Original article
DOI: 10.14529/food230310

METHODOLOGY FOR OBTAINING COMPLEX BIOACTIVE SUBSTENCES FROM PLANT RESOURCES

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Abstract. Natural biologically active substances obtained from various plant sources are highly valued for their low toxicity, wide range of sources and high yield of final components. It has been established that biologically active (antioxidant) properties are characterized by such biochemical substances as flavonoids, polyphenols, vitamins, especially carotenoids, and active polysaccharides. Rosemary contains significant concentrations of biologically active components, the most important of which are essential oils, carnosol, carnosic acid and rosemary acid. As a rule, there is a synergistic effect between different types of antioxidants. The antioxidant capacity of bioactive components after mixing can be significantly higher than that of the original antioxidants, and it is more effective, less energy-consuming, safer and less toxic. Rosemary leaf extracts can enhance their antioxidant activity when interacting with natural carotenoids. The purpose of this study was to design and analyze the optimized parameters for obtaining a complex of biologically active substances based on the principles of synergism of carrot vitamins and rosemary polyphenolic compounds. To confirm and comparatively analyze the bioactive properties of the obtained complex extracts, studies of antiradical activity, content of polyphenolic and flavonoid compounds were performed. The monoextracts based on 70 % ethanol as well as distilled water extract of rosemary were characterized by the highest DPPH activity and significant concentration of polyphenolic substances. The efficiency of complex plant ethanol extracts was established, the highest concentration of bioactive components and antioxidant activity in the range of 86.9–92.5 % was observed. As a result of this study, the feasibility of application of different extraction agents for the isolation of bioactive compounds from rosemary and carrots was scientifically substantiated, and bioactive components with proven antioxidant properties were developed.

***Keywords*:** bioactive components, polyphenols, antioxidant properties, rosemary leaves, extraction, synergistic effect

For citation: Merenkova S.P., Yan Huang, Wu Siyu, Chzhou Khenkhen. Methodology for obtaining complex bioactive substences from plant resources. *Bulletin of the South Ural State University. Ser. Food and Biotechnology*, 2023, vol. 11, no. 3, pp. 83–90. DOI: 10.14529/food230310

Научная статья
УДК 581.192+543.48
DOI: 10.14529/food230310

МЕТОДОЛОГИЯ ПОЛУЧЕНИЯ КОМПЛЕКСНЫХ БИОЛОГИЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ ИЗ РАСТИТЕЛЬНЫХ РЕСУРСОВ

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Аннотация. Натуральные биологически активные вещества, полученные из различных растительных источников, высоко ценятся за их низкую токсичность, широкий спектр источников и высокий выход конечных компонентов. Установлено, что биологически активными (антиоксидантными) свойствами характеризуются такие биохимические вещества, как флавоноиды, полифенолы, витамины, особенно каротиноиды, и активные полисахариды.

Розмарин содержит значительные концентрации биологически активных компонентов, наиболее важными из которых являются эфирные масла, карнозол, карнозиновая кислота и розмариновая кислота. Как правило, существует синергетический эффект между различными типами антиоксидантов. Антиоксидантная способность биоактивных компонентов после смешивания может быть значительно выше, чем у исходных антиоксидантов, и это более эффективно, менее энергозатратно, безопаснее и менее токсично. Экстракты листьев розмарина могут усиливать свою антиоксидантную активность при взаимодействии с природными каротиноидами. Целью данного исследования было разработать и проанализировать оптимизированные параметры для получения комплекса биологически активных веществ, основанного на принципах синергизма витаминов моркови и полифенольных соединений розмарина. Для подтверждения и сравнительного анализа биологически активных свойств полученных комплексных экстрактов были проведены исследования антирадикальной активности, содержания полифенольных и флавоноидных соединений. Моноэкстракты на основе 70 % этанола, а также водный экстракт розмарина характеризовались самой высокой DPPH активностью и значительной концентрацией полифенольных веществ. Установлена эффективность комплексных растительных этанольных экстрактов, наблюдалась наибольшая концентрация биоактивных компонентов и антиоксидантная активность в диапазоне 86,9–92,5 %. В результате этого исследования была научно обоснована целесообразность применения различных экстрагентов для выделения биологически активных соединений из розмарина и моркови.

