Phytochemical Compounds of Horseradish with Potential Application in Biotechnology

Magdalena Roșu, Andrea-Maria Malanca, Monica Dragomirescu

University of Life Sciences „King Mihai I" from Timisoara, Calea Aradului, 119, 300645, Timisoara, Romania

Abstract
Horseradish (*Armocia Rusticana*), is an edible plant from the *Basicacea* family. Horseradish is a very rich source of vitamins and minerals (vitamin C, vitamin B6, calcium, iron, magnesium, potassium), enzymes, isothiocyanates, glycosylates, flavonoids, pigments, oils, etc. Horseradish through its bioactive compounds provides antibacterial, anti-inflammatory and anti-carcinogenic properties. The biologically active compounds extracted from roots and leaves of horseradish can find applications in the food, pharmaceutical, chemical and agrochemical industries. Extraction from plant sources is a complex field involving various techniques to successfully obtain essential compounds and substances from live or dried plants. For the extraction of biologically active compounds, aqueous buffer systems are used (in a neutral, weakly basic environment - monosodium phosphate-disodium phosphate buffer solution, in an acidic environment citric acid-disodium phosphate buffer solution), organic solvents (tetrahydrofuran, benzene, methyl alcohol, etc.), eutectic solvents (ChCl: urea, ChCl: acetamide, ChCl: ethylene glycol, etc.), supercritical solvents (CO_2). Regardless of the solvent used, it must be nontoxic, biocompatible, biodegradable, and sustainable. The bioactive compounds from horseradish are versatile and are of particular importance in many industrial fields, but also in research.

Keywords: horseradish, phytochemical compounds, extraction, biotechnological applications

1. Introduction

Horseradish (*Armocia Rusticana*), is an edible plant from the *Basicacea* family. Horseradish is known for its strongly aromatic and spicy root, which makes this plant used for flavoring food in the cuisine of many peoples. Horseradish is rich in biologically active compounds with antioxidant properties such as polyphenols, flavonoids, vitamins, minerals and enzymes. Horseradish through its bioactive compounds provides antibacterial, anti-inflammatory and anti-carcinogenic properties. Thanks to these properties, horseradish is considered a healthy food, beneficial for health [1].
The biologically active compounds extracted from roots and leaves of horseradish can find applications in the food, pharmaceutical, chemical and agrochemical industries.
Extraction from plant sources is a complex field involving various techniques to successfully obtain essential compounds and substances from live or dried plants. The solvent used for extraction must be nontoxic, biocompatible, biodegradable, and sustainable [2].

2. Extraction of bioactive compounds from horseradish

Horseradish is known for its rich content of bioactive substances such as glucosinolates, sulfur compounds and antioxidants. The composition of the bioactive compounds in horseradish can vary depending on the species and the growing conditions [3].
In the root and leaf of horseradish a diversity of bioactive compounds can be find, such as

* Corresponding author: M. Dragomirescu
Email: mdragomirescu@animalsci-tm.ro

glucosinolates and their hydrolysis products represented in the largest proportion by isothiocyanates and thiocyanates, enzymes (peroxidase and myrosinase), vitamins - especially vitamin C and vitamin from the group B, fibers, phytonutrients, volatile oils, carbohydrates, proteins, etc [4].
To analyze the bioactive compounds in horseradish, it is necessary to extract them. The extraction process involves the separation of the bioactive compound of interest from the horseradish, both from the roots and from the leaves.
The extraction methods are carried out through different techniques, such as solvent extraction, supercritical carbon dioxide extraction, or other specific processes. After extraction the horseradish compounds are moved to the solvent, and the obtained extract is subsequently subjected to qualitative and quantitative analyzes to determine the chemical composition in specific bioactive compounds. Thus, it is possible to quantify the potentially beneficial compounds in horseradish and establish its biochemical profile from the point of view of its nutritional and medical properties [5].

