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The Pharma Innovation

ISSN: 2277- 7695
TPI 2017; 6(2): 15-17

www.thepharmajournal.com
Received: 04-12-2016
Accepted: 05-01-2017

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Study of nettle juice stability during storage

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Abstract
The nettle juice stability during storage in dark glass bottles at 2-4 °C for 27 months was studied. Deviations of organoleptic (color, odor), physical and chemical (density, pH, dry residue, identification and quantification of hydroxycinnamic acids, carotenoids and chlorophyll) parameters were within acceptable standards. Accordingly, the juice shelf life in these conditions was established – 2 years.

Keywords: Nettle juice, physical and chemical studies, shelf life

1. Introduction
Stinging nettle (*Urtica dioica* L., Urticaceae) is a plant that is widely used in cosmetics and medicines intended to stimulate hair growth. Leaves and aerial part contains organic (formic, ascorbic, pantothenic) and hydroxycinnamic (chlorogenic, caffeic) acid, carotenoids, chlorophyll, flavonoids, organically bound silicon, etc. [1]. Biologically active substances (BAS) of nettle improve blood circulation in the skin capillary system, stimulate metabolism and trophic processes, cause regenerating and growth stimulating properties of hair follicle cells [2].
In order to create the new medical cosmetic remedies, we worked out the laboratory method of nettle juice obtaining from its fresh aerial parts [3]. Required type of scientific research is the shelf life determination of a new drug or substance. Such studies allow to assess the change in product quality during storage under the influence of environmental factors (light, temperature, humidity, packing material etc.).
The aim of research is determination of the nettle juice stability according with organoleptic, physical and chemical quality parameters during storage for 27 months in dark glass bottles at 2-4 °C.

2. Materials and Methods
Nettle juice was obtained by pressing of plant fresh aerial parts and kept in dark glass bottles (100 ml) at 2-4 °C. Samples of fresh juice and every 3 months were subjected to analysis of the following parameters: description, density, pH, dry residue, identification and quantification of main BAS (hydroxycinnamic acids, carotenoids, chlorophyll).
The juice relative density was determined by pycnometric method accordance with Ukraine State Pharmacopoeia (USP) 2, Article 2.2.5; pH was measured potentiometrically in accordance with SPU 2, Article 2.2.3; the juice dry residue was determined in accordance with SPU 2, Article 2.8.6 [4].
For the hydroxycinnamic acids identification in the nettle juice the method of thin-layer chromatography (TLC) was used [1, 4, 5]. For the TLC, nettle juice was evaporated to dryness, the residue was dissolved in 50% ethanol and again evaporated. This residue was dissolved in methanol. Then chromatographic plates Merck Silica gel F254 and solvent system of anhydrous formic acid – water – methanol – ethyl acetate (2,5:4:4:50) were used. The solution of 10 g/l defineline acid aminoethylamide ether in methanol was used for chromatograms displaying. Results evaluations were performed with comparing color intensity and Rf zones size on the comparison solution and the test solution chromatograms. To prepare the comparison solution it was used the following marker substances: rutin, chlorogenic acid, caffeic acid.
The TLC analysis in ultraviolet light at 365 nm wavelength discovered blue fluorescence zones which were typical for hydroxycinnamic acids (Fig. 1). By Rf size and color zones equality on the chromatogram obtained with reference solution and the nettle juice test solution, chlorogenic and caffeic acid were identified.

For carotenoids and chlorophyll identification it was used spectrophotometric measurement of the nettle juice extract. In studying of the extract spectral characteristics it was observed the absorption maximum at the wavelength of 442 ± 2 nm that was typical for carotenoids (violo xanthine) and at the wavelength 667 ± 3 nm, which was characteristic for chlorophyll (Fig. 2).

The investigated BAS absorption maximums did not match, so it was possible to conduct the simultaneous determination of chlorophyll and carotenoids in the nettle juice with spectrophotometric method without previous separation.

