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Original Article

Phytochemical Study of Odorous Celery Root (*Apium graveolens* L.) Grown in the North Caucasus

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ABSTRACT

Context: Flavoring celery (*Apium graveolens* L.) from the umbrella family (Apiaceae) is a common food plant, its use as a spice has been known since Ancient Greece. Stems, leaves and root are widely used in cooking in various dishes: soups, salads and side dishes. Celery fragrant has mild laxative, diuretic, antiseptic, anti-allergic, anti-inflammatory, enveloping and wound healing properties. **Aims:** The aim of our research was the phytochemical study of celery root odorous, quantitative definition of flavonoid, carotenoid, polysaccharides, tannin, free organic acids, essential oils and lipid. **Methods and Material:** The research material was dried and chopped celery fragrant root passing through a 3 mm sieve. We carried out quantitative determination of biologically active substances in the celery root odorous by the following methods: flavonoids (In terms of rutin) by the spectrophotometric method; carotenoids by the photocolorimetric method; essential oil, polysaccharides, lipophilic substances by the gravimetric method; free organic acids and tannins by the titrimetric method. **Statistical analysis used:** The results of the studies are given in the table. Statistical data processing was performed according to the method "Statistical processing of the results of a chemical experiment and biological tests" presented in State Pharmacopoeia XI [1]. The sample size (*n*) is 6, the confidence level (*P*) is 95%, the student criterion (*t* (*P*, f) is 2.57). **Results:** For the first time, we carried out a quantitative assessment of the content of seven groups of biologically active compounds of celery root odorous, grown in the North Caucasus: essential oil - 2.04 ± 0.01%, flavonoids - 1.44 ± 0.01,% lipophilic substances - 4, 05 ± 0.01%, carotenoids - 3.03 ± 0.02%, free organic acids - 2.38 ± 0.02%, tannins - 8.06 ± 0.01%, water-soluble polysaccharides - 8, 33 ± 0.01%, pectin substances - 3.45 ± 0.01%, hemicellulose A - 1.54 ± 0.01%, hemicellulose B - 1.47 ± 0.01%. **Conclusion:** Celery root odorous grown in the North Caucasus is a source of biologically active compounds.

Key words: Flavonoids, Tannins, Carotenoids, Essential oil, Free organic acids, Polysaccharides.

Key Messages: Celery root odorous grown in the North Caucasus is a source of biologically active compounds: essential oil - 2.04%, flavonoids in terms of rutin - 1.44%, lipophilic substances - 4.05%, carotenoids - 3.03 %, free organic acids - 2.38%, tannins - 8.06%, water-soluble polysaccharides - 8.33%, pectin substances - 3.45%, hemicellulose A - 1.54%, hemicellulose B - 1, 47%.

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INTRODUCTION

Flavoring celery (*Apium graveolens* L.) from the umbrella family (Apiaceae) is a common food plant, its use as a spice has been known since Ancient Greece (**Figure 1**). Stems, leaves and root are widely used in cooking in various dishes: soups, salads and side dishes. Celery fragrant has mild laxative, diuretic, antiseptic, blood-purifying, anti-allergic, anti-inflammatory, enveloping and wound-healing properties.

In scientific medicine, it is practically not used. But in folk medicine, he has long found wide application. It is applied with diseases of the kidneys, bladder, gout, gastritis, gastric ulcer and duodenal gout, diabetes, obesity, dermatitis, rheumatism, nephritis and inflammation of the prostate gland. The active ingredients of celery fragrant are: essential oil, flavonoids, furanocoumarin, acids (Ascorbic, oxalic, organic, glutamine), mineral salts, vitamins B1, B2, PP, A, B6, B9, E, K.

During the phytochemical study of the water extraction of the aerial part of celery fragrant, the content of flavonoids, coumarins, phenolcarboxylic acids, tannins, organic acids, polysaccharides was determined.[1]

The aim of our research was the phytochemical study of celery root odorous, quantitative definition: flavonoids, carotenoids, polysaccharides, tannins, free organic acids, essential oils and lipids.

MATERIALS AND METHODS

The research material was dried and chopped celery fragrant root passing through a 3 mm sieve.