Ключевые слова: биологически активные компоненты, полифенолы, антиоксидантные свойства, листья розмарина, экстракция, синергетический эффект

Для цитирования: Merenkova S.P., Yan Huang, Wu Siyu, Chzhou Khenkhen. Methodology for obtaining complex bioactive substences from plant resources // Вестник ЮУрГУ. Серия «Пищевые и биотехнологии». 2023. Т. 11, № 3. С. 83–90. DOI: 10.14529/food230310

Introduction

Natural bioactive compounds that are derived from various plants are highly praised for their low toxicity, wide range of sources, and huge yields. It has been established that biologically active (antioxidant) properties are characterized by such biochemical substances as flavonoids, polyphenols, vitamins, specially carotenoids, and active polysaccharides [1]. Different natural antioxidants have different antioxidant mechanisms, so there are different interactions among them, including additive, antagonistic and synergistic effects. Among them, the synergistic effect has attracted the most attention, because it can produce a variety of compound antioxidant. The mechanism of synergistic effects includes several types. There are many related studies on the synergy between polyphenols and vitamins and the synergy between flavonoids and polysaccharides [2].

Plant polyphenols refer to the general group of polyhydric phenolic compounds that widely exist in plants. They are secondary metabolites synthesized by plants during normal development or under stress conditions, In their structure, there is at least one aromatic ring with one or more hydroxyl groups. Polyphenolic compounds react with excess free radicals in the human body to generate relatively more stable phenolic oxygen free radicals, which can protect the human body from damage [3]. Currently, more than 8,000 polyphenols have been isolated and identified. In recent years, the active function of polyphenolic compounds has gradually attracted researcher's attention. With the discovery of biologically active functions such as slowing down the aging, preventing cardiovascular and cerebrovascular diseases, eliminating free radicals in the body, counteracting lipid oxidation, anti-radiation and anti-cancer protection, – the research and application of polyphenols have been paid more and more attention.

Rosemary is an evergreen, winter-hardy plant that can be grown in Mediterranean climates and often appears on people's tables as a spice. As a natural preservative and natural antioxidant, rosemary has played an important role in food preservation since ancient times. Rosemary contains significant concentrations of biologically active components, the most important of which are essential oils, carnosol, carnosic acid and rosmarinic acid [4]. Rosemary has been shown to be pharmacologically active and contains components that are potential for medical applications. Thus bioactive components of rosemary inhibit the development of malignant cells in tissues, melanoma cells and leukemia cells. Based on bioinformatics, rosemary can be used for biological targeting therapy of cancer, and the different molecular targets regulated by the active components of rosemary are useful indicators of success in clinical trials of cancer chemoprevention. The table 1 lists active ingredients of rosemary, their applications and advantages. Figure 1 shows the molecular structure of rosemary BAS.

There is generally a synergistic effect between the different types of antioxidants. According to a certain proportion, the antioxidant capacity of the bioactive substances after compounding can be significantly increased, and become more efficient, less energy-consuming, and less toxic. At present, researchers study the antioxidant synergy between vitamins and polyphenols; flavo-

