

## Study on herbal actions of horseradish (*Armoracia rusticana*)

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Received: 07 February 2013; Accepted: 09 March 2013

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### Abstract

Horseradish - a strong spice which was successfully applied in phytotherapy for its valuable effects on health. Although used for a long time in traditional medicine, its therapeutic properties had not been documented until recently, confirming in part the utilization of this vegetal product in indigenous medicine of different nations. The volatile oil, by its components and sinigrin transmits its revulsive, rubefacient, diuretic and antiseptic properties.

Horseradish consumption normalizes the arterial pressure and prevents the risk of blood clots formation. Simultaneously, sulfurous substances from horseradish enhance the elasticity of cerebral and coronary blood vessels, thus reducing the risk of an infarct or cerebrovascular accident.

The present article shows the investigation results of antioxidant potential in fresh, lyophilized and extract obtained by supercritical fluid extraction from horseradish. Fresh horseradish has the highest antioxidant capacity, followed by supercritical fluid extraction and lyophilized horseradish.

**Keywords:** horseradish, supercritical fluid extract, antioxidant capacity

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### 1. Introduction

Horseradish, a strong spice which was successfully applied in phytotherapy for its valuable effects on health, is little demanding regarding the environmental factors, being resistant to both low temperatures and long periods of drought. Perennial vegetable plant of *Brassicaceae* family, the horseradish is native to southeastern Europe and western Asia.[1]

Therapeutic properties of the horseradish had been recently documented, although it has been used in traditional medicine for a long time, confirming in part the use of this vegetable product in traditional medicine of different nations. Thus, due to the vitamins and especially vitamin C, it has antiscorbutic action. The volatile oil, by its components and sinigrin transmits its revulsive and rubefacient properties; the diuretic and antiseptic properties of horseradish are also due to these products, eliminated through the respiratory and renal systems.

Certain substances that horseradish contains have beneficial effects on peripheral blood flow. Horseradish consumption normalizes the blood pressure and prevents the risk of thrombosis. Also, sulfurous substances from horseradish improve the elasticity of cerebral and coronary blood vessels, therefore reducing the risk of an infarct or cerebrovascular accident.

Horseradish root is rich in vitamin C and B1, minerals (iron, potassium, calcium and magnesium), phytoncide and essential oils, a glycoside (sinigrin) which releases a volatile aglycone (allyl isothiocyanate) identical with the essence of mustard plant.[2] The pungent smell of the roots is due to allyl sulfide, a substance present in garlic and onion.

Likewise, compounds showing antimutagenic and / or anticarcinogenic activity were identified. Sinigrin, the main component present in horseradish and vegetables from *Brassicaceae* family, is degraded by the myrosinase enzyme complex to the allyl isothiocyanate which has antimicrobial effects

against *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium* și *Staphylococcus aureus*. [3].

Recent research has shown that hydrolyzed form sinigrin causes apoptosis in tumor cells in vitro and suppresses the formation of NO in macrophages. [4].

Some researchers have shown that derivatives of isothiocyanate type 2-thiohydantoin can form during cooking or even in the gastrointestinal tract, although myrosinase enzyme complex is thermally labile and is inactivated during cooking processes like boiling, steaming, baking. [5].

However, the use of high concentration of horseradish is limited by the strong organoleptic properties that can lead to serious side effects. [4]

Horseradish peroxidase is one of the most used peroxidase due to wide application in various fields such as analytical chemistry, environmental chemistry or clinical trials. The enzyme is used for many purposes and applications are found at reasonable prices.

Generally, the enzyme shows a number of features that make its use beneficial to the common catalysts, namely the ability to operate under conditions of mild reactions, as the processes are ecological in terms of environmental development. However there are a number of constraints in using the enzyme, being sensitive, unstable and having to be used in water, features that are ideal for a catalyst but undesirable in most syntheses. [6]

Great importance has to be granted to functional supplements based on horseradish used in cardiovascular diseases because cardiovascular diseases are the leading cause of death and disability worldwide, accounting for 17 million deaths each year. Globally, Romania stands in the first 4 places in terms of cardiovascular mortality.

In horseradish, seven isoenzymes were identified of horseradish peroxidase (HRP), among which the c isoenzyme of HRP (HRPc) is the most abundant and has been successfully isolated, purified and characterized [7]. It has a cardiogenic effect and is recommended to the people that suffer from high blood pressure.

Considering all these, the paper presents the results of our research related to the antioxidant activity of horseradish, which are part of a larger study that aims to obtain dietary supplements with anticholesterol and hypoglycemic action for people with cardiovascular diseases.

## 2. Materials and Methods

*Materials.* The horseradish used in the experiments was purchased from producers in Galați. It was further submitted to lyophilization process and supercritical fluid extraction. The equipment adapted for the experiment was designed, installed and operated by Natex Prozesstechnologie GmbH Ternitz, Austria.