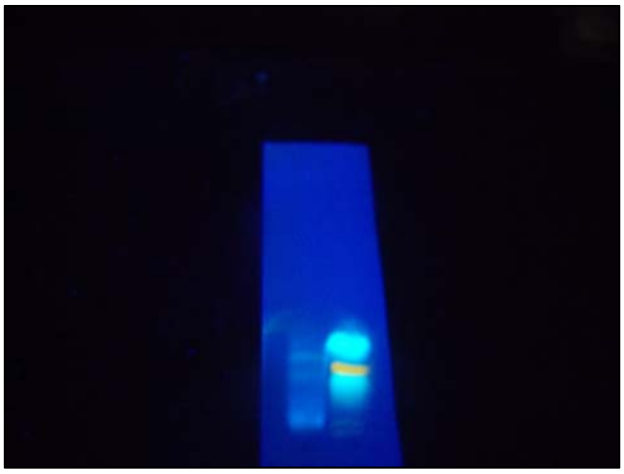

Fig 1: The nettle juice thin-layer chromatography.

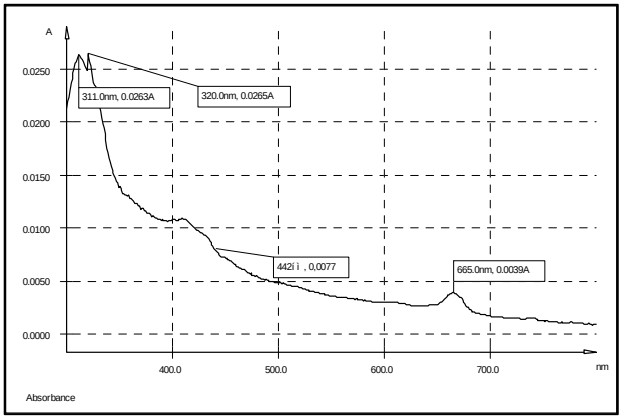

Fig 2: UV-spectrum of the nettle juice in hexane.

Quantitative content assessment of the nettle juice main BAS with spectrophotometric method was performed on a spectrophotometer Specord 200. All used reagents met the USP 2 requirements [1].

Hexane was used for lipophilic substances (chlorophyll and carotenoids) extraction. The juice 1, 5 g was filled with hexane 10 ml, shaken for 10 minutes. The hexane extract was separated and filtered. Optical density of the obtained hexane extract was measured: for chlorophyll at a wavelength 666 nm, for carotenoids – 442 nm.

Quantitative content of chlorophyll (X) was calculated with the formula:

$$X = \frac{D1 \times 10 \times 100}{m \times 944{,}5}$$, where (1)

D_1 – hexane extract optical density at a wavelength 666 nm; m – the juice sample weight, g; 944, 5 – specific absorption rate of chlorophyll at 667 ± 3 nm; 10-dilution; 100-percentage recalculation.

Quantitative content of carotenoids (X) equivalent to violoxanthine was calculated with the formula:

$$X = \frac{D1 \times 10 \times 100}{m \times 2500}$$, where (2)

D_1 - hexane extract optical density at a wavelength 442 нм; m-the juice sample weight, g; 2500-specific absorption rate of violoxanthine at 442 ± 2 nm; 10-dilution; 100-percentage recalculation.

The quantitative content of the nettle juice hydroxycinnamic acids was determined according to the method described in USP 2, monograph "Nettle leaves" [2]. The method was based on the coordination complex reaction with sodium molybdate and sodium nitrite solution, resulting in pinkish-orange solution formation in an alkaline environment, which color depended on the ratio of cinnamic acid derivatives in raw materials. The wavelength measurement depended on the maximum absorption of the standard substance complex in terms of which calculated the cinnamic acids quantitative content.

The sum of hydroxycinnamic acids quantitative determination in recalculating on caffeic acid.

The initial solution. The juice 20 g was placed in a bottle, added 10 ml 96% alcohol, heated with reflux in a water bath for 1 hour, cooled and filtered.

Test solution. 1, 0 ml of the initial solution was placed in a volumetric bottle 10 ml, sequentially added with stirring 2 ml of 0, 5 M hydrochloric acid solution, 2 ml of freshly prepared solution (10 g of sodium nitrite and 10 g of sodium molybdate in 100 ml of water), 2 ml of sodium hydroxide dilute solution, diluted with water to the mark and mixed.

Compensation solution. 1,0 ml of the initial solution was placed in a volumetric bottle 10 ml, sequentially added with stirring 2 ml of 0,5 M hydrochloric acid solution, 2 ml of sodium hydroxide dilute solution, diluted with water to the mark and mixed.

