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# ANTIOXIDANT PROPERTIES OF HORSERADISH (*ARMORACIA RUSTICANA*) – PILOT STUDIES

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**Abstract:** The aim of the study was to evaluate the antioxidant properties of horseradish extracts (Armoracia rusticana). The study's material consisted of wild, horseradish samples coming from three different regions of Poland (Mazowsze, Wielkopolska and Podlasie). Antioxidant activity was investigated using DPPH$^{\bullet}$ free radical, and the final result was converted into the equivalent of the antioxidant activity of vitamin C. Studies have shown that one of the samples had significantly higher antioxidant activity than the other two, whose antioxidant potential was similar from a statistical point of view. It was calculated that 1 g dry substance of horseradish samples had an antioxidant activity corresponding to 4.31 – 8.78 mg of vitamin C.

**Key words:** horseradish, extracts, antioxidant properties, DPPH$^{\bullet}$.

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## Introduction

Horseradish (*Armoracia rusticana*) is one of many plants belonging to a group of medicinal plants known from antiquity. Most probably it originated in the areas of northern Europe, and the area of Poland is listed as its natural environment [1]. Descriptions of the beneficial effects of horseradish on the human body can be found in older, e.g. Czarnowski [2] and newer [3] herbal guides. In the abovementioned publication from 1939, horseradish was characterized as an agent acting "excitingly and soothingly"which facilitates digestion after "using it moderately". It was recommended to stimulate the work of the stomach as a diuretic and treat scurvy, gout and water ascites. Rubbed into flakes or in the form of a spirit solution (as a wrap or otherwise used externally), it was recommended for the relief of rheumatic toothaches, headache, tinnitus, chest and stomach cramps, cruciate pain, freckles and spots (most likely bleaching– supposition of the Authors). Freshly squeezed horseradish juice could be a dietetic agent; added to milk, it was supposed to prevent its souring (e.g. during a storm) [2].

In a publication from 1987 [3], horseradish was still recommended as a good anti-scurvy agent due to the content of ascorbic acid in it (100 mg%). In addition, information was given that it contains a significant amount of B vitamins, carotene, calcium, potassium and phosphorus. It has already been known that it contains a sinigrin glycoside, which is responsible for the characteristic acute flavor characteristics. Even then, the bactericidal and fungicidal activity of the sinigrin degradation products was known. In addition, you can read that the horseradish stimulates the appetite and promotes digestion. Fresh horseradish juice and its aqueous solutions increase the secretion of hydrochloric acid in the stomach, which is why they cure gastritis caused by its achlorhydria. It is also a diuretic, healing water canker, urolithiasis and rheumatism; it can also be used for inflammation of the nerves, in back pain and in the waist (in the form of external compresses). With colds, you can cover the feet and toes with horseradish. Festering wounds, ulcers and ear inflammations are treated with mucus or infusion from the roots.

The information contained in popular literature and folk medicine experience increased over the centuries has been verified in recent years by the results of experiments. This also applies to horseradish. Already at the beginning of the twenty-first century, horseradish was included in the group of plants in which anticancer activity was examined and confirmed [4]. Hence, science has confirmed the beneficial effects of the ingredients contained in horseradish on the human body.

## Active compounds in horseradish

The active compound in horseradish is the aforementioned sinigrin belonging to a group of compounds called glucosinolate (glycoside subgroup).

These are sulfur compounds (thioglycosides), which contain a glucose molecule, an amino acid residue as well as sulfur [5,6]. Glucosinolates have been studied for several years because they have been shown to lower the risk of developing many types of cancer [7]. For example, they cause carcinogenic compounds to be bound by enzymes and removed

from the body via the respiratory tract, or they are excreted together with urine. In addition, glucosinolates have an effect on hormone modification. Therefore, it is possible to counteract the emergence of cancer of hormonal origin, an example of this is breast cancer [8].

Sinigrin occurs in cruciferous plants. Horseradish is one of the richest sources of this compound; hence, it is obtained industrially just from horseradish, which contains about 95.5 $\mu$mol/g of s.s. [7].

Sinigrin is a crystalline substance that decomposes in the presence of water under the influence of the enzyme mirosinase [9]. As a result of this decomposition, allyl isothiocyanate (allyl isothiocyantes) is formed [5,10]; phenylethyl isothiocyanate and others [10], are directly responsible for the sharp flavor characteristics. Mirosinase is released as a result of the plant structure disturbance [11] and during thermal processing as well as in the intestines.

