



## Determination of Standard Parameters of Acorn Squash Leaves

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### Abstract

One of the priority tasks of pharmacy is to find new sources of raw materials for the medicinal products. The purpose of this work is to determine a number of indicators of the quality of promising medicinal raw materials that are the leaves of acorn squash *Cucurbita pepo* (*Cucurbitaceae*) for the creation of regulatory and technical documentation. For our research one of the most competitive kind - "Ukrainian multi-plant" was chosen. This kind is not sensitive to climatic conditions (drought-resistant). It has been grown in many countries with moderate climate and the most parts of Ukraine (Polissya, forest-steppe, steppe) and other since 1950. In this way, for the first time, 7 series of acorn squash leaves of "Ukrainian multi-plant" were studied. The main diagnostic features of morphological structure, including morphometric indicators, the main diagnostic microscopic features of the powder of acorn squash leaves were determined. The work presents the limit values of the main figures of 7 series of raw materials: weight loss during drying (not more than 8.5 %), total ash content (not more than 3.7 %), extractive substances content (not less than 20.0%), phenolic compounds content (not less than 2.0 %). According to the results obtained the regulatory and technical documentation "Methods of quality control of" Acorn squash leaves "has been developed.

**Keywords:** *Acorn squash, Leaf, Morphological structure, Microscopic diagnostic signs, Plant raw materials.*

### Introduction

In the 21st century, increasingly stringent requirements continue to be imposed on the quality of medicinal plant materials [1]. Decision of the Council of the Eurasian Economic Commission dated January 26, 2018 No. 15 approved the "Rules of good practice for growing, collecting, processing and storing raw materials of plant origin", which allow for clear planning due to proven agricultural practices, easily regulated volumes of raw material harvesting and low toxicity [2].

Representatives of the *Cucurbita* genus, which belongs to the *Cucurbitaceae* family, attracted our attention. According to different sources, this family consists of from 13 to 27 genuses [2, 3, 4]. Due to its chemical composition, *Cucurbita* is one of the valuable vegetable plants. The fruits and seeds of

plants have a great economic and therapeutic importance. According to literature, *Cucurbita* is one of the representatives of the oldest vegetable crops, whose homeland is the countries of South America and Mexico [5]. Archaeological studies in Mexico have found evidence of the use of *Cucurbita pepo* in a culture from 7000 BC [6]. In Europe, the vegetable became known after the completion of the Columbus expedition [7]. Representatives of the genus are one- and perennial plants with long creeping shoots, often ribbed and pubescent stems.

The leaves are simple, regular and large often with a rugged leaf blade, of various shades of green, sometimes with white spots in the corners of the branching veins. The axillary metamorphoses of the shoot - tendrils, like all aboveground organs, are

pubescent. Monoecious plants. Flowers unisexual, single, axillary, perianth double, corolla bell yellow or white, five-lobed. In male flowers, 5 stamens with fused, almost sessile, anthers. Female flowers have a coenocarpous gynoecium, (a pistil with a lobed sedentary stigma), formed by 3 carpels, the lower ovary is 3-5-nested. A fruit is a pumpkin, the size, shape and color of which has diagnostic feature at the level of the species or sort [3, 8]. Three species of this genus are very popular for cultivation: *Cucurbita pepo* L., *Cucurbita maxima* Duch., *Cucurbita moschata* Duch. ex Poir., which practically do not interbreed with each other and which can be easily distinguished by the shape of leaves, stems, fruits and seeds [8, 9].

It is widely used in official medicine *Cucurbita* seeds as anthelmintic and fatty seed oil as an anti-inflammatory, anti-allergic, anti-sclerotic, antioxidant, wound healing and antiulcer medicine [10]. This medicine is recommended for treating hepatitis, prostatic hyperplasia and helminthes [10-12]. Food industry, apart from seeds, also uses *Cucurbita* fruits as a source of juice, jam, dietary fiber and like an industrial source of carotene [13-15].

Folk medicine uses the over ground part of the plant: flowers, leaves, fruits. Flowers containing carbohydrates, amino acids, volatile and phenolic compounds are used as anti-inflammatory, wound healing and antioxidant agents [9, 16]. Fruits containing terpenoids, carbohydrates, phytosterols, ketones, aldehydes, fatty acids and vitamins [10, 17, 18] are widely used as urinary, choleric and blood-purifying agents, as well as in cardiovascular and cancerous diseases and diabetes [6, 15, 19]. Leaves containing phenolic compounds, elements, polysaccharides are used as a medicine to reduce the signs of fever, nausea and to increase hemoglobin in the blood and as an anesthetic [10, 20, 21]. There is information about the antioxidant properties of leaves [15].