Extraction methods can vary, the main extraction methods used are:
1. The classic extraction method in aqueous solution
The horseradish is ground and then mixed with water in a certain ratio of plant material: extraction medium. Depending on the compound of interest, specific pH and ionic strength buffer solutions, saline solutions, etc. can be used as aqueous extractive media. The bioactive compounds in horseradish are released into the solution under gentle stirring. Considering that some of the compounds are thermolabile, in order to avoid their degradation, the process is carried out at room temperature, avoiding high temperatures. To increase the extraction efficiency, the process can be assisted by ultrasound to fragment the plant cells and accelerate the diffusion of the compounds into the aqueous medium. The solution is then filtered or centrifuged at low temperature to separate the extraction medium containing the biologically active compounds from the solid residues. The concentration of aqueous extractive media can be done by lyophilization, this being a very suitable method for preserving the biological activity of thermolabile compounds [5-7].

2. Extraction in organic solvents
The ground plant material is treated with an organic solvent, such as chloroform, ethyl alcohol or methanol. The solvent extracts the bioactive compounds from the horseradish. Organic solvents are chosen based on the solubility of the compounds in these solvents. Organic solvent extraction can be a more efficient method to extract a wider range of compounds, including lipid-soluble or polar ones. This method requires extra care to avoid toxic solvents. The resulting solution is evaporated to obtain a concentrated form of the extracted compounds [6].
3. Extraction in green solvents
In recent years, the planet has been affected by a lot of factors, one of them being the pollution. In order to reduce pollution, more and more physical and chemical environmentally friendly methods have been used. In these methods classic organic solvents, toxic to humans and the environment, have been replaced with green solvents. [7].

The selection of a suitable solvent is done taking into account the safety of those working with that solvent, the safety of the process, the protection of the environment and the sustainability of the process. In order to be included in the green solvent category, the solvent must have a series of characteristics:

- Chemical and physical stability
- low volatility
- recyclability
- reusability

Taking into account these characteristics, green solvents are:

- water
- solvents based on organic salts - ionic liquids and deep eutectic solvents
- biosolvents - ethanol, glycerol, fatty acid methyl esters of vegetable oil, terpenes
- supercritical fluids (CO_2 and water) [8].

Eutectic solvents have been studied and used to achieve a more environmentally friendly, faster and economically efficient extraction of bioactive components from natural sources.
Eutectic solvents are mixtures whose boiling points are lower compared to the boiling points of the components from which they were

synthesized. These solvents fall into three categories, namely ionic liquids (IL), deep eutectic solvents (DES) and natural deep eutectic solvents (NaDES), each consisting of other components and having different properties [9].
The first category, i.e. IL can be synthesized by using an organic cation and an anion of either inorganic or organic nature (Figure 1.). The advantages of IL are: the solubilization, extraction capacity and also the fact that these solvents are recyclable. On the other hand, there are also some disadvantages, including their toxicity, but also the high costs for their production. [7]

