic extract plants on DPPH radicals

The annihilation of superoxide anions.

Superoxide is generated *in vivo* by several oxidative enzymes. In PMS-NADPH-NBT system, superoxide anion derived from dissolved oxygen by PMS-NADPH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicated the consumption of superoxide anion in the reaction mixture. Figure 2 illustrates the percentage inhibition superoxide radical generated by alcoholic plant extracts.

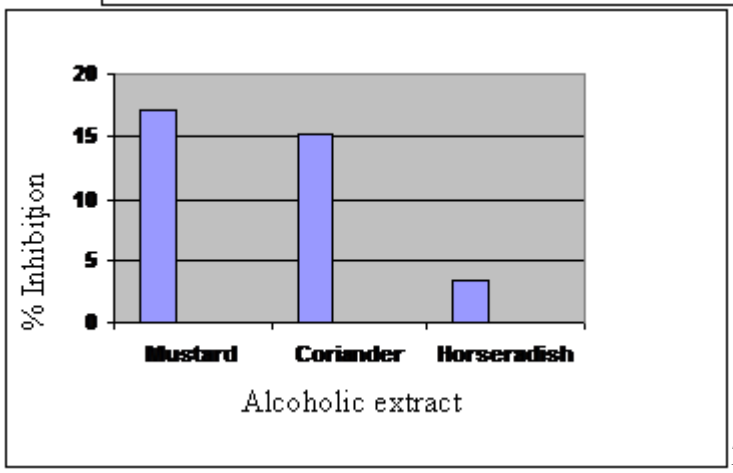

Figure 2. Effect of alcoholic extracts on superoxide anions

The annihilation of hydroxyl radicals.

The deoxyribose method evaluates the ability of hydroxyl radicals to damages carbohydrates. Highly reactive hydroxyl radicals ($HO^{\cdot}$) are generated by mixture of ascorbate and $FeCl_3$-EDTA at pH 7.4. Figure 3 shows the results of the deoxyribose damage by hydroxyl radicals, in the presence of alcoholic plant extracts studied. This attack is partially inhibited in the presence of the compounds tested. Mustard seeds exhibited the strongest protective action, 37.3%, coriander fruits 32.5% and horseradish 2.7%.

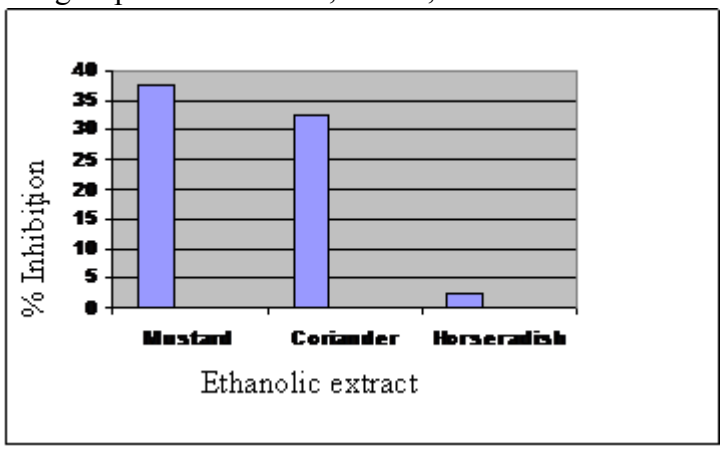

Figure 3. Effect of alcoholic extracts on hydroxyl radicals

The inhibition of the lipid peroxidation process. Brain unsaturated acyl-lipids undergo rapid non-enzymatic peroxidation when incubated with $FeCl_3$ and ascorbic acid at pH 7.4. At low concentration, ascorbate accelerates lipid peroxidation through its ability to reduce iron into the active ferrous state, while, at high concentrations, ascorbic acid inhibits lipid oxidation by inactivating free radicals [decker]. Figure 4 shows the inhibition of lipid peroxidation in the presence of mustard seeds, coriander fruits and horseradish alcoholic extracts. Mustard had the highest antioxidant activity (63.8%), followed by coriander fruits (55.7%) and horseradish roots (3.3%).

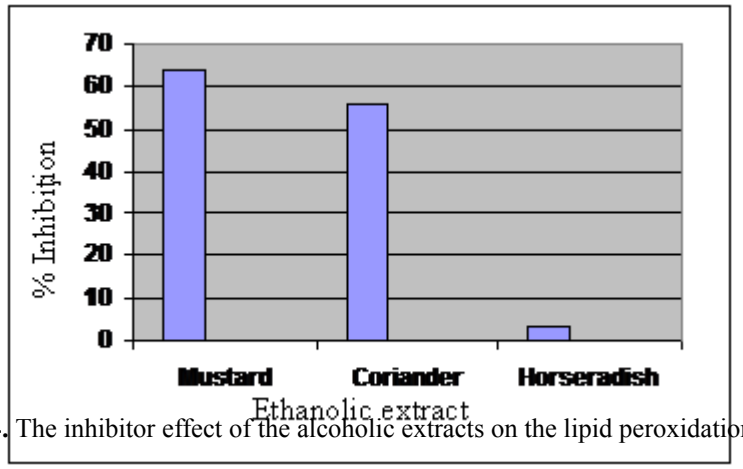

Figure 4. The inhibitor effect of the alcoholic extracts on the lipid peroxidation process from brain homogenate

The antioxidant activity shown by mustard seeds and coriander fruits in this study was as expected according to previous reports (Wangesteen, Huang). Low antioxidant activity of horseradish roots alcoholic extract is due of low levels of amount of total phenolics, reported by Kähkönen and col.

Conclusions

1. The alcoholic extracts of mustard seeds and coriander fruits scavenging DPPH radicals, superoxide anions and hydroxide radical.
2. The alcoholic extracts of mustard seeds and coriander fruits significantly reduced the malondialdehyde content, with is a measure of lipid peroxidation and show antioxidant activity.
3. Mustard seeds and coriander fruits extracts have potential as natural antioxidants in food.
4. The antioxidant activity of horseradish roots alcoholic extract was 15 to 20 times lower than mustard seeds and coriander fruits alcoholic extracts.

Acknowledgement

This research was supported by CNCSIS Research Project No. ID 256.

References

1. KÄHKÖNEN M. P., HOPIA A. I., VUORELA H. J., RAUHA J. P., PIHLAJA K., KUJALA T. S, *J. Agric. Food Chem, 1999, 47, 3954-3962*
2. SHIMADA K., FUJIKAWA K., NAKAMURA T.,*J. Agric. Food Chem.,* 1992, **40:945-948.**
3. WANG., S. Y., KUO Y. H., CHANG H. N., TSAY H. S., LIN K. F., YANG S. H., SHYUR L. F., 2002, J. Agric. Food Chem., **50,** p. 1859.
4. HALLIWELL B., GUTTERIDGE J. M., ARUOMA O.I., Anal. Biochem., 1987, **165**, p. 215
5. WANGENSTEEN H., SAMUELSEN A. B., MALTERUD K. E., *Food chemistry, 2004,* **88, 293-297.**
6. HUANG Z., WANG B., EAVES D. H., *Food Chemistry, 2007, 103, 1395-1402.*
7. DECKER E. A., XU Z., *Food Tech.* **52**: 54-59.