Considering the availability of raw materials base and chemical composition, a wide range of pharmacological activity and application in both official and traditional medicine, further study of *Cucurbita* as a source of raw materials for medicines is timely. Based on the foregoing, one of the promising types of raw materials for creating drugs, the

producer of which are representatives of this species, are the leaves of *Cucurbita pepo*. With all its components, one should take into account the rate of reproducibility of the plant, which depends on the growing places, climatic conditions, and sort. Therefore, the sort of "Ukrainian Multiple" attracted our attention for the further pharmacognostic study of the leaves of *Cucurbita pepo*. This sort is unpretentious to climatic conditions (drought tolerant).

It has been grown in many countries with moderate climate, including Ukraine (Polissya, Forest-Steppe, Steppe) since 1950. Earlier, we obtained polysaccharide, alcohol and lipophilic fractions from *Cucurbita pepo* sort of "Ukrainian Multiple" and determined the spectrum of their antimicrobial activity in relation to six reference cultures: *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus subtilis*.

It was determined that the alcohol fraction of *Cucurbita pepo* leaves has antagonistic activity against yeast-like fungi of the genus of *Candida* (*C. albicans*) [22]. The aim of the work is to determine a number of quality indicators of perspective medicinal raw materials *Cucurbita pepo* leaves to create regulatory and technical documentation.

## Experimental Part

The objects of research were a series of *Cucurbita pepo* leaves sort of "Ukrainian Multiple". Raw materials were cultivated on private plantations and harvested in the phase of mass flowering in May-June 2018 (series 1-6) and May 2019 (series 7). The raw materials were prepared in: the Poltava region Lohvitsky district village Iskovets (series 1) and village Sokirikha (series 2), the Dnipropetrovsk region Sofievsky district village Ordo-Vasilyevka (series 3), the Kharkov region: Valkovsky district village Volunteer (series 4), Olshansky district village Protopopovka (series 5), Dergachevsky district village Bezruki (series 6) and Chuguevsky district village Pechenegi (series 7).

To conduct research from raw materials of 7 series by the method of quartering average samples were taken. The morphological features of the raw materials were determined visually using a ruler, caliper, magnifier x10, x20 and using a

stereomicroscope MBS-10 (Russia) (16x, 32x). For the preparation of micro preparations were used fixed in a mixture ethanol-glycerol-water (1: 1: 1) raw materials and dry crushed raw materials, which were previously soaked according to generally accepted methods [23]. In microscopic studies were used a Granum microscope (Austria) with magnification x40, x100, x400. A Sony DSC-W80 camera, followed by processing in Adobe Photoshop CS3, recorded the results [24].

Definition of a number of numerical indicators: loss in mass upon drying, determination of total ash and quantitative content of extractives substances and the sum of phenolic compounds was carried out according to SPU 2.0 methods [25]. Statistical processing of the research results was carried out using Student's criterion and one-way analysis of variance.

## Results and Discussion

A certain specificity of morphometric studies of leaves is consist of that the leaves of plants of the same species and sort and even of one plant as the evolutionarily most plastic organ have some difference in morphological and

morphometric characteristics from each other which can change under the influence of environmental factors. A severe pubescence noticeable to the naked eye was characteristic for the whole leaves of all series of *Cucurbita pepo*. The leaves are simple 1 with long petiole and without stipules, stiff-haired. The leaf blade is heart-shaped, 3-5-lobed, the tips of the blades are sharp, coarse and uneven, the base is heart-shaped (Fig. 1 A).

The color of the leaves is from light to dark green, the color is lighter on the underside of the leaf blade. Air-dry raw materials change color to olive green (Fig. 1 B). Venation palmate, veins depressed on the adaxial side of the leaf blade light yellow in color and convex well pronounced on the abaxial side (Fig. 1 C), greenish-yellow in color, copiously pubescent with rigid hairs (Fig. 1 D).