The quantitative determination of the essential oil was carried out according to the State Pharmacopoeia of the 11th edition by the method 1.[1,2] A portion of crushed root of odorous celery, (About 25

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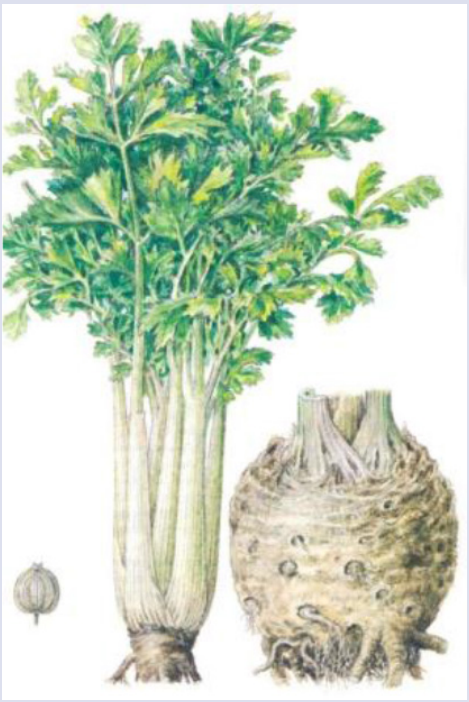

Figure 1: *Apium graveolens* L.

g) was placed in a 1000 ml round-bottomed flask. We added 300 ml of water and hung a graduated receiver with a thin wire on it. The flask with its content was heated and boiled for 2 h. After the end of the distillation and cooling, the volume of the settled layer of essential oil was measured and its percentage was calculated in terms of absolutely dry raw materials using the formula:

$$X = \frac{V.100fter}{m(100er}$$

where **V** is the volume of the essential oil in milliliters; **m** is the mass of raw material in grams; **W** is the mass loss during the drying of raw materials in percent.

The quantitative determination of flavonoids was carried out according to the method developed for arnica flower.[3]

An analytical sample of the raw material (About 1 g) of crushed root of odorous celery was placed in a conical flask with a volume of 200 ml volume; 100 ml of 70% alcohol was added and weighed (With an error of ± 0.01). The flask was attached to a reflux condenser and heated in a boiling water bath for 30 min. Then the flask was cooled at room temperature, weighed again and if necessary, 70% alcohol was added to get the original weight. This mixture is filtered through a paper filter in a flask of 100 ml volume, discarding the first 20 ml of filtrate (Solution A). 1 ml of solution A is placed in a measuring flask of 25 ml volume, 5 ml of 2% alcohol solution of aluminum chloride are added and the volume of the solution is brought to the mark by adding 95% alcohol. After 30 min, the optical density of the solution was measured on a spectrophotometer at a wavelength of 410 nm in a cuvette with a layer thickness of 10 mm. A solution consisting of 1 ml of solution A and 0.1 ml of a solution of concentrated acetic acid, brought to the mark with 95% alcohol in a 25 ml volumetric flask, was used as a reference solution. In parallel, the optical density of the solution of the State standard sample of rutin in 95% alcohol was measured. The content of the sum of flavonoids in terms of rutin and absolutely dry raw materials in percent was calculated by the formula

$$X = \frac{D.mond\ absol}{Dond\ absolut}$$

Where D is the optical density of the test solution; D0 is the optical density of the solution of the State standard sample of rutin; m is the mass of raw material in grams; m0 is the mass of the State standard sample of rutin in grams; W is the mass loss during the drying of raw materials in percent.

Determination of the content of lipophilic substances was carried out according to the method developed for seed of Siberian cedar pine.[3]

About 10 g (Exact weight) of crushed odorous celery root was ground in a mortar, quantitatively transferred from 100 ml of hexane to a 200 ml round bottom flask and heated under reflux in a water bath at about 60°C for 15 min. The solution was filtered into a dry weighed flask for distillation with a capacity of 250 ml through a filter paper. To the residue in the flask was added 100 ml of hexane, extracted and filtered through the same filter into a flask for distillation. The filter was washed with 15 ml of hexane. The combined extracts were evaporated under vacuum at a temperature of about 60°C until complete removal of the solvent, the flask was cooled to room temperature, closed with a stopper and weighed.

The content of lipophilic substances in the celery root odorous in% was calculated by the formula

$$X = \frac{(m2 - m0).100\%}{m1}$$

Where m0 is the mass of the empty flask for the distillate, in grams; m1 is the weight of seeds in grams; m2 is the mass of the flask with content, in grams.

Quantitative determination of carotenoids was carried out according to the method developed for the fruits of the sea buckthorn.[3]

About 1 g of crushed odorous celery root (Exact weight) was ground in a porcelain mortar with a small amount of anhydrous sodium sulfate until a homogeneous mass. The contents of the mortar were quantitatively transferred to a conical flask with a capacity of 250 ml, then 50 ml of petroleum ether were added and infused for 30 min with occasional stirring. The resulting extract was carefully decanted into another flask with a capacity of 250 ml. The extraction volume in a volumetric flask was adjusted to the mark by adding a solvent. 100 ml of the obtained extract was transferred to a round-bottom flask and evaporated in a water bath at 40–500°C under vacuum to a volume of 20 ml, quantitatively transferred to a 25 ml volumetric flask, the solution was brought to the mark by adding a solvent and stirred. The optical density of the obtained solution was measured on a photoelectric colorimeter at a wavelength of about 450 nm (Blue filter) in a cell with a layer thickness of 5 mm. Petroleum ether was used as a reference solution.