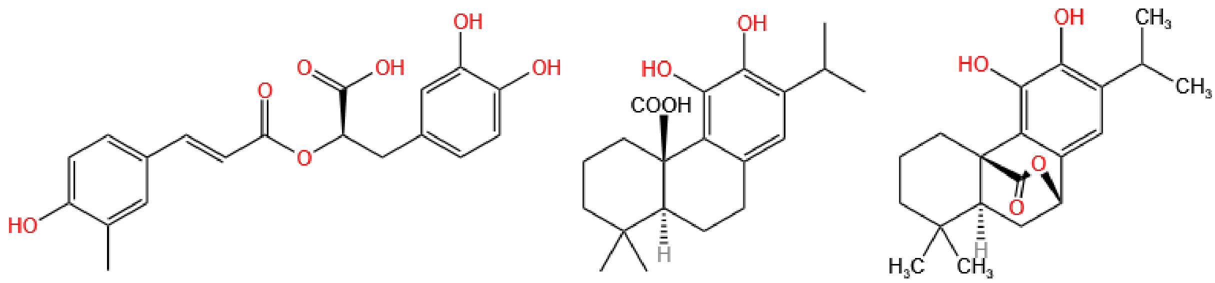

Fig. 1. Main bioactive substances of rosemary

Table 1

List of active ingredients of rosemary and their applications and advantages

Bioactive compounds	Direction of application	Beneficial effect	Reference
Carnosic acid, rosemic acid and carnosol	Antioxidant	Safe and efficient free radical scavengers can effectively prevent the oxidation and rancidity of oil and prevent the fading or discoloration caused by oxidation	[5]
2-pinene, camphor and 1,8-eucalypt hormone	Antimicrobial	Can inhibit the growth and survival of insects and eggs, trace and efficient	[6]
Carnosic acid caffeic acid, and Luteolin-7-O-glucuronide	Anti-depression	By regulating catecholamines, it can also reduce the toxic damage of corticosterone and protect nerve cells	[7]
Carnosic acid , carnosol	Regulation of metabolism	Rosemary extract improved plasma total cholesterol, LDL, and triglycerides in mice fed on a high-cholesterol diet, as well as decreased fasting plasma glucose, plasma total cholesterol, and free fatty acids	[8]
Luteolin, carnosic acid and rosmarinci acid	Anti-nerve injury	Can reduce the expression of Hsp 47 in nerve cells, thereby improving the damage degeneration of nerve cells in a stressful environment	[9]
Carnosic acid	Antiinflammatory	Lower the factors involved in inducing the development of inflammation, such as myeloperoxidase, interleukin 1- β, tumor necrosis factor- α, and reduced leukocytes	[10]
Carnosic acid、carnosol salviol	Antineoplastic	Tumor cell survival is affected by promoting cell apoptosis and inhibiting the phosphatidylinositol-3-kinase / protein kinase (PI3K / Akt) pathway	[11]

noids and polysaccharides. The biochemical reaction mechanisms of these various types of compound antioxidants have been clarified and have showed excellent efficiency. According to the antioxidant complex chain theory, multiple antioxidants can act in combination [12]. The authors' research has found that rosemary polyphenolic compounds obtained by extraction enhance their antioxidant properties when combined with vitamin compounds, especially carotenoids [13].

Both traditional and advanced extraction methods are used to extract bioactive components from plant sources. Polyphenol extraction schemes include ultrasonic-assisted extraction, microwave-assisted extraction, ultrasonic-microwave-assisted extraction, supercritical fluid extraction and others. Table 2 describes the extraction methods of bioactive compounds from plant raw materials.

Table 2

Extraction methods for obtaining plant bioactive substances

Extraction Method	Extraction principle	Benefit	Reference
Maceration	Conducted by soaking plant materials in solvent at room temperature to obtain plant extract.	Simple operation and low facility requirements	[14]
Percolation	Conducted by passing solvent through plant materials at controlled rate	Simple operation, continuous operation	[%]
Decoction	Boiling plant materials in water to obtain plant extracts.	Good separation effect and easy operation	[16]
Heat reflux extraction and Soxhlet extraction	Utilize reflux technique but with different apparatus to extract active compounds from plant materials.	The extract has high purity and is easy to operate	[17]
Ultrasound-assisted extraction	Utilizes cavitation caused by ultrasound effects in solvent to enhance the Extraction of active compounds from plant materials	Higher yields, shorter extraction times, less energy and solvent consumption	[18]
Supercritical fluid extraction	Supercritical fluid has strong penetrating power, high density and good solubility	Does not destroy active ingredients, no organic solvent residue	[19]
Microwave-assisted extraction	Microwave energy is absorbed by polar substances inside the cell, generating a lot of heat, which creates holes and cracks in the cell wall	Wide adaptability, low temperature, high extraction efficiency	[20]
Pressurized liquid extraction	In pressurized liquid extraction, the solvent is heated above its atmospheric boiling point and maintained at high pressure down so that they remain liquid. Use temperatures and pressures below the critical point of the solvent	Can dissolve more active substances, replacing organic solvents with water	[21]
Enzyme-assisted extraction	Some enzymes are able to hydrolyze plants cell wall to promote the release of active compounds from plant matrixes	Can be used to enhance the extraction of active compounds from plant material	[22]
High-voltage-assisted extraction	When pulses of high voltage at very short time are applied to cells, lipid bilayers of cell membrane are disrupted, forming temporary pores that significantly increase mass transfer between inside and outside of the cells	The ability to extract active compounds from plant material can be improved	[23]

Table 2 (End)

Extraction Method	Extraction principle	Benefit	Reference
Ionic liquids or deep eutectic solvents-based extraction	Solvent properties significantly affect the extraction of active compounds. Therefore, if the solvent properties can be tailored, will facilitate extraction	Solvent properties can be tailored which will facilitate extraction	[24]
Adsorption	Adsorption technique makes use of solid adsorbents to adsorb targeted active compounds in crude extracts to achieve the purpose of extraction and isolation	Consumption of organic solvents, extraction time, costs, waste generated can be reduced, and no pretreatment is required	[25]

The purpose of this study was to design and analyze the optimized parameters for obtaining a complex of biologically active substances based on the principles of synergism of carrot vitamins and rosemary polyphenolic compounds.