Petiole rounded, grooved on the adaxial side, pubescent with stiff hairs. The basal part of the petiole has a more or less expanded leaf pad. The veins of brown-yellow color and brown-green petioles with more pronounced ribs are clearly visible in the air-dry raw materials. The hard pubescence of air-dried leaves becomes scratchier perceptible (Fig. 1).

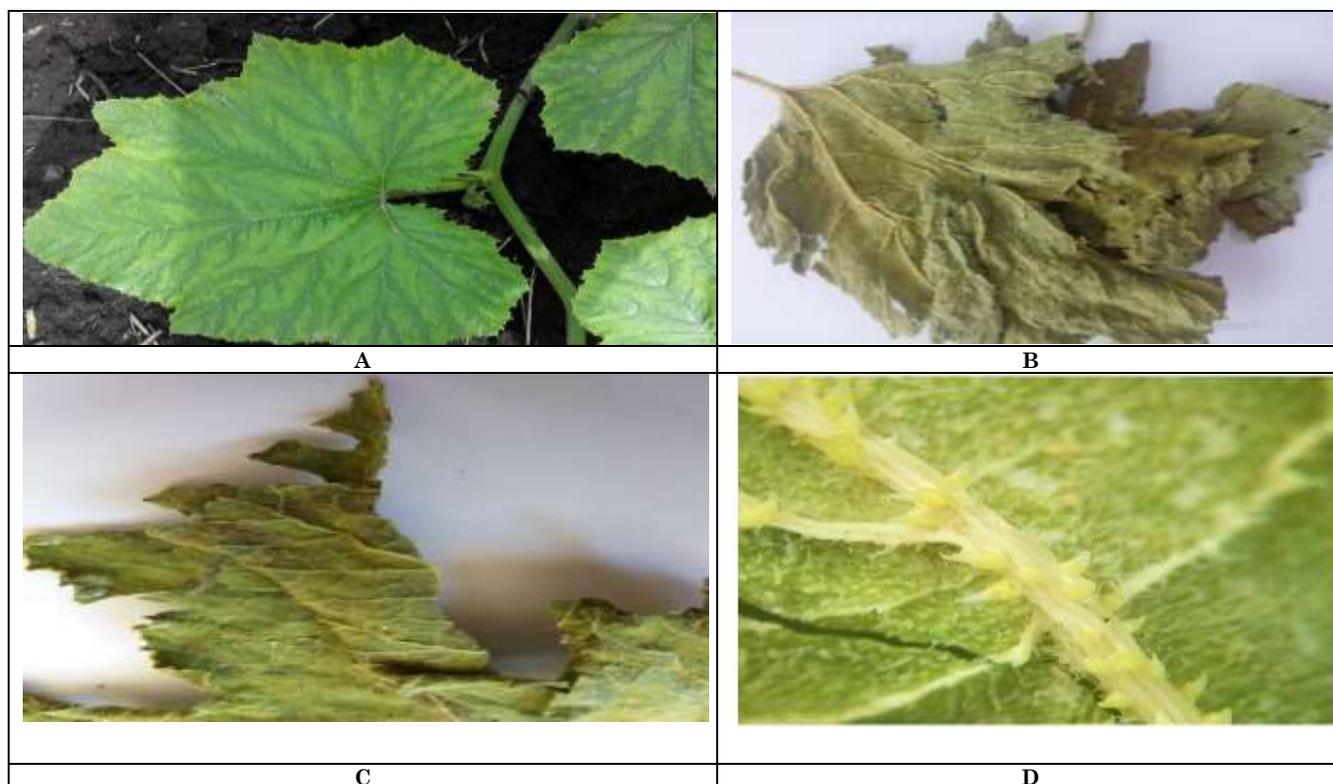


Fig. 1: Appearance of *Cucurbita pepo* leaf: fresh (A) and air-dry (B), the upper and lower sides of the leaf blade (C), pubescence of the central vein on the lower side of the leaf blade (D)

The results of determining the morphometric parameters of

*Cucurbita pepo* leaves in analyzed series are shown in Fig. 2-5.