Isothiocyanates, including allyl isothiocyanate, have chemopreventive properties [12]. The allyl isothiocyanate isolated from horseradish roots is characterized by strong antibacterial activity – it destroys fungal and bacterial pathogens on seeds, fresh products, bread, meat and cheese [10, 13]. This compound could be used as a natural antibiotic because it acts as an antibacterial against *Escherichia coli* O157: H7, *Listeria monocytogenes*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Staphylococcus sp.* as well as against lactic acid bacteria [10].

Horseradish roots are the main source of horseradish peroxidase enzyme, which is used for the production of enzyme immunoassays [14]. This enzyme is also a biosensor used for the determination of harmful substances in the environment such as phenols and aromatic amines. Horseradish peroxidase oxidized by hydrogen peroxide becomes a hydrogen donor, and then it can react with other compounds (e.g. with phenolic substances and aromatic amines), thus, constituting an indicator of their presence [15]. This enzyme is involved in redox reactions [16].

Despite many important functions, already confirmed, played by the active ingredients contained in horseradish, there is still little information about its antioxidant effects. The active antioxidant compounds are isothiocyanate, especially allyl- and phenyl-. These substances could be natural antioxidants of food. However, the obstacle is the acute flavor of isothiocyanates, [17] and they are most likely the reason that horseradish is rarely considered as a natural antioxidant in food production in the world.

Because in North-Eastern Europe the use of horseradish has a centuries-old tradition, and its use as a food additive is relatively common, perhaps using it as a natural antioxidant in Poland would be acceptable among consumers. Therefore, at the beginning, an attempt was made to determine the antioxidant power of extracts derived from horseradish. Wild horseradish was used for the study, and its antioxidant activity was compared to the antioxidant properties of vitamin C.

These studies should be treated as preliminary, but perhaps they will inspire further experiments leading to the use of horseradish extracts as a natural antioxidant in industrial food production.

## Material

The material for research was horseradish roots from various regions of Poland (Mazowsze, Wielkopolska and Podlasie). The horseradish came from private gardens and was a wild, common plant used by the owners as a spice. The roots were dug in September and analyzed in October-November. Horseradish samples were marked as 1, 2 and 3.

## Methods: Preparation of test samples

The individual horseradish roots were washed, peeled, cut and ground to a pulp in the type DJE241 Illico grinder (Moulinex producer). Samples prepared in this way were placed in glass vessels, frozen at -30°C, and then thawed in batches for analysis.

## Determination of antioxidant properties

From the crushed horseradish, a sample was prepared with three different masses: about 0.5 g, 1 g and 2 g with an accuracy of 0.0001 g. All samples were prepared in triplicate. A different mass of samples reduced the possible measurement error associated with different degrees of extraction of active compounds from the material. The weighed samples were placed in centrifuge tubes; into which methanol and deionized water were then added at a volume ratio of 1: 2, respectively. The tubes were placed in a HS501 digital shaker (producer IKA-WERKE) with a shaking number of 230/minute for 1 hour. Samples after shaking were placed in a High Speed Brushless Centrifuge MPW-350R centrifuge (producer MPW MED. Instruments) and centrifuged for 15 minutes at 4500 rpm.

After centrifugation, the supernatant was filtered through a filter paper directly into 50 $cm^3$ graduated flasks, and the rest of the volume of the flask was supplemented with deionized water (up to 50 $cm^3$).

Various dilutions were made from the prepared samples, containing a known volume of extract from a sample of known mass, and they reacted with a free radical DPPH$^\bullet$ (2,2-diphenyl-1-picryl-hydrase, Sigma-Aldrich Chemie GmbH, Germany). The reaction was carried out for 30 minutes in a dark place. The result of the reaction was determined spectrophotometrically using an EVOLUTION 220 UV Visible Spectrophotometer (producer Thermo SCIENTIFIC)

at wavelength $\lambda$=517 nm. The blank sample was DPPH$^\bullet$ solution with water.

### Comparison of antioxidant properties of horseradish extracts with vitamin C antioxidant activity

On the basis of the curves of the relationship between the amount of extract from the known mass of the sample (converted to dry substance), a reducing force (mass of reduced DPPH$^\bullet$) corresponding to 1 g of dry horseradish substance was calculated. Then the mass of the reduced DPPH$^\bullet$ was converted into the antioxidant equivalent of vitamin C according to the formula:

$$Vit.C[mg] = 4.6341x + 1,4059(R^2 0,9936),$$

where x is the reduced amount of DPPH$^\bullet$ [$\mu$g].
This formula was a modification of the dependence developed by Biller and Ekielski [18].

