

Leaves of Common Nettle (*Urtica dioica* L.) As a Source of Ascorbic Acid (Vitamin C)

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Abstract: The article presents the results of the determination of ascorbic acid in the leaves, the infusion of common nettle and herbal vitamin preparation. Dried and grinded nettle leaves (industrial sample of raw material), infusion of the nettle leaves obtained by the method of general State Federal pharmacopoeia XI "Infusions and decoctions", herbal vitamin preparation (fruits of brier, mountain ash and sea-buckthorn, nettle leaves and grass lemon balm) served as the study objects. The concentration of ascorbic acid was determined by the three methods: titration by 2,6-dichlorophenolindophenol sodium salt, iodometry and iodimetric titration. It was determined that the concentration of vitamin C was $0,032 \pm 0,001$ mg%. The use of quantitative analysis method revealed that the leaves of common nettle contained $36,402 \pm 0,017$ mg% of ascorbic acid and infusion of nettle was $0,45 \pm 0,02$ mg%.

Key words: Vitamin C • Common nettle • Herbal vitamin preparation • Ascorbic acid • Iodometry • Iodimetric titration

INTRODUCTION

At the present time, the majority of the population of Russia reveals the multivitamin and mineral deficiency. Regardless of balanced diet, the deficit of important vitamins in the diet may reach 30 %. The deficiency of vitamins B is identified in 30-40 % of diagnosed people, β -carotene in more than 40 % and vitamin C in 70-90 % [1]. Continuous stress, unfavorable environmental conditions and poor nutrition and other factors contributed to the reduction of immunity of an organism and increase of morbidity of the population [2, 3].

Vitamins are a special group of organic substances that perform important biological and biochemical functions in living organisms. These organic compounds possess various chemical natures and are synthesized mainly by the plants and microorganisms [4, 5]. The organisms of a man and animal are not able to synthesize require external amount of vitamins in minimal doses compared with other substances such as proteins, carbohydrates and fats.

Specific role of vitamin-mineral complexes in maintaining of the human health and importance for treatment of diseases reveal the significance of traditional approaches in the health care and prevention of diseases [6, 7]. Vitamins are especially effective at the reduction of immunity and high loads on the organism [8]. Numerous studies proved that vitamins are absolutely necessary for normal life of a person due to their biological catalytic activity affecting the metabolism and ensuring protection from adverse environmental factors [9-11].

Ascorbic acid (vitamin C) is important for maintenance of immunity and stimulation of the interferon synthesis, thus participating in immune-modulating processes. Vitamin C is a powerful antioxidant, protects lipoprotein oxidation and required for detoxification in hepatocytes with the involvement of cytochrom P450 [12-14]. Vitamin C neutralizes superoxide anion radical to hydrogen peroxide, inhibits the glycosylation of hemoglobin and converts glucose to sorbitol.

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The plants are one of important sources of vitamin C [8]. At the present time, there is sufficient volume of knowledge on chemical structure and pharmacological properties of medicinal plants accumulated, which provides a new look at the possibilities for their use in medicine [15]. Medical importance and efficiency of biologically active substances (BAS) of the plants is that BASs are containing in the plant raw material after prolonged biological balance filter and coherent to a man. Sufficient advantage of the medicinal plants is low toxicity that makes them suitable of continuous uptake course (for months or years) [5, 9, 16].

Valuable plant source of vitamins is common nettle, which is used in traditional and officinal medicine as haemostatic and vitamin substances [17-19]. It is included into the herbal vitamin preparations and medicines used for treatment of the prostatitis, diabetes and vitamin deficiency. The nettle leaves are used mostly for preparation of infusions. Nettle leaves contain ascorbic acid (270 mg%), carotenoids (50 mg%), vitamins B, K (200 mg%) and E, coumarins, flavonoids, phenolic acids, tannins, phytoncides, glycoside urticin, organic acids, sterols, chlorophyll (up to 5%), alkaloids and minerals [20-22].

Ascorbic acid in nettle leaves is in two forms - ascorbic acid and dehydroascorbic acid synthesizing by its oxidation. The content of the first form increases until certain maximum and then reduces during vegetation period of nettle. The content of the second form during vegetation is usually high, then reduces and reaches a minimum at the maximum content of reduced form [12]. Interconversion of ascorbic acid and dehydroascorbic acid in the plants are closely related with enzymatic interconversions of oxidized and reduced glutathione.

Both ascorbic and dehydroascorbic acids are physiologically active and promotes the synthesis of collagen, serotonin from tryptophan, catecholamines and corticosteroids. Ascorbic acid is also involved in the conversion of cholesterol into bile acids [4].

The purpose of this study is to evaluate the leaves, herbal preparation containing the nettle leaves and infusion of nettle for the content of ascorbic acid.

MATERIALS AND METHODS

Dried and grinded nettle leaves (industrial sample of raw material), infusion of the nettle leaves obtained by the method of general State Federal pharmacopoeia XI "Infusions and decoctions", herbal vitamin preparation

(fruits of brier, mountain ash and sea-buckthorn, nettle leaves and grass lemon balm) served as the study objects.