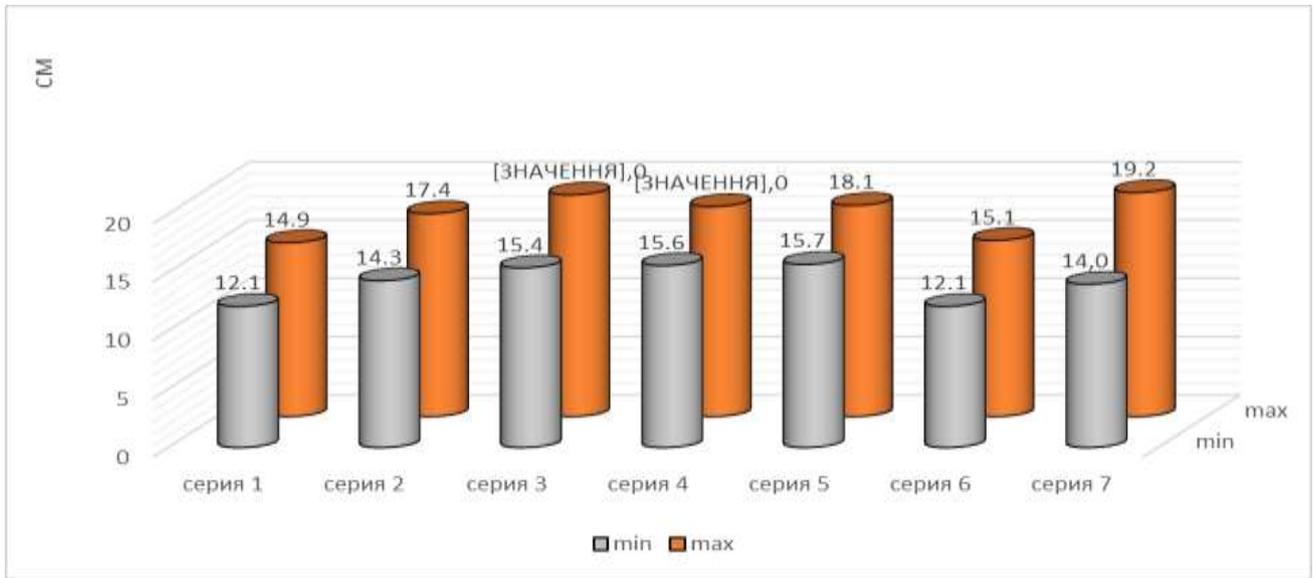


Fig. 2: The variability of the length of leaf blade of 7 series of Cucurbita pepo leaves