### Determination of the dry substance of horseradish

In all types of horseradish, the dry mass substance was determined in a Binder FD-53 laboratory dryer. The horseradish was used immediately after crushing. Drying was carried out to obtain a constant mass of samples. The dry substance of horseradish ranged from 13 ± 0.8 to 30 ± 0.7%.

### Statistical Analysis

The results were subjected to statistical analysis. A student's test was carried out (STATISTICA 10.0, StatSoft) to assess the significant differences between the antioxidant activity of individual horseradish samples from different regions of Poland.

### Results and discussion

Figures 1-3 present the relationship between the known amount of horseradish extract of known mass [mg] and the amount of reduced DPPH$^\bullet$ [$\mu$g]. Samples from different regions of Poland were called horseradish 1 (from Mazowsze), 2 (from Wielkopolska) and 3 (from Podlasie) respectively.

The horseradish samples tested had a different dry substance content, ranging from 13 ± 0.8 to 30 ± 0.7%. One of the samples had a significantly higher water content than the other two. The content of dry substance in horseradish was also determined by Agneta et al. (2014), and they received values ranging from 24.9 to 36.4%.

The reference to the antioxidant properties of the examined samples to the dry substance of the samples from which the extract was made enabled the comparison of the

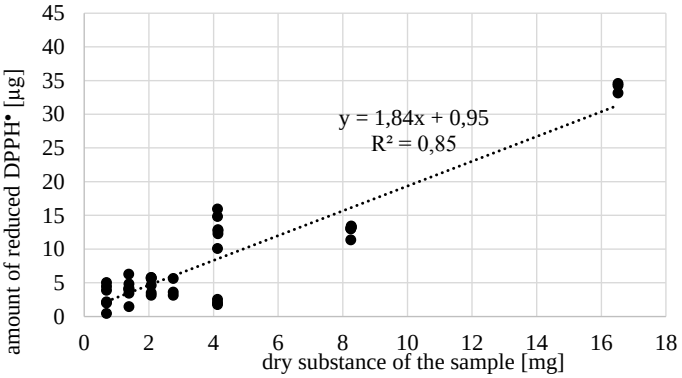


Fig. 1: The relationship between the known amount of extract from horseradish 1 (from Mazowsze) with known mass [mg of dry substance] and the amount of reduced DPPH$^\bullet$ [$\mu$g].

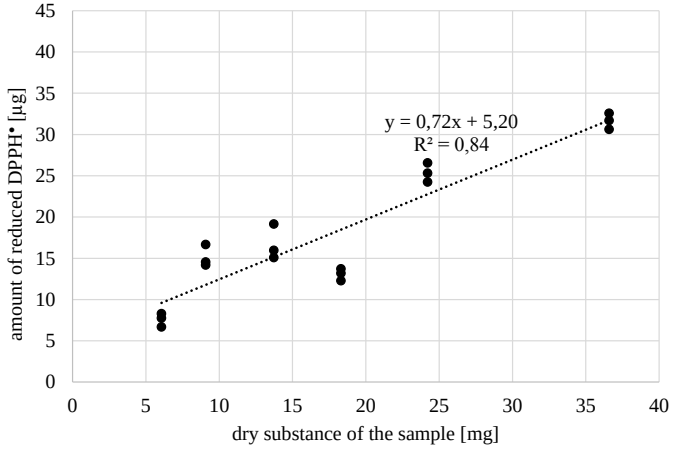


Fig. 2: The relationship between the known amount of extract from horseradish 2 (from Wielkopolska) with known mass [mg of dry substance] and the amount of reduced DPPH$^\bullet$ [$\mu$g].

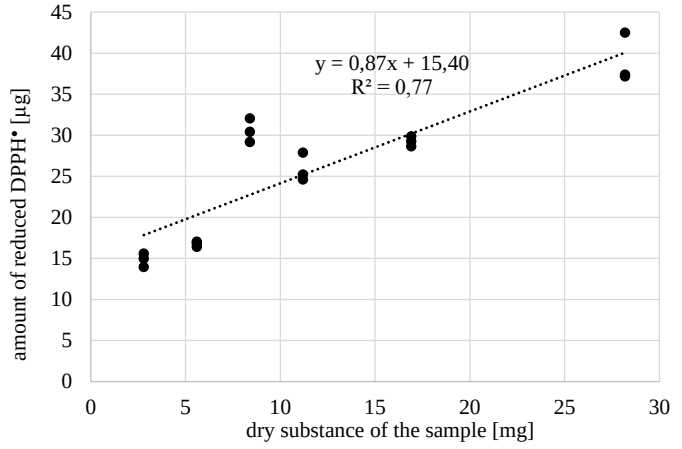


Fig. 3: The relationship between the known amount of extract from horseradish 3 (from Podlasie) with known mass [mg of dry substance] and the amount of reduced DPPH$^\bullet$ [$\mu$g].

antioxidant power between the examined roots. From the equations of the curves shown in Figs. 1-3, the antioxidant

properties of individual horseradish samples corresponding to 1 g of dry substance were calculated (Table 1).