*Analysis methods* performed on three samples of horseradish (fresh, lyophilized and extract) were as follows:

- determination of the water content according to the AOAC - 1995 method
- determination of polyphenols - Folin Ciocalteu method
- determination of flavonoids – spectrophotometric method, reference substances are rutin and quercetin
- determination of antioxidant capacity by DPPH method.

## 3. Results and discussion

Using horseradish for therapeutic purposes involves getting a horseradish extract in which the bioactive compounds remain in native state and in a concentration as large as possible.

Our research aims to establish a method to fulfill these conditions, knowing that any thermal processing would distort the biologically active principles.

Thus, after horseradish extract by supercritical fluids extraction (CO<sub>2</sub>) three categories of horseradish were studied: fresh horseradish (FH), lyophilized horseradish for supercritical fluid extraction (LyH) and horseradish extract (EH) in terms of antioxidant compounds content and antioxidant activity.

Phenolic compounds, the most important antioxidants, include two groups of substances which show strong antiradical action: flavonoids and phenolic acids, which are both present in horseradish.

The results obtained from the analysis of horseradish samples on flavonoid content are presented in Figure 1.

From Figure 1 it is noticeable that, depending on the condition of horseradish samples, the content of flavonoids increases except lyophilized sample, which indicates an even lower level than the fresh sample. According to research conducted by Abascal et al. (2005), who tested several methods of drying plants for preserving bioactive compounds, the use of microwave drying and lyophilization gives a greater stability to the antioxidants than other conventional methods. But in economic terms, lyophilization is not recommended, being an energy intensive technique.[8]

Horseradish extract obtained by supercritical fluid extraction showed the highest values for both flavonoids (rutine) and flavones (Quercetin). This was explained by Huang Z., et al. (2012) as the result of the mechanical action that the material undergoes before extraction (mincing) and of the higher surface extraction which is created this way [9].

These pretreatments promote releasing the solutes from cells, facilitating solvent flow through the packed bed, increasing substrate extraction quantity into units and improving the rate and yield of the extraction process. As a consequence, superficial horseradish cells may be considerably broken, rendering the free analyte to be extracted on the surface of the solid material.

The content of polyphenols in the samples followed the same trend as flavonoids (Fig. 2).

As it can be seen, in horseradish polyphenols are found more as tannic acid than gallic acid, the highest content being recorded in the case of horseradish extract obtained by supercritical fluid extraction.

The antioxidant capacity of horseradish samples, illustrated in Figure 3, shows a similar trend in antioxidant compounds content, higher values being recorded on the horseradish extract.

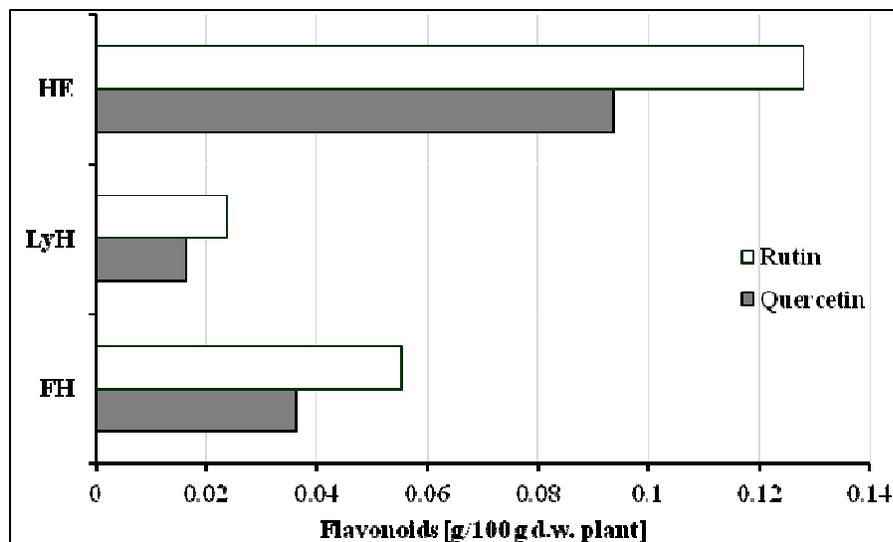


Figure 1. Evolution of flavonoids in the obtaining of horseradish extract  
 FH - fresh horseradish; LyH - lyophilized horseradish, HE - horseradish extract