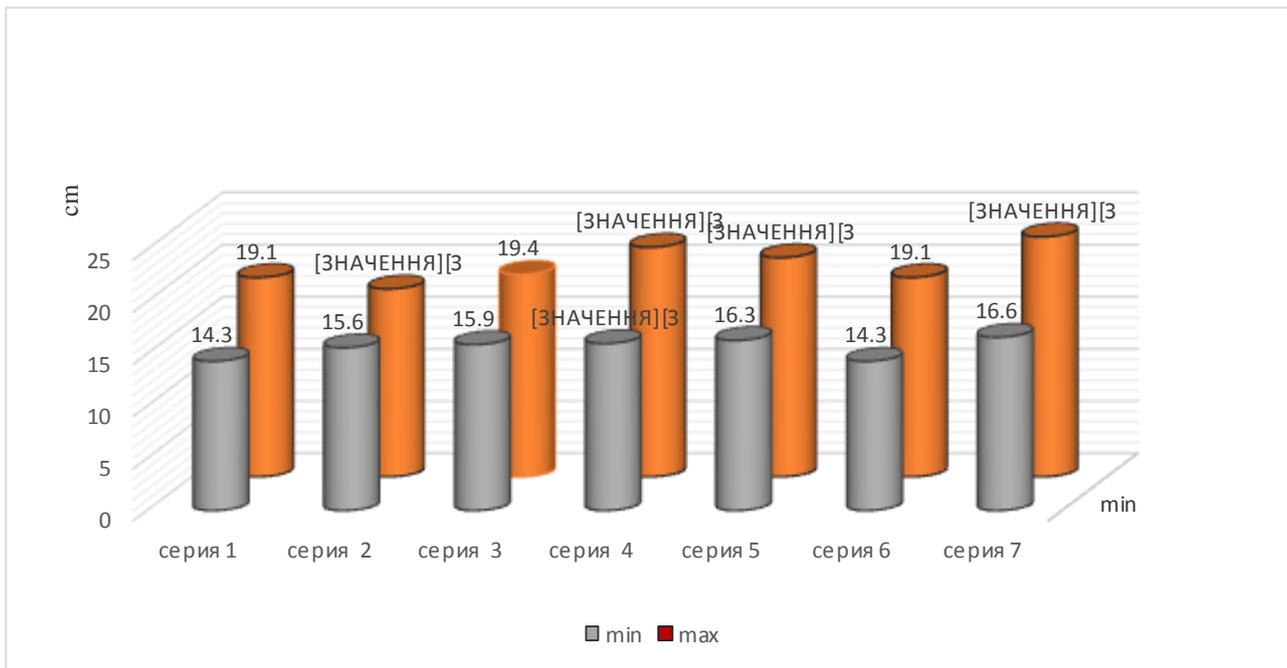


Fig. 3: The variability of the width of the leaf blade of 7 series of Cucurbita pepo leaves