Table 1: Antioxidant properties of horseradish per 1 g of dry substance.

| Number of the sample | Dry substance of the sample [g] | Amount of reduced DPPH• [µg] |
|---|---|---|
| **1** | 1 | 1840,25 ± 286 |
| **2** | | 730,79 ± 113,5 |
| **3** | | 890,29 ± 201,5 |

Using the values in Table 1 and the formula given in the methodology, one could express antioxidant activity of 1 g dry mass of individual horseradish samples as an equivalent of antioxidant properties of vitamin C (Fig. 4).

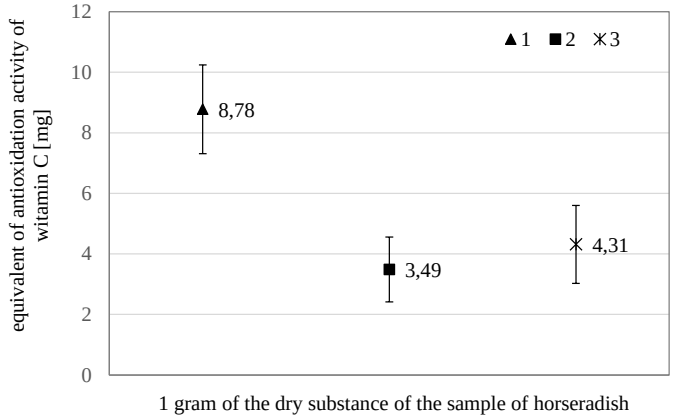


Fig. 4: Differences in antioxidant activity 1 g dry substance of horseradish test samples converted into the equivalent of antioxidant properties of vitamin C..

The experiment confirmed that all wild horseradish samples tested had antioxidant properties that could be expressed as an equivalent of the antioxidant power of vitamin C. These properties varied between samples, with test-t showing that horseradish marked as 1 (from Mazowieckie region) had significantly higher antioxidant activity than horseradish 2 and 3. Horseradish from the Wielkopolska and Podlasie regions showed similar antioxidant properties from the statistical point of view. This result was in line with the observations of other authors [19], who showed that the place of origin may affect the quality characteristics of horseradish. Agneta et al. [19] studied the genotypic diversity of horseradish growing in one of the regions of Italy and analyzed the effect of variety diversity on the content of glucosinolates. The research material consisted of various horseradish samples, which grew half wild in various villages of the Policoro region, then they were planted in an experimental field and subjected to research after a period of growth in the plantation. The authors confirmed that, depending on the genotype of plants, they contained different glucosinolate content and a different profile (glucosinolates are a precursor of isothiocyanins – active antioxidant compounds as previously stated). This means that the antioxidant differences of samples 1 – 3 could also be due to differences in their growth environment and the varieties that were used for the study. In Italy, most of the horseradish is also slow growing, as in Poland, and their varieties are not known, as in the case of this study.

Similar information on the relationship between glucosinolate content and the environment was provided by Szwejda-Grzybowska [5]. The following factors influence the glucosinolate content: species, variety, climate, soil conditions.

Differences in antioxidant activity in the tested samples could also come from the varying intensities of synagrynia to allyl isothiocyanate transformance under the influence of myrosinase [9], the activity of which could be different between particular plants.

Based on the obtained results, it was found that 1 g dry substance of horseradish had an antioxidant potential equal to 4.31 to 8.78 mg of vitamin C.

## Conclusions

The pilot studies carried out have confirmed that the horseradish extracts have antioxidant properties. Their antioxidant activity per 1 g of dry substance was equivalent to the antioxidant activity of 4.31 to 8.78 mg of vitamin C. One of the horseradish samples tested (from the Mazowieckie region) had a significantly higher antioxidant potential than the two other samples.

The results of the study showed that the potential of biologically active compounds contained in horseradish could be but is not fully used.

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