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STANDARDIZATION OF ZIZIPHUS JUJUBE FRUIT (JUJUBE)

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ABSTRACT

This paper presents studies on the standardization of local raw materials - the fruits of jujube. Reliable morphological and anatomical features characterizing the authenticity of the raw materials have been determined, and methods for the qualitative and quantitative determination of the main active substances have been developed.

KEYWORDS: standardization, high quality, numerical indicators, microscopy, authenticity.

INTRODUCTION

The modern concept of treatment and prevention of hypertension, based on the principles of long-term and continuous use of antihypertensive drugs, is associated with the search for new drugs that, along with high specific activity, have minimal toxic effects on the human body.

Among medicinal plants with hypotensive activity, jujube is very promising (Chinese date, Ziziphus jujube Mill.) - a fruit plant growing in natural conditions in the south of our country The therapeutic effectiveness of jujube is largely due to its biological structures (leaves, flowers, fruits) of a unique complex of biologically active substances.[1]

A phytochemical study has found that tannins, catechins, flavone glycosides, coumarins, saponins, sugars, organic acids, resins, essential oils, and vitamins are contained in different parts of jujube. Fruits of the plant are of special biological value; they are richer in the content of ascorbic acid, rutin and potassium than other fruit and berry cultures, and in terms of the content of organic iodine, they are second only to indian feijoa. It has been established that aqueous extracts and dry preparations of jujube have a high hypotensive, cardiotropic and diuretic effect.[1,2]

Given the above, for the introduction into medicine of drugs based on the fruits of jujube, it is necessary to solve issues related to standardization.

We aimed to develop methods for quality control and standardization of jujube fruits.

Experimental Part

The authenticity of recommended raw materials was established by external, anatomical and diagnostic features, as well as the results of qualitative reactions.[2,3]

In order to determine the characteristic external signs, small amount of raw material was laid out on glossy paper (40x50), examined with normal eyes and under magnifying glass with a tenfold increase in various positions. When studying the external signs of the fruits of the jujube paid attention to the structure of the fetus, its characteristics, size (length and diameter of the fruit). The dimensions were determined using a measuring ruler or graph paper.

The color of the raw material was determined in daylight, the smell when rubbed, the taste of trying a piece of dry raw material and its water decoction.

Fruits are whole, spherical or oblong in shape, length up to 5 cm, diameter 3-5 cm. The color of fruits is brown or reddish-brown. The smell is absent. The fruit consists of fleshy, nutritious, mealy pulp and the fruit concluded in its cavity - a nut of oblong shape. The walls of the dried fruit are hard, brittle; the outer surface is shiny, less often matte, more or less wrinkled. The color of the pulp is light brown; the taste is sweet, slightly astringent. In order to determine the diagnostic features of the raw material, the anatomical structure of the seed oats has been studied.

Microscopic analysis was performed on both fresh and fixed (cold maceration in a glycerin-water-ethanol mixture, 1:1:1) material in accordance with the requirements of articles GF XI “Fruits” and “Technique for microscopic and microchemical studies on medicinal raw materials”. Microscopes Ergaval Carl Zeiss Jena (Germany) were used for anatomical diagnostics; photographs were made with a micro-attachment with a digital camera.

Microscopic examination of the fruits of the jujube studied longitudinal and transverse sections of the raw jujube.

Cross sections are made through the middle of the fruit. Fetal pericarp in cross section of parenchymal-beam type. The pericarp of the fetus consists of exocarp, mesocarp, and endocarp. Exocarp includes epidermis and hypodermis. Epidermis is single-row, round oval with thickened outer walls (cuticle).

Hypodermis 4-5 line, tangentially elongated, pigmented by brown substance (Figure 1).

The mesocarp is fleshy, consists of loosely connected cells of the parenchyma, there are intercellular spaces, the size of which increases from the periphery to the center. Parenchymal cells are round-oval, thin-walled, of various sizes, and some crystals are localized in them. The conductive beams are closed, collateral, numerous, consisting of phloem and xylem, which are located in the parenchyma around the periphery and in the central part of the fetal mesocarp - randomly (figure 1).

The endocarp is stiff, parchment-like consistency, since it consists of mechanical tissue.