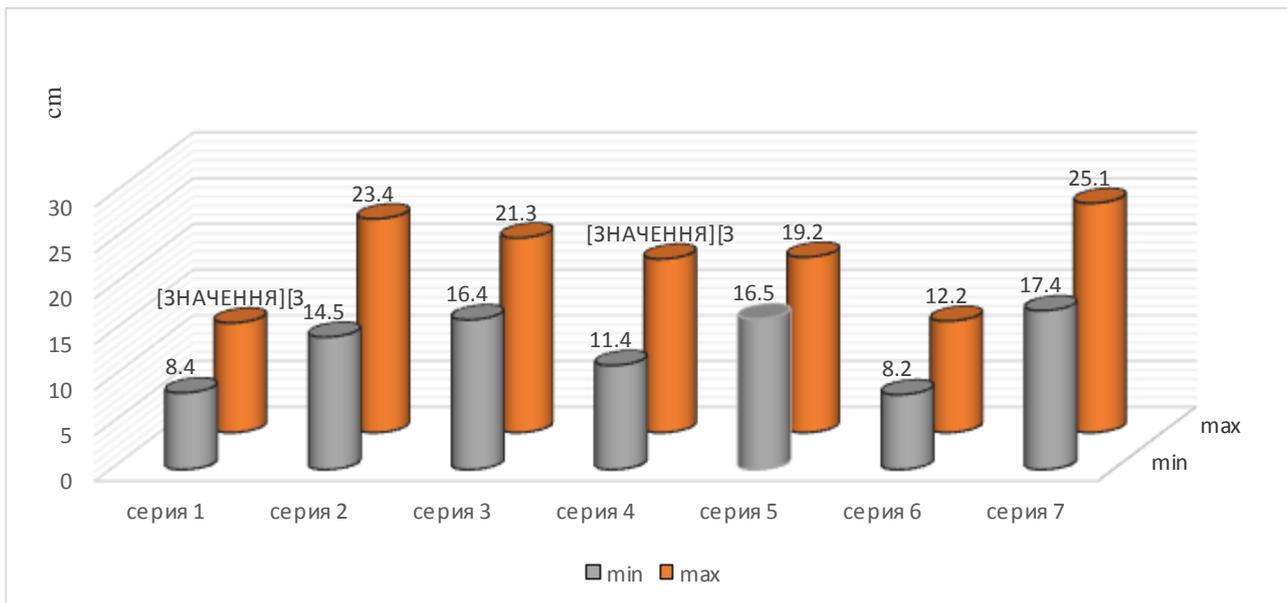


Fig. 4: The variability of petiole length of 7 series of Cucurbita pepo leaves

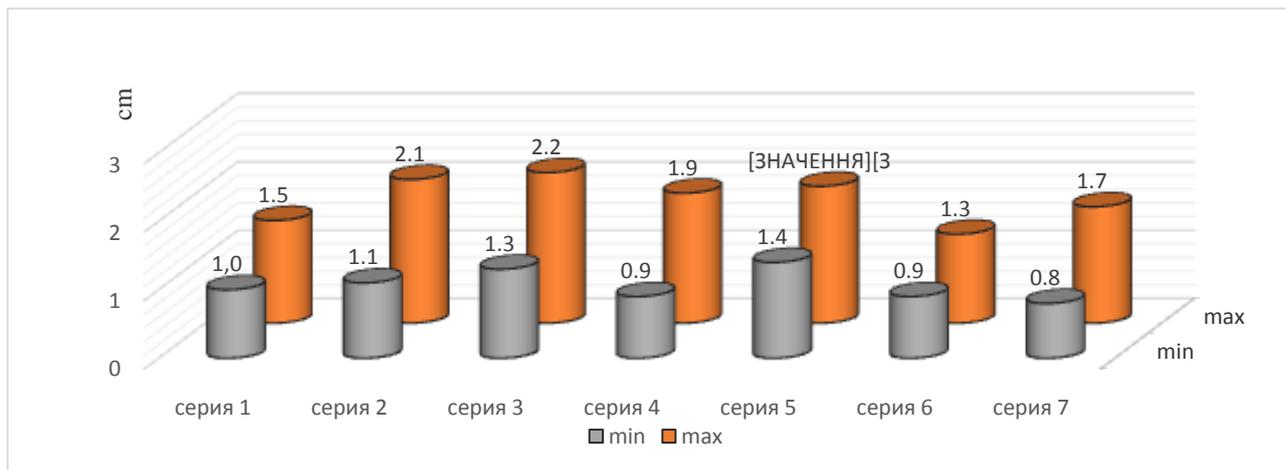
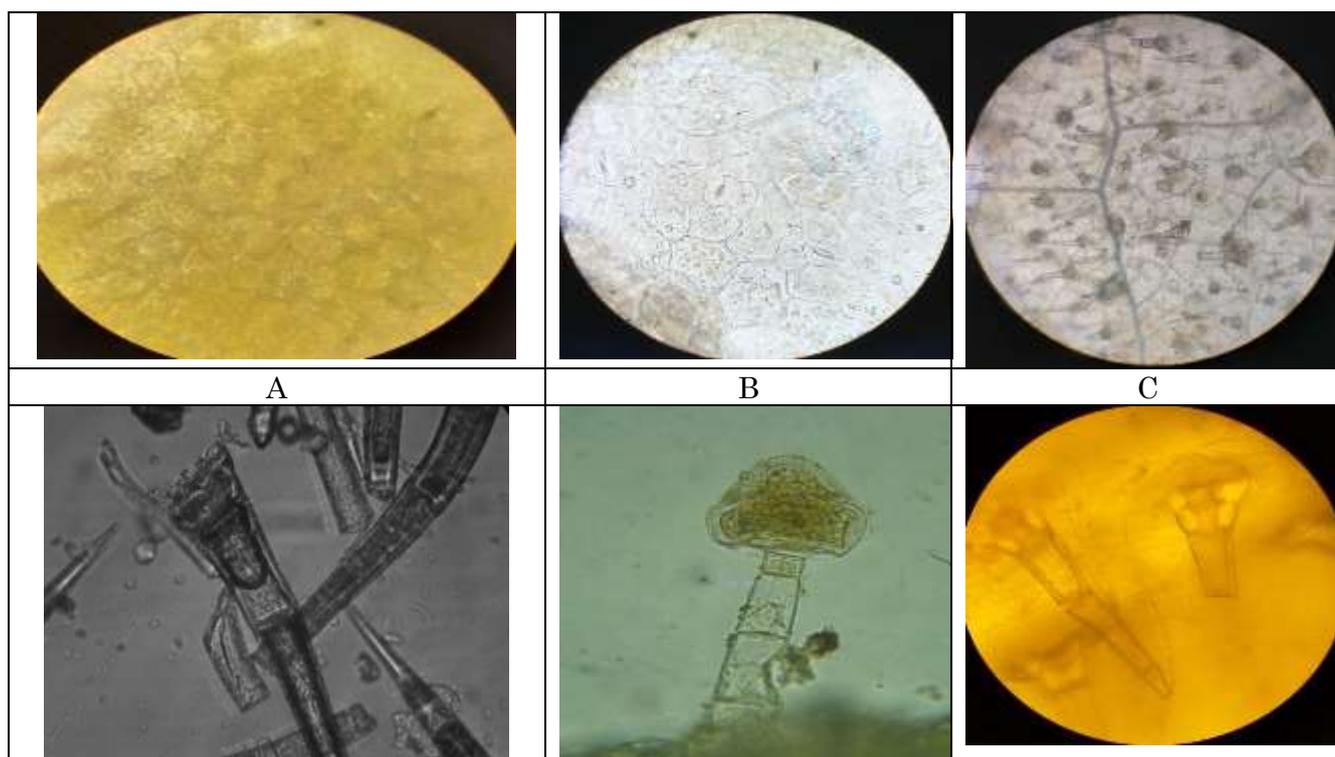