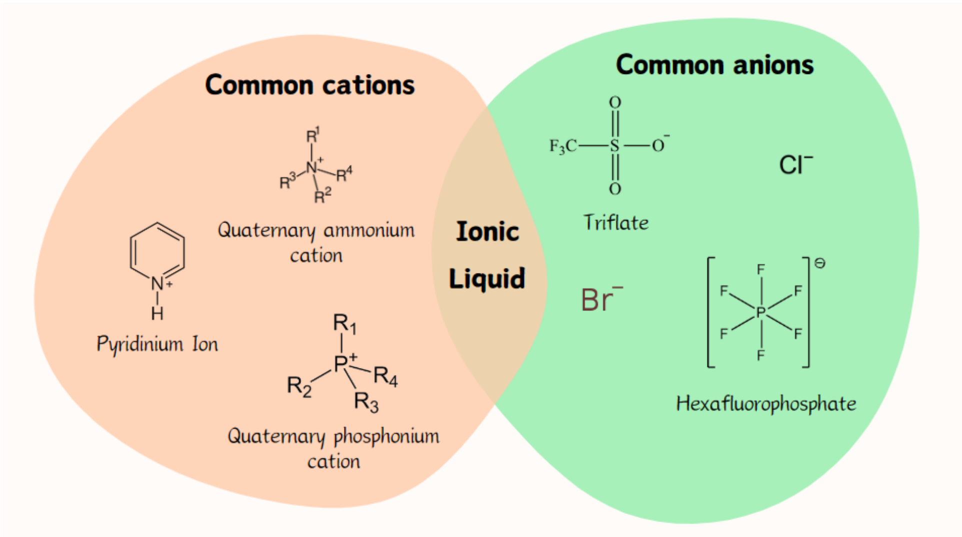

Figure 1. Synthesis of ionic liquids

Unlike ILs, DESs have only been obtained for a few years, via a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA) (Figure 2.). The advantages of DES are the ability of solubilization, recycling and extraction, ease of synthesis, biodegradability and low production costs. An important disadvantage in terms of environmental protection is the toxicity of some DES due to the content of metal salts. [7]

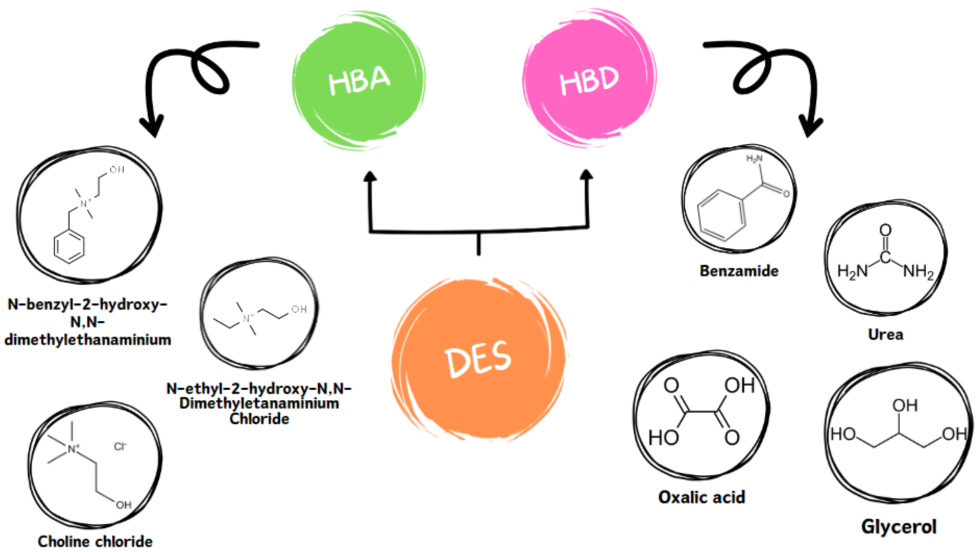

Figure 2. Synthesis of deep eutectic solvents

Natural deep eutectic solvents (NaDES) are obtained by using only natural components. Although NaDES are very useful for reducing the environmental impact, they have a major problem, they are difficult to remove from the extracted mixtures. This shortcoming can be solved by adding an anti-solvent, by crystallization, by solid-liquid and liquid-liquid extraction, by centrifugation so as to separate the molecules according to density, etc. (Figure 3.). [10].

