

QUANTITATIVE DETERMINATION OF TANNIN IN NETTLE
BY SPECTROPHOTOMETRIC AND CHROMATOGRAPHIC METHODSS. H. APOYAN ^{1*}, S. M. VARDAPETYAN ^{2**}, A. M. HOVHANNISYAN ^{1***},
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The amount of tannins in the leaves of stinging nettle was determined depending on the phenological phases (budding phase, mass flowering phase and fruiting phase). UV spectrophotometric and high performance liquid chromatography methods were used to quantify tannins in the plant. It was found that the maximum accumulation of this group of substances in nettle leaves occurs in the fruiting phase. Based on the results of the study, the chromatographic method is optimal for the analysis of tannins in nettle leaves.

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Introduction. The beneficial properties of medicinal plants depend on the content of biologically active substances, such as alkaloids, glycosides, saponins, essential oils, organic acids, vitamins. The quantity of these substances depends on the phase of plant development. The maximum amount of these substances in the aboveground green parts of plants is usually reached during the period of flowering and the beginning of fruiting of the plant. This determines the collection time for each type of raw material.

Tannins are known as biologically active compounds. They are produced by plants during their life and are intermediate metabolic products. Tannins are widely used in pharmacy, as they possess astringent, antiphlogistic, hemostatic and bactericidal action, which is based on the ability to bind to proteins to form dense albuminates. In medicine, tannins are used in the treatment of diseases such as stomatitis, gingivitis, pharyngitis, tonsillitis, colitis, enterocolitis and dysentery. The breadth of medical applications makes this group of biologically active substances interesting for the search and study of new sources of raw materials containing tannins [1]. The content of tannins in plants depends on environmental factors and the type of plant. The object of our research is stinging nettle (*Urtica dioica* L.). Stinging nettle is a perennial plant and is widespread in Armenia. The chemical composition of this medicinal plant depends on the state of the environment, habitat

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and climatic conditions. Stinging nettle leaves contain vitamins K, B2, C, carotene, pantothenic acid, phytoncides, proteins, sugars, chlorophyll, tannins, silicic and formic acids, macro- and microelements (iron, vanadium, manganese, chromium, copper, aluminum) and other substances [2]. The purpose of the study was to determine the quantitative content of tannins depending on the phenological phase (budding phase, mass flowering phase and fruiting phase). Stinging nettle blooms from May to late autumn, fruits ripen at different times. Nettle leaves were collected around Stepanavan for two years (2019–2020).

Experimental Part. For the quantitative determination of tannins in the plant, UV spectrophotometric (Scilogex UV-1800PC) and high performance liquid chromatography (HPLC-Waters Separationsmodule 2695) methods were used [3]. For the analysis, nettle extracts were prepared from raw materials, which were collected in the phase of budding, mass flowering and fruiting. The extraction of raw materials was carried out with ethyl alcohol of various concentrations (70%, 50%) at a temperature of 20°C [4]. For the analysis of tannins 1 g of crushed raw material was poured into 100 mL of boiling water and heated in a water bath for 30 min. Then, within 30 min, the extraction was defended at room temperature and filtered through a folded paper filter into a 100 mL flask and made up to the mark with water. For the quantitative determination of tannins, 5 mL of the obtained extract was placed in a volumetric flask with a capacity of 50 mL. Then it was brought to the mark with 70% ethyl alcohol. Then 2.5 mL of the resulting solution was placed in a 25 mL volumetric flask and brought to the mark with 70% ethyl alcohol. The optical density of the resulting solution was measured on a spectrophotometer at a wavelength of 275 nm relative to 70% ethyl alcohol. The optical density of a solution of a standard tannin sample was also determined. The total content of tannins (X , %) was determined by the formula

$$X = \frac{DxMt \cdot 100 \cdot 50 \cdot 25 \cdot 2.5 \cdot 100}{Dt \cdot Mx \cdot 5 \cdot 2.5 \cdot 50 \cdot 100},$$

where Mt is the mass of tannin, g; Mx is the mass of raw material, g; Dt is the optical density of tannin; Dx is the optical density of the test solution [5].

Results and Discussion. The research results are presented in Tab. 1.