The test solution optical density at 510 nm wavelength was measured immediately using a compensation solution as a comparison solution. The sum of hydroxycinnamic acids in recalculating on caffeic acid was calculated using the formula:

$$X = \frac{D1 \times 10 \times 30 \times m0 \times 0{,}2 \times 98}{m1 \times 1 \times 10 \times 10 \times D0}$$, where (3)

D_1 – test solution optical density; m_1 – juice sample weight, г; 30 – dilution of juice; 10 – dilution of juice; m_0 – caffeic acid standard sample (SS); D_0 – caffeic acid SS optical density; 0,2, 10 – dilution of SS; 98 – coffee acid quantitative content (%) in SS.

3. Results and Discussion

The experimental results of nettle juice stability are given in table. 1. The juice is dark brown transparent liquid with specific odor. During the experiment the external changes were not observed. Qualitative reactions on the main groups of biologically active substances (phenol carbonic acids, carotenoids, chlorophyll) also confirmed the juice stability. Variabilities in density (from 1, 0002 to 1, 0022 g/ml), pH

(from 6, 6 to 7, 0) and dry residue (not less than 3, 5%) in the research samples were within the limits of permissible deviations. Spectrophotometric determination of the BAS quantitative content in the juice samples during 24 months' storage in comparison with the initial data showed that the content of hydroxycinnamic acids was in the range of 10,05 to 8,09 mg% (at the rate of not less than 8 mg%), carotenoids-from 2,14 to 2,05 mg% (at the rate of not less than 2 mg%), chlorophyll-from 3,106 to 3,017 mg% (at the rate of not less than 3 mg%).

Table 1: Results of the nettle juice stability during storage in dark glass bottles at 2-4 °C

Term of storage (months)	Appearance (description)	Density g/cm^3	pH	Dry residue, %	Content of hydroxy cinnamic acids, mg%	Content of carotenoids, mg%	Content of chlorophyll, mg%
Fresh	Greenish-brown transparent liquid with a specific odor	1,0024 ± 0,00088	6,60 ± 0,04	3,78 ± 0,064	10,0502 ± 0,0327	2,139 ± 0,017	3,106 ± 0,028
3	-//-	1,0022 ± 0,00064	6,62 ± 0,064	3,74 ± 0,048	10,0502 ± 0,0327	2,128 ± 0,017	3,078 ± 0,034
6	-//-	1,0012 ± 0,00064	6,70 ± 0,080	3,70 ± 0,040	9,9957 ± 0,0436	2,112 ± 0,017	3,078 ± 0,034
9	-//-	1,0012 ± 0,00032	6,74 ± 0,072	3,70 ± 0,040	9,6826 ± 0,0436	2,112 ± 0,017	3,092 ± 0,023
12	-//-	1,0002 ± 0,00104	6,78 ± 0,064	3,66 ± 0,048	9,5463 ± 0,0436	2,075 ± 0,017	3,035 ± 0,028
15	-//-	1,0002 ± 0,00136	6,82 ± 0,064	3,64 ± 0,048	8,9460 ± 0,0480	2,053 ± 0,011	3,063 ± 0,034
18	-//-	1,0004 ± 0,00152	6,84 ± 0,088	3,60 ± 0,040	8,4432 ± 0,0545	2,053 ± 0,011	3,023 ± 0,023
21	-//-	1,0010 ± 0,00080	6,90 ± 0,080	3,58 ± 0,064	8,1845 ± 0,0436	2,048 ± 0,017	3,023 ± 0,023
24	-//-	1,0006 ± 0,00088	6,92 ± 0,064	3,58 ± 0,064	8,0889 ± 0,0439	2,048 ± 0,017	3,017 ± 0,015
27	-//-	1,0008 ± 0,00112	6,92 ± 0,096	3,54 ± 0,064	7,7078 ± 0,0708	2,043 ± 0,012	2,795 ± 0,034

4. Conclusion

Results of the certain series analysis according with organoleptic and physical-chemical parameters indicate that the nettle juice is stabile for 2 years of storage in dark glass bottles at 2-4 °C. Currently, research continues on determination of microbiological purity of this herbal substance.

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