Materials and methods

Material of research was fresh rosemary (LLC "Green Paradise"), which was treated according to the protocol: wash the rosemary leaves, remove impurities and excess dust. Separate the rosemary leaves from the stems. Material of research was fresh carrots (*Daucus carota var. sativa Hoffm.*). According to the experimental plan, the carrots were treated as follows: wash the carrots and remove the indicated soil; crush the carrots and dry at 40 °C to a moisture content of 6 %.

Three types of solvent were used to prepare the extracts: distilled water; petroleum ether; and 70 % ethanol Each type of plant material was extracted separately in three types of solvents. Subsequently, the extracts obtained were mixed in a 1:1 ratio. A total of 8 samples of plant extracts were obtained, depending on the type of plant material (rosemary or carrot) and the type of solvent (distilled water, petroleum ether or ethanol) (Table 3).

Results and Discussion

To confirm and comparatively analyze the bioactive properties of the obtained complex extracts, studies of antiradical activity, content of polyphenolic and flavonoid compounds were performed. The results of the analysis are presented in Figures 2–4.

The monoextracts based on 70 % ethanol as well as distilled water extract of rosemary were characterized by the highest DPPH activity and significant concentration of polyphenolic sub-

Table 3

Composition of plant extracts samples

Group cod	Solution composition	Proportion of extracts
R-1	Rosemary Extract (distilled water)	1.0
R-2	Rosemary Extract (ethanol)	1.0
R-3	Rosemary Extract (petroleum ether)	1.0
C-1	Carrot Extract (petroleum ether)	1.0
C-2	Carrot Extract (ethanol)	1.0
RC-1 ethanol	Rosemary Extract (distilled water)+ Carrot Extract (ethanol)	1 : 1
RC-1 petrol	Rosemary Extract + Carrot Extract (Petroleum Ether)	1 : 1
RC-3 ethanol	Rosemary Extract + Carrot Extract (ethanol)	1 : 1