At the same time, the optical density of a standard solution of potassium bichromat is measured under the same conditions.

The content of the sum of carotenoids in terms of β-carotene in raw materials in mg% is calculated by the formula.

$$X = \frac{D.0,00208.250.100}{D0.m.25}$$

Where D is the optical density of the test solution; D0 is the optical density of a potassium bichromate standard solution; 0,00208 - the amount of

β-carotene corresponding to 1 ml of a standard solution of potassium bichromate in mg; m - the mass of raw materials in grams.

Quantitative determination of the content of free organic acids was carried out according to the State Pharmacopoeia of the 11th edition.[4]

25 g (Exact weight) of odorous celery crushed root was placed in a flask with a capacity of 250 ml, poured 200 ml of water and kept for 2 h in a boiling water bath, then cooled, quantitatively transferred into a measuring flask with a capacity of 250 ml, then brought the volume of water extraction to the mark and regularly was stirred. 10 ml of the extraction was taken, placed in a flask with a capacity of 500 ml, then added 200-300 ml of freshly boiled water and 1 ml of 1% alcohol solution of phenolphthalein and 2 ml of 0.1% methylene blue solution and titrated the mixture with caustic soda solution (0.1 mol / l) until there is lilac-red color the foam appeared.

The content of free organic acids in terms of malic acid in absolutely dry raw materials in percent was calculated by the formula:

$$X = \frac{V \cdot 0{,}0067 \cdot 250 \cdot 100 \cdot 100}{m \cdot 10 \cdot (100 - W)},$$

where 0.0067 is the amount of malic acid, average 1 ml of sodium hydroxide solution (0.1 mol / l), in grams; V is the volume of caustic soda solution (0.1 mol / l), followed by titration, in milliliters; m is the mass of raw materials in grams; W is the mass loss during the drying of raw materials in percent.

Quantitative determination of the content of tannins according to the State Pharmacopoeia of the 11th edition.[4] About 2 g (Exact weight) of odorous celery crushed root, sieved through a sieve with 3 mm diameter holes, placed in a 500 ml conical flask, poured 250 ml of boiled water and boiled under reflux on an electric hotplate with a closed helix for 30 min with periodic stirring. The resulting liquid was cooled to room temperature and filtered through cotton wool into a 250 ml volumetric flask so that raw material particles did not fall into the flask and made up to the mark with adding water. Then, a pipette of 25 ml of an aqueous oil solution, 250 ml was taken, 500 ml of water, 25 ml of indigo sulfonic acid solution were added and titrated with constant stirring of 0.02 M potassium permanganate solution until golden yellow. Then we take 25 ml of the obtained water extract with the pipette and added it into another 750 ml conical flask, the added 500 ml of water, 25 ml of indigo sulfonic acid solution and titrated with constant stirring with 0.02 M potassium permanganate solution until golden yellow color.

In parallel, a control experiment was carried out: 525 ml of water, 25 ml of indigo sulfonic acid solution were added to a 750 ml conical flask and was titrated with constant stirring by 0.02 M potassium permanganate solution until golden yellow color.

1 ml of 0.02 M solution of potassium permanganate corresponds to 0.004157 g of tannins in terms of tannin.

The content of tannins (X) in percent in terms of tannin and absolutely dry raw materials was calculated by the formula.

$$= \frac{(V - V1).0{,}004157.250.100.100}{m.25.(100 - W)}$$

Where V is the volume of a 0.02 M solution of potassium permanganate consumed for titration of a single extract, in milliliters; V1 is a volume of 0.02 M potassium permanganate solution consumed for titration in the control experiment, in milliliters; 0.004157 - the amount of tannins corresponding to 1 ml of 0.02 M potassium permanganate solution (In terms of tannin), in grams; m - weight of raw material or medicinal plant preparation, in grams; W - weight loss during drying of raw materials in percent; 250 - the total volume of water extraction, in milliliters; 25 is the volume of water extraction, taken for titration, in milliliters.

The quantitative determination of polysaccharides was performed by a gravimetric method.[5]

10 g (Exact weight) of odorous celery crushed root was poured with 200 ml of purified water and extracted with stirring for 1.5 h at 20°C. The extraction was repeated and the raw material with the extractant was left for 12 h. Then it was filtered. Extraction was dialyzed through a semi-permeable membrane and Water-soluble Polysaccharides (WSP) were precipitated with a double volume of 96% ethyl alcohol. The solution was decanted, the precipitate was centrifuged at a speed of 5000 rpm for 30 min. The precipitate was dried in a vacuum dryer, the residue was weighed. The precipitate was a greenish-gray amorphous powder consisting of water-soluble polysaccharides.