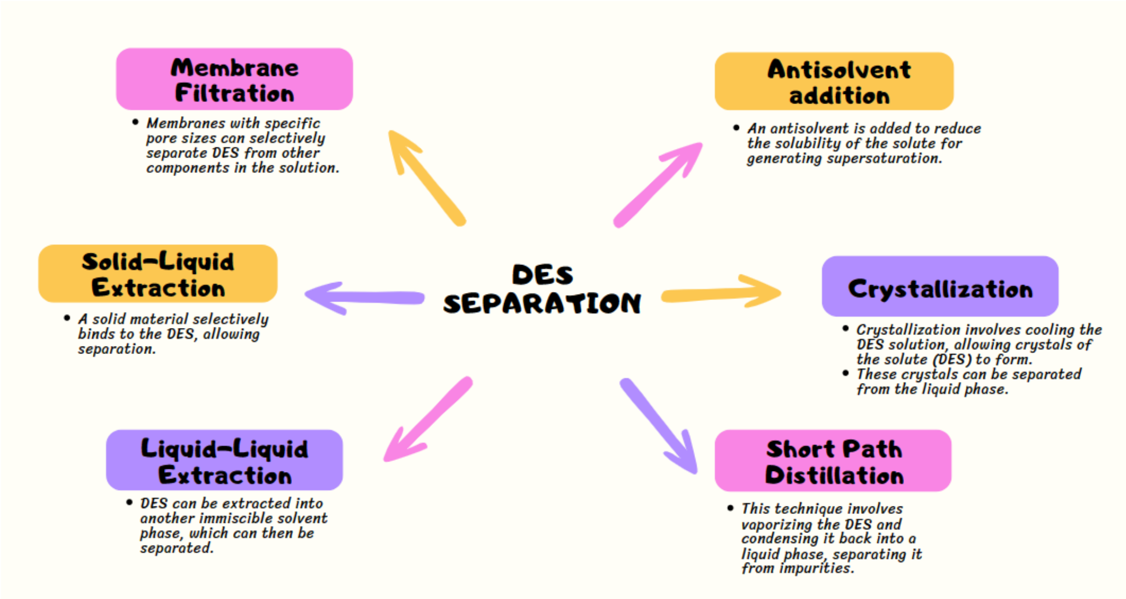

Figure 3. DES separation from extraction mixture

As previously mentioned, horseradish contains a high diversity of bioactive compounds important for their applications in biotechnology, and DES and ecological methods can be used to extract them. For example, an isothiocyanate found particularly in horseradish is allyl isothiocyanate (AITC), derived from sinigrin (GSL).

Rootstock extraction can be done by atmospheric solid-liquid extraction (ASLE) or hot pressurized liquid extraction (HPLE). ASLE is a common method used for the extraction of bioactive components from solid materials, at atmospheric pressure (101.3 kPa) and moderate temperature (40-80°C) and generally using protic solvents (ethanol, glycerol, isopropanol) or their aqueous mixtures to enhance polyphenol extraction [11]. HPLE is used for solid or semi-solid materials and consists of extraction at high temperature and pressure, with the aim of keeping the solvent in a liquid state [12].

In the case of horseradish, these methods could be used to extract horseradish peroxidase (HRP), an enzyme found in the roots of the horseradish plant [13].

Extraction from horseradish can be influenced by several factors that can affect the efficiency and quality of the extract. Among these factors can be mentioned the granulometry of the plant material, the nature of the solvent, the temperature and time of extraction, the pH at which the extraction is done (Table 1.) [4]. Optimizing these factors in the extraction process from horseradish can lead to obtaining an extract rich in enzymes and other bioactive compounds.

Table 1. The factors influencing extraction efficiency

The factors influencing the extraction	The way by which the extraction is influenced
The degree of grinding of the horseradish	Large contact surface
The solvent used for extraction	Increases the solubility of compounds
Temperature	It increases the solubility of compounds and the yield of extractives, the activity of enzymes
Duration of the extraction	The amount of bioactive substances extracted
pH	Solubility and selectivity of compounds, enzyme activity

3. The properties of bioactive compounds from horseradish and their biotechnological applications

Horseradish contains enzymes such as peroxidase and myrosinase, with potential health benefits. Studies have shown that these enzymes can have antimicrobial and antioxidant effects, helping to protect cells against oxidative stress. In addition, the enzymes in horseradish are involved in digestion processes, facilitating the breakdown of nutrients in the body. However, research continues to reveal in depth how these enzymes can influence human health and to explore potential medical or food applications 14, 15].

Horseradish peroxidase is an enzyme that plays an important role in the plant's physiological responses to various conditions, including oxidative and infectious stress. Peroxidase catalyzes oxidation-reduction reactions, using hydrogen peroxide as a substrate [16]. In horseradish, peroxidase is involved in defense mechanisms against pathogens. When the plant is exposed to microbial invasions, peroxidase helps generate reactive oxygen species that contribute to the destruction of the invading microorganisms [1].