Table 1

The content of tannins in nettle leaves depending on the phenological phase

Method	Phase	Content in mg/mL
Chromatographic	budding phase	0.26
	mass flowering phase	0.44
	fruiting phase	0.634
Spectrophotometric	budding phase	0.11
	mass flowering phase	0.32
	fruiting phase	0.52

Based on the data in Tab. 1, both methods have showed the maximum content of tannins in nettle leaves in the fruiting phase. As can be seen from the Tab. 1, the chromatographic method is optimal, in contrast to the spectrophotometric one. The content of flavonoids and pigments was also investigated in alcoholic extracts, since their amount also depends on the phenological phases. Raw materials were analyzed

according to generally accepted methods [6, 7]. We used the complexation reaction with a solution of aluminum chloride, for a quantitative assessment of flavonoids. This reaction is selective for phenolic compounds and gives a bathochromic shift in the spectrum towards longer wavelengths, which allows the separation of flavonoid compounds. In order to select an analytical wavelength, we studied the UV spectrum of nettle extraction with 50% and 70% ethyl alcohol, as well as a standard sample of rutin with the addition of aluminum chloride. It was found that the absorption maximum of colored reaction products of the sum of phenolic compounds with rutin is $410 \pm 2 \text{ nm}$. Therefore, rutin we used as a standard sample.

Table 2

Chemical composition of 70% alcohol extracts from nettle

Name	Budding phase	Mass flowering phase	Fruiting phase
Chlorophyll A, mg/mL	15.14	10.82	16.57
Chlorophyll B, mg/mL	5.24	4.46	10.15
Carotene, mg/mL	2.42	1.69	3.05
Flavonoids, mg/mL	5.8	5.5	5.9

As can be seen from Tab. 2, the highest content of extractives is observed in the fruiting phase. The content of chlorophylls A, B and carotenoids in the budding and fruiting phases is higher than in the mass flowering phase. The amount of flavonoids in the extracts is almost unchanged.

Table 3

Chemical composition of 50% alcohol extracts from nettle

Name	Budding phase	Mass flowering phase	Fruiting phase
Chlorophyll A, mg/mL	5.24	1.14	11.84
Chlorophyll B, mg/mL	2.16	0.47	7.19
Carotene, mg/mL	0.83	0.21	0.68
Flavonoids, mg/mL	6.5	7.0	6.9

The yield of nettle extract with 50% ethyl alcohol varies considerably. The data in Tab. 3 show that the content of chlorophylls A, B and carotenoids is lower compared to the extracts obtained earlier. The amount of flavonoids prevails in 70% of the extracts. As in the case of 50% alcoholic extract, the highest content of extractives is observed in the fruiting phase.

Conclusion. Thus, using various methods, we determined the content of tannins in nettle leaves in different phenological phases, and it was found that the maximum accumulation of this group of substances in nettle leaves occurs in the fruiting phase. Based on the results of this study, the chromatographic method is optimal for the analysis of tannins in nettle leaves, in contrast to the spectrophotometric method. Basically, nettle leaves are harvested during the flowering period. Based on research, it is recommended to harvest nettle leaves at the fruiting stage.

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ՏԱՆԻՆԻ ԶԱՆԱԿԱԿԱՆ ՈՐՈՇՈՒՄԸ ԵՂԻՆՋԻ ՏԵՐԵՎՆԵՐՈՒՄ
ՍՊԵԿՏՐՈՖՈՏՈՄԵՏՐԱԿԱՆ ԵՎ ՔՐՈՄԱՏՈԳՐԱՖԻԱԿԱՆ
ՄԵԹՈԴՆԵՐՈՎ

Քանակապես որոշվել է տանինի պարունակությունը երկտուն եղինջի տերևներում կախված զարգացման փուլերից, որը ներառում է բողբոջման, ծաղկման և պտղագոյացման փուլերը: Դաբաղանյութերի քանակական որոշման համար կիրառվել է ՈւՄ-սպեկտրոֆոտոմետրական և բարձր արդյունավետությամբ հեղուկ քրոմատոգրաֆիական մեթոդները: Պարզվել է, որ այս խմբի նյութերի առավելագույն կուտակումը եղինջի տերևներում տեղի է ունենում պտղագոյացման փուլում: Դաբաղանյութերի քանակական որոշման համար քրոմատոգրաֆիական մեթոդը ավելի արդյունավետ է քան սպեկտրոֆոտոմետրական մեթոդը:

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КОЛИЧЕСТВЕННОЕ ОПРЕДЕЛЕНИЕ ТАНИНА В КРАПИВЕ
СПЕКТРОФОТОМЕТРИЧЕСКИМ И ХРОМАТОГРАФИЧЕСКИМ
МЕТОДАМИ

Определено количество танина в листьях крапивы двудомной в зависимости от фенологических фаз (фазы бутонизации, массового цветения и плодоношения). Для количественного определения танина в растении были использованы УФ-спектрофотометрический метод и метод высокоэффективной жидкостной хроматографии. Установлено, что максимальное накопление дубильных веществ в листьях крапивы происходит в фазу плодоношения. На основании результатов исследования для анализа количества танина в листьях крапивы оптимальным является хроматографический метод.