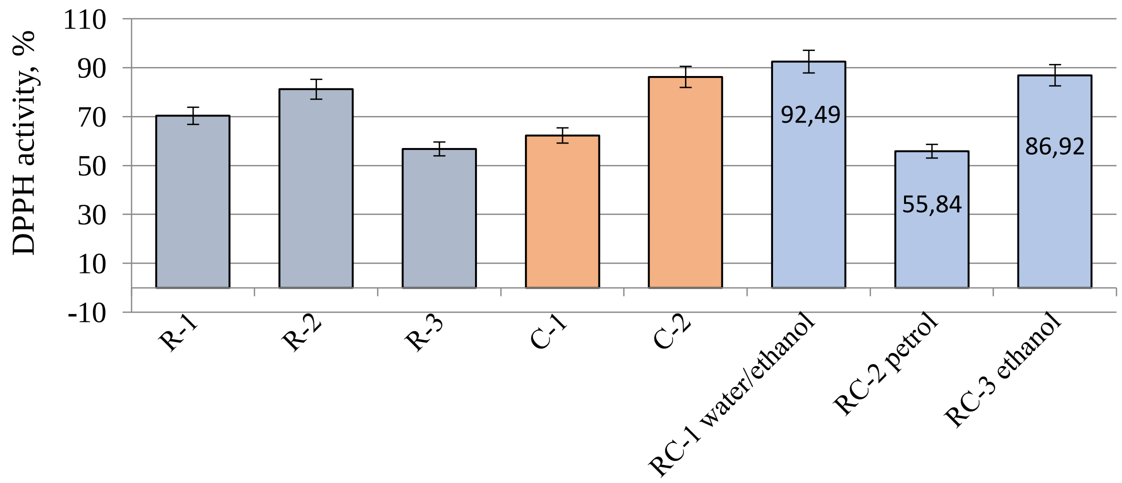

Fig. 2. Antiradical (DPPH) activity of bioactive substance extracts

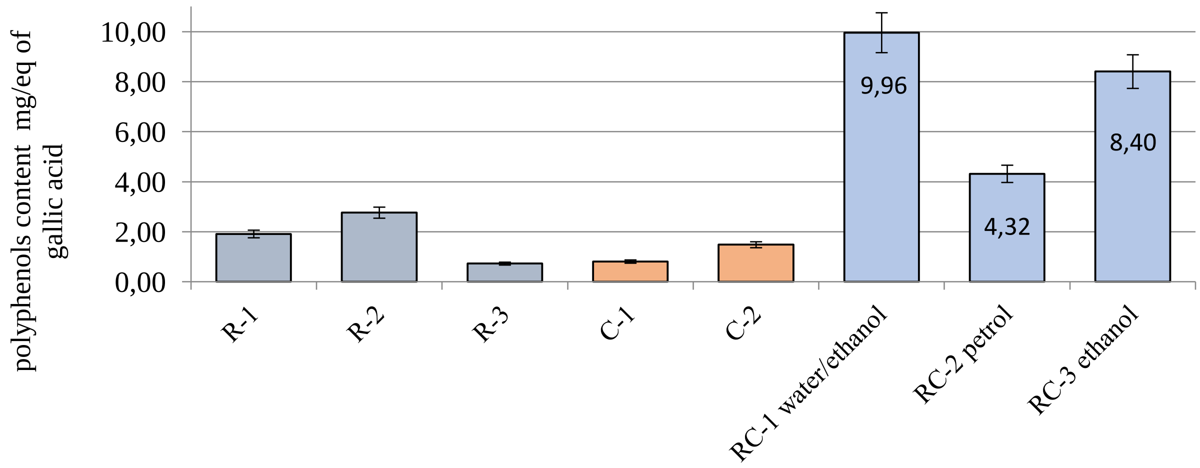

Fig. 3. Polyphenols content in bioactive substance extracts

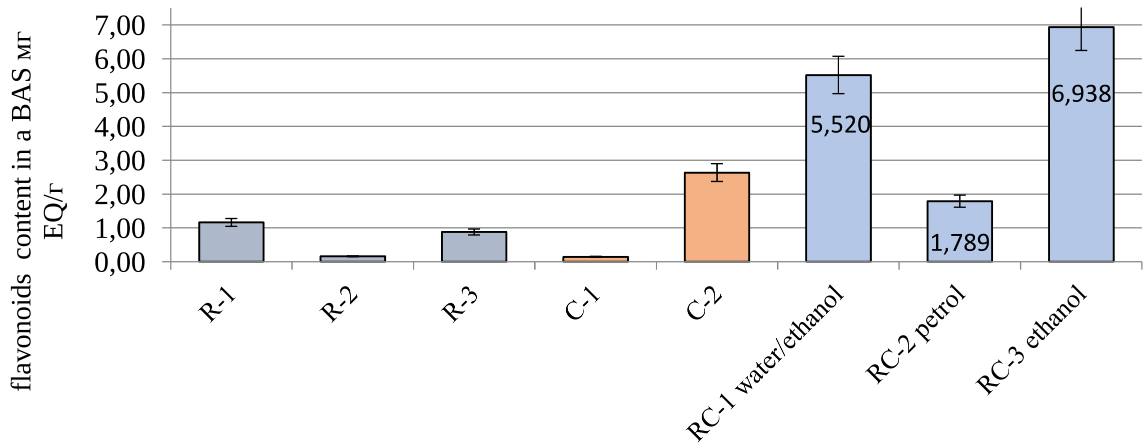

Fig. 4. Flavonoids content in bioactive substance extracts

stances. The efficiency of complex plant extracts based on distilled water and ethanol was established, the highest concentration of bioactive components and antioxidant activity in the range of 86.9–92.5 % was observed.

Conclusion

Bioactive components, that have been extracted from natural plant sources are characterized by pronounced bioactive and antioxidant properties, which depend on the extraction method. The biological activity of the plant substances is depending on the content of polyphenolic and vitamin compounds, as well as their synergistic interaction. Rosemary leaf extracts can enhance their antioxidant activity when interacting with natural carotenoids. As a result of this study, the feasibility of application of different extraction agents for the isolation of bioactive compounds from rosemary and carrots was scientifically substantiated, and bioactive components with proven antioxidant properties were developed.

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The article was submitted 14.04.2023
Статья поступила в редакцию 14.04.2023