Myrosinase plays a significant role in the generation of organosulfur compounds responsible for horseradish's characteristic aroma. This enzyme is involved in the breakdown of glucosinolates, such as sinigrin, present in horseradish cells, into volatile organic compounds. When horseradish cells are damaged or cut, myrosinase comes into contact with sinigrin and catalyzes its conversion to compounds such as allyl isothiocyanate, responsible for horseradish's pungent flavor. This process of enzyme activation and release of volatile compounds occurs in response to mechanical stress or damage to the plant. These bioactive substances make horseradish a valued food in gastronomy and traditional medicine [5].

Enzyme extraction from horseradish involves specific steps to obtain enzymes in an active form. Methods such as fine grinding and the addition of buffer solutions are used to maintain optimal conditions for the enzymes to function [2].

Isothiocyanates are responsible for the pungent taste and various beneficial health effects.

They have antibacterial and antioxidant properties, helping to maintain the immune system and reduce the risk of inflammatory conditions. Horseradish is often associated with benefits for the respiratory and circulatory systems, with potential in the prevention of cardiovascular disease [5].

Besides the bioactive compounds discussed previously, all other biomolecules, minerals and fibers have beneficial effects for health (Table 2.) [4].

Table 2. The properties of bioactive compounds from horseradish

Bioactive compounds	Role/properties
Glucosinolates	Antioxidants and anticancer
Vitamin C	Antioxidants
Enzymes	Antimicrobials and antioxidants
Fibers	Digestive activity
Potassium	Blood pressure regulation
Calcium	Bone health
Isothiocyanates	Spicy taste, antibacterial and antioxidant properties
Flavonoids	Cardiovascular and immune system
Volatile oils	Specific aroma, beneficial properties for health

6. Applications of horseradish extracts in the biotechnological industry

The bioactive compounds in horseradish have numerous biotechnological applications due to their unique properties [1, 5, 6].

In the **food industry** they can be integrated into spices, sauces or preserved products due to their strong flavor and potential health benefits. Also, the antimicrobial properties of isothiocyanates help preserve food by inhibiting the growth of pathogenic bacteria and fungi.

In **medicine**, horseradish extracts have been investigated for their possible anti-inflammatory, antioxidant and anti-carcinogenic effects, participating in the development of supplements or drugs. The antibacterial properties of horseradish compounds are being explored for the development of natural antibiotics that can be used in the treatment of bacterial infections resistant to conventional antibiotics. Due to its expectorant effects, horseradish is used in the formulation of cough syrups and other products intended to relieve respiratory symptoms.

In the **pharmaceutical** field horseradish extracts can play an important role due to its bioactive compounds with potential health benefits. Glucosinolates and isothiocyanates have demonstrated anticancer potential by inducing apoptosis (programmed cell death) and inhibiting the proliferation of cancer cells. Horseradish contains substances with anti-inflammatory properties, which makes it a candidate for the development of natural anti-inflammatory drugs. Due to the high content of vitamin C and other antioxidants, horseradish extracts are used for the development of dietary supplements and drugs aimed at combating oxidative stress.

In the **cosmetic industry**, horseradish extracts are used in the formulation of cosmetic products due to their antimicrobial and antioxidant properties, helping to fight acne and protect the skin against premature aging.

In the **agrochemical industry**, horseradish compounds are used in the formulation of natural biopesticides for the protection of agricultural crops against pests and diseases.

In the environment protection, horseradish isothiocyanates can be used in bioremediation for the detoxification of soils contaminated with heavy metals and other harmful chemicals, due to their ability to mobilize and degrade pollutants.

Conclusions

The bioactive compounds from horseradish are versatile and are of particular importance in many industrial fields, but also in research. Continued research and development in biotechnology has the potential to further expand the uses of these compounds in innovative solutions for health, agriculture and the environment.

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