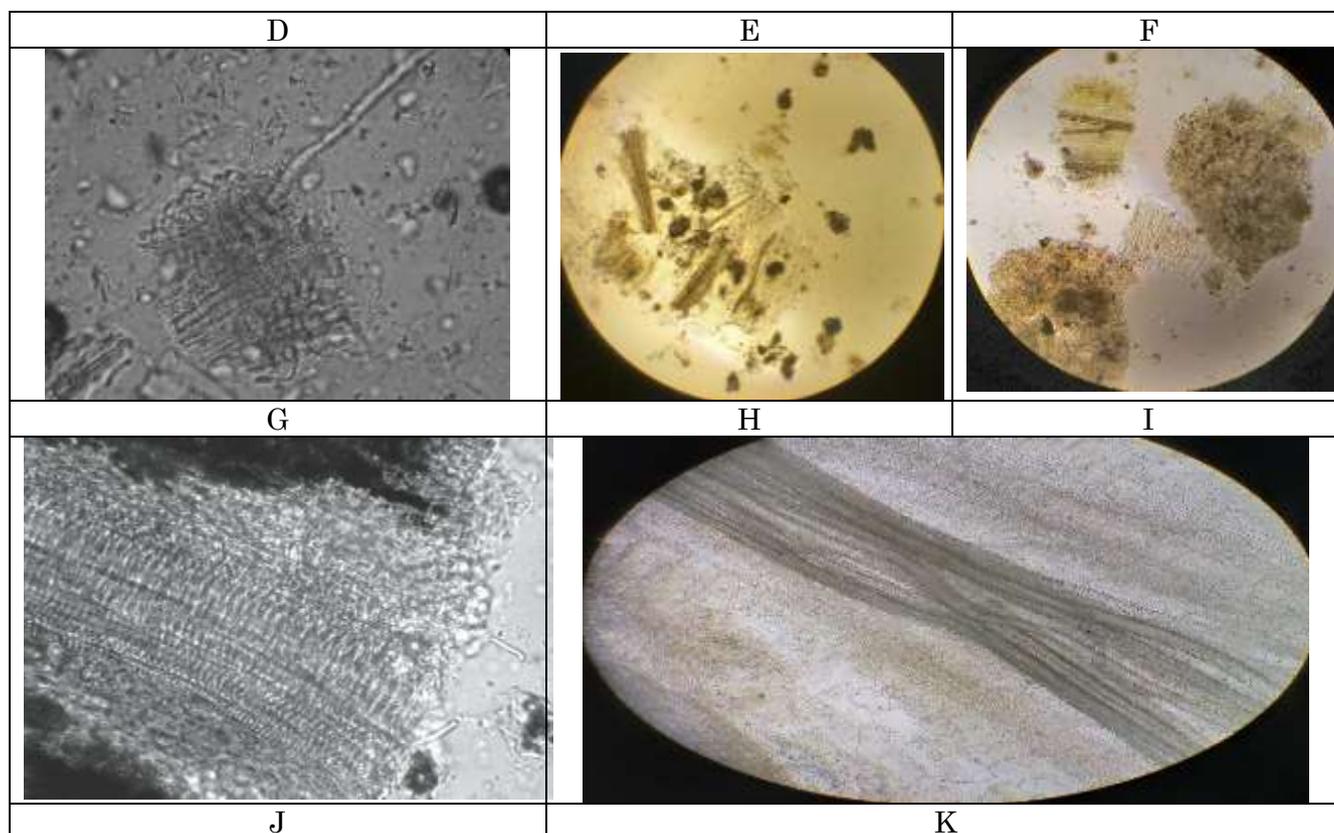
Fig. 5: The variability of petiole diameter of 7 series of *Cucurbita pepo* leaves

The lower and upper limit boundaries of the dimensional characteristics of the sheet were established based on the morphometric measurements. The length of leaf blade is 12.1-19.2 cm, width is 14.3-23 cm. Petiole length ranges from 8.2-25.1 cm, and its diameter is 0.8-2.2 cm. The next step in our research was the establishment of diagnostic microscopic features of the powder of *Cucurbita pepo* leaves (Fig. 6).