Fruit is with sincarp, oligospermous, fleshy that non-opening when ripe, refer to the drupes; pericarpium of the fetus - parenchymal-beam type; pericarpium of the fetus consists of exocarp, mesocarp and endocarp; single-row epidermis with thickened outer walls; multi-row hypodermis, tangentially elongated, pigmented by brown substance; parenchymal cells are round-oval, thin-walled; closed conductive beams, collateral.

The specific activity of the fruits of jujube is characterized by a number of biologically active substances, in particular, due to the content of tannins and flavonoids. Therefore, chemical standardization of raw materials was carried out for this group of natural compounds.

Authenticity

0.1 g of the crushed raw material is placed in a volumetric flask with a capacity of 25 ml, add 10 ml of purified water, mix and bring the volume to the mark with water and filter through a filter paper. To 2 ml of the obtained filtrate add 5-6 drops of a 5% alcoholic solution of iron (III) - chloride; green staining (flavonoids) appears.

To 5 ml of solution A add 2 ml of a solution of iron oxide chloride; a black and green color (tannins) appears.

When developing criteria for the benignity of the fruits of jujube, in accordance with the indications of the article GF XI "Fruits", the following numerical indicators were determined:

- Content of active ingredients;
- Humidity;
- Total ash content and ash insoluble in 10% hydrochloric acid solution;
- Content of impurities.