Three methods were used for determination of concentration of ascorbic acid in the leaves and the infusion of nettles: titration by 2,6-dichlorophenolindophenol sodium salt (according to article 38 "Fruits of brier" of Federal State XI Pharmacopoeia), iodometry and iodometric titration used for analysis of substances and preparations of ascorbic acid modified for the analysis of medical plants and infusion [23-25].

RESULTS

The Content of Ascorbic Acid in the Infusion of Nettle was determined as following: 5 ml of infusion prepared according to the method of General article of State Pharmacopoeia XI "Infusions and decoctions" was placed into the conical flask added with 5 ml of purified water, 5 ml of 2% hydrochloric acid, mixed and titrated from microburette solution by 2,6-dichlorophenolindophenol sodium salt (0,001 M) until stable pink dying for 30-60 s.

Iodometric Technique modified for the determination of ascorbic acid content in the raw materials and the nettle infusion included following steps: approximately 5 g (accurate weight) of the ground material was placed in 250 ml flask with section and added with 100 ml of water. The flask was attached to backflow condenser was heated on a water bath at maximum temperature 40°C for 20 minutes, periodically shaking to remove particles of raw materials from the walls. Then, water extraction was cooled until room temperature and filtered through the cotton wool into 100 ml volumetric flask, added until maximal volume and mixed. A conical flask was added with 5 ml of aqueous infusion, 5 ml of purified water, 1 ml of 2 % solution of hydrochloric acid, 0,2 g of crystalline potassium iodide, 5 drops of starch solution and titrated by potassium iodate solution (0,01 M) until stable blue dying for 3 min.

The determination of ascorbic acid in the infusion, 5 ml infusion prepared by the method of General article of State Pharmacopoeia XI "Infusions and decoctions" was added to a conical flask and further proceeded according to the method described above for raw material.

In the British Pharmacopoeia, the substance of ascorbic acid is quantitatively evaluated by iodometric titration [26, 27]. To achieve our objectives, we used suggested method with some modifications.

Table 1: Content of ascorbic acid in the leaves and infusion of nettle ($n=10$; $p=0,95$)

Analyzed object	\bar{x} , mg %	S ²	S	$\pm X$	ϵ , %
Titration by 2,6-dichlorophenolindophenol sodium salt					
Leaves	30,153	2,424	1,557	0,031	0,102
Infusion	0,030	0,001	0,005	0,001	0,341
Iodatometry					
Leaves	36,402	0,765	0,875	0,017	0,047
Infusion	0,032	0,001	0,004	0,001	0,260
Iodometry					
Leaves	34,351	1,026	1,013	0,020	0,058
Infusion	0,033	0,001	0,009	0,001	0,524

Iodometric Determination of Ascorbic Acid in Raw

Material: Approximately 5 g (accurate weight) of the ground material was placed to 250 ml flask with section, added with 100 ml of water, attached to the backflow condenser and heated on a water bath at maximum temperature 40°C for 20 min, periodically shaking to remove particles of raw materials from the walls. Then, water infusion was cooled until room temperature and filtered through cotton wool to 100 ml volumetric flask, added until maximum volume and mixed. 5 ml of aqueous infusion were placed to a conical flask, added with 5 ml of purified water, 1 ml of 0,1 M of sulfuric acid solution, 5 drops of starch solution and titrated by iodine solution (0,1 M), until stable blue dyeing for 3 minutes.

Infusion of nettle for determination of ascorbic acid was prepared according to General article of State Pharmacopeia XI "Infusions and decoctions". The conical flask was added with 5 ml of infusion and further proceeding according to the method described above.

The results of determination of ascorbic acid in the nettle leaves and infusion using three methods are shown in the table and applicable for quantitative determination of ascorbic acid in the raw material and the infusion of nettle.

The content of vitamin C in herbal vitamin preparation (fruits of brier, mountain ash and seabuckthorn, nettle leaves and grass lemon balm) was determined using modified pharmacopoeial method.

Determination of Ascorbic Acid in Vitamin Herbal Preparation:

A 20 g sample of herbal preparation was carefully ground in a porcelain mortar with glass powder (about 5 g), gradually added with 300 ml of water, infused for 10 min, stirred and filtered. 1 ml of the filtrate was placed to a 100 ml conical flask, added with 1 ml of 2% hydrochloric acid solution, 13 ml of water, mixed and titrated by 0,001 M 2,6-dichlorophenolindophenol sodium salt until stable pink dyeing for 30-60 s (titration continued less than 2 min). In case of intensive dyeing of filtrate or

high content of ascorbic acid (consumption of 0,001 M 2,6-dichlorophenolindophenol sodium salt exceeded 2 ml) detected by control titration, the initial extract should be diluted by water two times or more. It was found using this method that the analyzed herbal vitamin preparation contains $0,45 \pm 0,02$ % of ascorbic acid.

CONCLUSIONS

- It is shown that the content of ascorbic acid in the leaves and the infusion of nettle can be determined by three methods: titration by 2,6-dichlorophenolindophenol sodium salt, iodometric and iodometric titration.
- It was established that the nettle leaves contains $36,402 \pm 0,017$ mg% of ascorbic acid and nettle infusion contains $0,032 \pm 0,001$ mg% of vitamin C.
- A method for quantitative determination of ascorbic acid in herbal vitamin preparation has been developed and the concentration of vitamin C reached $0,45 \pm 0,02$ %.

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