As a result of microscopic analysis was determined the following diagnostic features: fragments of the epidermis of the leaf blade; cells of the upper epidermis of the parenchyma form with slightly sinuous membranes without stomata (Fig. 6 A); base cells of the lower epidermis with more sinuous membranes and stomatal complexes

of the anomocytic, less often anisocytic type with 3-5 subsidiary cells (Fig. 6 B); fragments of the epidermis with hairs, including broken ones, as well as rosettes of epidermal cells in the place of attachment of the hairs (Fig. 6 C); fragments of simple multicellular hairs with a pointed apical cell, the cell body constituting the body of the hair is often filled with air (Fig. 6 D) and capitated hairs (Fig. 6 E); a bright diagnostic features of *Cucurbita pepo* leaf powder is simple 1-3- cell hairs with thickened a shell and a powerful stand, they can be completely or partially broken off (Fig. 6 F); fragments of the palisade mesophyll parenchyma upper and lower epidermis and elements of conductive tissues (Fig. 6 G-I); fragments of conducting tissues of vein bundles in the form of narrow-lumen spiral, annular vessels and staircases of larger diameter (Fig. 6 J, K).





**Fig. 6:** Microscopy features of powder leaves of *Cucurbita pepo* (A - a fragment of the upper epidermis without stomata; B - a fragment of the lower epidermis with anomocytic stomatal apparatus; C - a fragment of the epidermis with simple hairs, partially broken off hairs and rosettes of epidermal cells at the base of the hairs; D - fragments of mesophyll and vessels; E - fragments of simple hairs, with air bubbles in the cells; F - capitated hair with a unicellular head and 4-cell stem; G - simple hairs with a powerful stand, consisting of 3-6 cells; H - scraps of conductive tissues and palisade parenchyma; I - scraps of the upper and lower epidermis, J-K - elements of the xylem of the conducting bundle of the vein (annular, spiral vessels, ladders)

During the study of 7 series of *Cucurbita pepo* leaves was determined the following quality indicators: loss in mass upon drying,

total ash content, quantitative content of extractives and the amount of phenolic compounds. The results are shown in Table 1.

**Table 1:** Numeric indicators of series *Cucurbita pepo* leaves

No series	Loss in mass upon drying, %	Quantitative content, (n=5, in %, in terms of absolutely dry raw materials)		
		total ash	extractives	amount of phenolic compounds
1	7,90±0,04	3,24±0,10	20,35±0,01	2,13± 0,03
2	8,03±0,04	3,43±0,12	23,26±0,01	2,15± 0,03
3	7,95±0,04	3,45±0,12	24,18±0,02	2,35± 0,05
4	7,58±0,03	3,03±0,09	22,31±0,01	2,01± 0,02
5	7,43±0,03	3,67±0,14	21,15±0,02	2,09± 0,02
6	7,12±0,02	3,56±0,14	24,05±0,01	2,46± 0,06
7	8,04±0,04	3,37±0,11	24,09±0,02	2,33± 0,05

It was established that the loss in mass during drying of raw materials of the 7 series was not more than 8.5 %, the total ash content was not more than 3.7 %, the content of extractives was not less than 20.0 %, the content of the sum of phenolic compounds was not less than 2.0 %.

The obtained result of the study was used to create the draft regulatory and technical documentation "Quality Control Methods" *Cucurbitae Folia Cucurbita Leaves* ".

## Conclusions

- It was studied 7 series of *Cucurbita pepo* leaves sort "Ukrainian Multiple" at the first time.
- The main diagnostic features of the morphological structure including morphometric of raw material were determined: the type and size of the leaf blade and presence of hard pubescence.
- The main diagnostic microscopic features of powder *Cucurbita pepo* leaves were determined: simple multicellular hairs

with a pointed apical cell, capitated hairs, simple hairs with a multicellular stand at the base, the presence of rosettes at the base of the hairs or fragments of the body of hairs.

- It was determined the limiting values of the main numerical indicators of 7 series of raw materials: weight loss during drying (not more than 8.5%), total ash content (not more than 3.7%), extractives content (not less than 20.0%), the content of the amount of phenolic compounds (not less than 2.0%).
- On the base of the results obtained a draft regulatory and technical documentation "Quality Control Methods" Cucurbitae Folia Cucurbita Leaves" was developed [26].

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