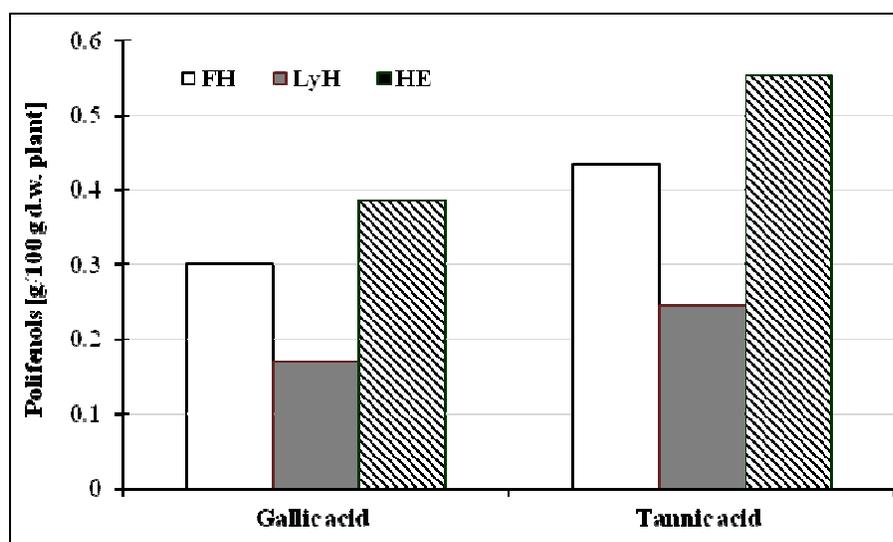


Figure 2. Evolution of polyphenols in the obtaining of horseradish extract  
 FH - fresh horseradish; LyH - lyophilized horseradish, HE - horseradish extract

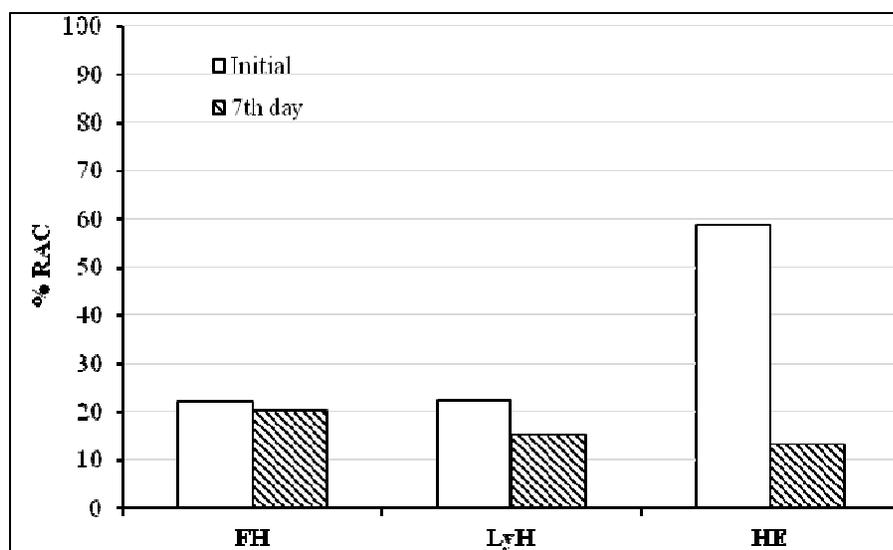


Figure 3. Evolution of antioxidant capacity in horseradish extract  
 FH - fresh horseradish; LyH - lyophilized horseradish, HE - horseradish extract

To test the perishability of the antioxidant capacity in horseradish, the samples were stored at 2-4°C for 1 week before being analyzed. It was found that antiradical power of horseradish decreased, the variation being 8.46% for fresh horseradish, 32.13% for lyophilized horseradish and 77.68% for horseradish extract. This drastic reduction in antioxidant capacity may be related to supercritical fluid extraction method, the data being consistent with those found in the specialty literature [10].

#### 4. Conclusion

Horseradish (*Armoracia rusticana*), a plant so common and yet so important to our health, using the most varied fresh or preserved as is customary romanian cuisine, herbal medicine is recommended for the treatment of many diseases and can replace successful drug treatment for bronchitis and other respiratory conditions.

This paper intended to highlight a class of bioactive polyphenols and flavonoids in horseradish (*Armoracia rusticana*) by qualitative and quantitative determinations and their antioxidant capacity.

Following the results we can conclude the following:

- increase flavonoid content, except lyophilized sample shows a lower even than the fresh sample;
- horseradish extract obtained by supercritical fluid extraction showed the highest values for both flavonoids (rutin) and the flavones (quercetin);
- polyphenols are found more than tannic acid, gallic acid form, the highest content was recorded in the case of horseradish extract obtained by supercritical fluid extraction;
- Horseradish antioxidant capacity of the samples shows a similar trend in antioxidant compounds content, higher values being recorded horseradish extract.

In addition, the storage stability of horseradish samples was very low. The minimum value observed with horseradish extract obtained by supercritical fluid extraction, within a week of its antioxidant capacity decreasing significantly. The results, however, allow us to seek other methods of obtaining a stable horseradish extract for use in therapeutic purposes.

#### Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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