The rest of the raw material was treated with 50 ml of boiling water with the addition of 0.2 ml of concentrated chloride. Then it was heated in a water bath for 30 min with a reverse freezer and treated in the same manner as WSP. At the same time, a precipitate of Pectic Substances (PS), representing a light-gray, bleaching powder, was obtained.

Then the residue of the raw material was extracted with 50 ml of a 10% aqueous solution of sodium hydroxide at 20°C, with stirring on a magnetic stirrer for 12 h. Then we added 20 ml of 50% acetic acid to precipitate hemicellulose A (HC A). To precipitate hemicellulose B (HC B), a twofold volume of 96% ethyl alcohol was added to the filtrate. The solution was decanted, the precipitate was washed several times with portions of 20 ml of 96% ethyl alcohol.

The obtained precipitates of HC A and HC B were dried in a vacuum drying apparatus to constant weight and were weighed. HC A is a grayish-brown crystalline powder and HC B is a yellowish-gray amorphous powder.

RESULTS

For the first time, we carried out a quantitative assessment of the content of biologically active substances of celery root odorous, grown in the North Caucasus (**Table 1**). The results of the studies are given in the table. Statistical data processing was performed according to the method "Statistical processing of the results of a chemical experiment and

Table 1: Quantitative content of biologically active substances in the celery root odorous, grown in the North Caucasus.

Biologically active compounds	$\bar{x}$, %	Metrological characteristics			
		S	Sx	ΔX	ε, %
Essential oil	2,0383	0,000137	0,0048	0,0123	0,60
Flavonoids in terms of rutin	1,4400	0,000080	0,0037	0,0095	0,66
Lipophilic substances	4,0542	0,000082	0,0037	0,0095	0,23
Carotenoids	3,0295	0,000389	0,0081	0,0208	0,69
Free Organic Acids	2,3800	0,000440	0,0086	0,0221	0,93
Tannins	8,0633	0,000041	0,0026	0,0067	0,08
Polysaccharides:					
WSP	8,3283	0,000057	0,0031	0,0079	0,09
PC	3,4483	0,000137	0,0048	0,0123	0,36
HCA	1,5367	0,000147	0,0049	0,0126	0,82
HCB	1,4667	0,000147	0,0049	0,0126	0,86

biological tests" presented in State Pharmacopoeia XI.[4] The sample size (n) is 6, the confidence level (P) is 95%, the student criterion (t (P, f) is 2.57.

DISCUSSION

Celery root odorous grown in the North Caucasus is a source of biologically active compounds: essential oil - 2.04%, flavonoids in terms of rutin - 1.44%, lipophilic substances - 4.05%, carotenoids - 3.03 %, free organic acids - 2.38%, tannins - 8.06%, water-soluble polysaccharides - 8.33%, pectin substances - 3.45%, hemicellulose A - 1.54%, hemicellulose B - 1, 47%.

CONCLUSION

Celery root odorous grown in the North Caucasus is not only a food aromatic plant, but also a valuable source of a complex of biologically active compounds.

CONFLICT OF INTEREST

The author declares no conflict of interest.

ABBREVIATIONS

WSP: Water-soluble polysaccharides; **PC:** Pectin substances; **HCA:** Hemicellulose A; **HCB:** Hemicellulose B.

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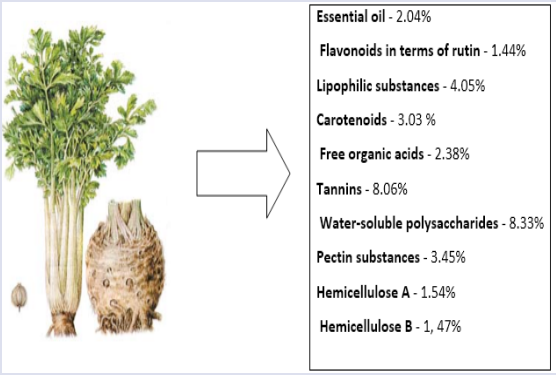
GRAPHICAL ABSTRACT



SUMMARY

- Celery root odorous grown in the North Caucasus is a source of biologically active compounds: essential oil - 2.04%, flavonoids in terms of rutin - 1.44, lipophilic substances - 4.05%, carotenoids - 3.03 %, free organic acids - 2.38%, tannins - 8.06%, water-soluble polysaccharides - 8.33%, pectin substances - 3.45%, hemicellulose A - 1.54%, hemicellulose B - 1, 47%.

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