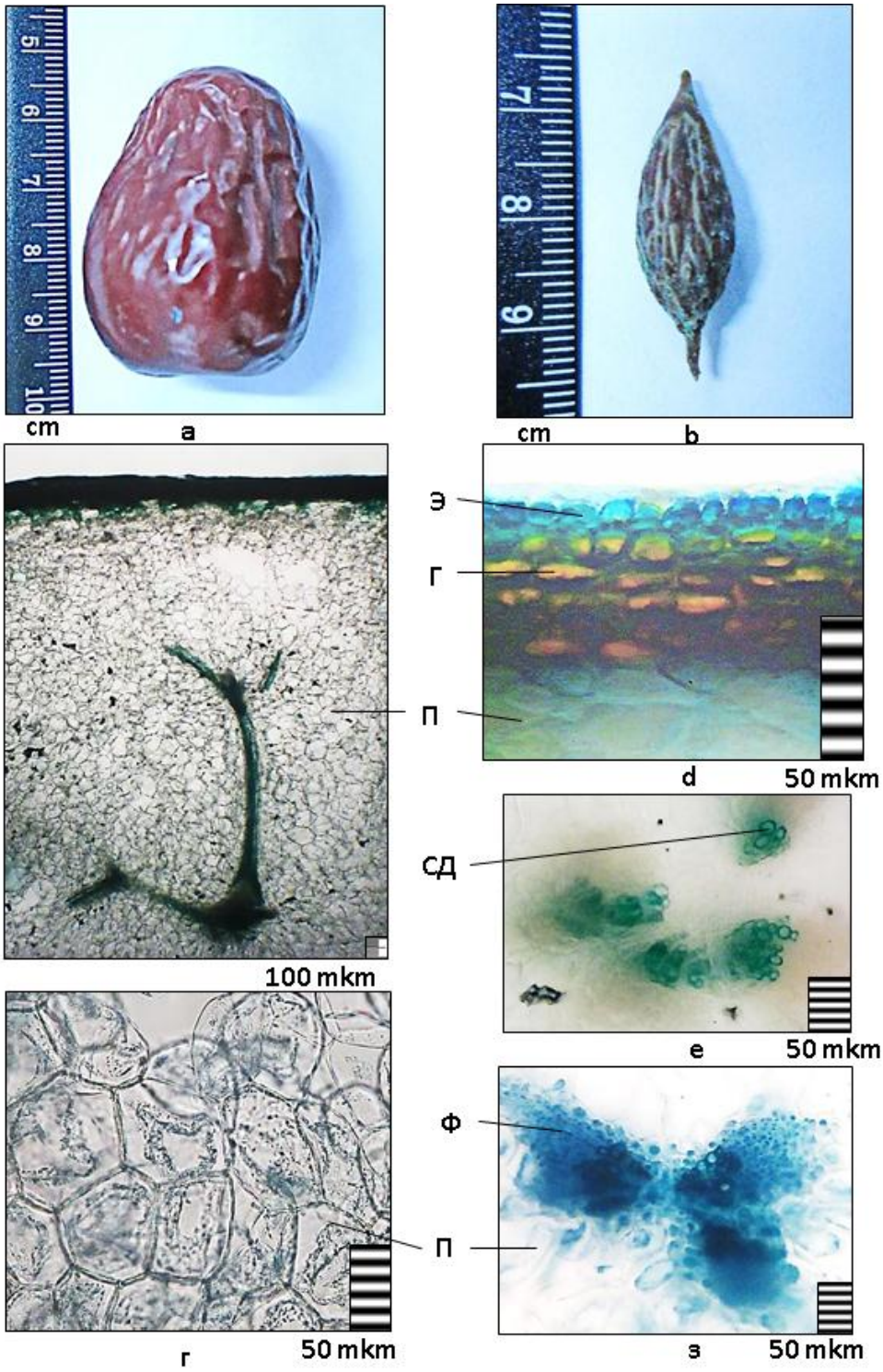

Figure 1: Micromorphological structure of the fetus Ziziphus jujube. a - the fetus, b - the seed (drupe), in the general form of the fruit in cross section, d - parenchyma, d - the epidermis (E), hypodermis (D) and parenchyma (P), e-z - conductive beams - DM - vessels, F - phloem.

Quantitative Determination Of Active Substances

1. Tannins. About 5 g (exact weight) of the crushed raw material is placed in a measuring flask with a capacity of 250 ml, add 100 ml of water, dissolve, and bring to the mark with water, mix. 25.0 ml of the solution obtained are transferred to a liter conical flask, 750 ml of water, 25 ml of indigosulfonic acid solution are added and titrated with stirring 0.02 mole/l of $KMnO_4$ solution until golden yellow.

In parallel, conduct a control experiment. 750 ml of water, 25 ml of indigosulfonic acid. 1 ml of 0.02 mole/l solution of $KMnO_4$ corresponds to 0.004157 g of tannins in terms of tannin.

The content of the amount of tannins (X), in percentage, in terms of tannin in absolutely dry raw materials is calculated by the formula:

$$X = \frac{(V - V_1)x0{,}004157x250x100x100}{mx25x(100 - W)},$$

Where,
V – volume of potassium permanganate solution (0.02 mole/l) consumed for the extraction titration in ml;
V_1 is the volume of potassium permanganate solution (0.02 mole/l) consumed for titration in the control experiment, in ml;
0.004157 – the amount of tannins corresponding to 1 ml of potassium permanganate solution (0.02 mole/l) (in terms of tannin), in g;

m – the mass of test sample, in g;

W – the mass loss during the drying of raw materials, in %.

The results of quantitative determination of tannins are presented in table 1.

Table 1: Metrological characteristics of quantitative determination results of tannins in fruits by using jujube titrimetric method.

f	*x*	$\bar{x}$	S^2	*S*	*t(pt)*	$\Delta\bar{x}$	$E_1^{\%}$	$E^{\%}$
5	5,5 5,3 5,4 5,6 5,7	5,5	0,02500	0,15811	2,78	0,07	7,9	3,6

As the results of the study showed, the content of polysaccharides in the fruits of oat seed varies from 5.3 to 5.7 %. Based on the data obtained, the rate of tannins is set at least 4 %.

2. Contents of total flavonoids. About 5 g (exact weight) of the crushed raw material, sieved through a sieve with a hole diameter of 3 mm, is placed in a conical flask with a capacity of 500 ml, filled with 250.0 ml of 50% ethyl alcohol and refluxed in a boiling water bath for 30 min. with occasional stirring. The liquid is cooled to room temperature and filtered through a white ribbon filter paper, discarding the first 10 ml of filtrate.

3.0 ml of the filtrate is placed in a volumetric flask with a capacity of 25 ml, add 3 ml of 2% aluminum chloride solution, 1 drop of diluted acetic acid and bring the volume of the solution with 96% ethyl alcohol to the mark. The solution is stirred and placed in a dark place. After 40 min, the optical density of the solution obtained is measured on a spectrophotometer at a wavelength of 400 nm in a cuvette with a layer thickness of 10 mm. A solution prepared in the same way, but without the addition of an aluminum chloride solution, is used as a reference solution.

In parallel, the optical density of the solution of the working standard sample is measured.

The total content of flavonoids (X), in %, in terms of rutin in absolutely dry raw materials is calculated by the formula:

$$X = \frac{D_1xa_0x250x25x1x100x100}{D_0xa_1x3x100x25x(100 - W)} = \frac{D_1xa_0x250x100}{D_0xa_1x3x(100 - W)},$$

Where
D_1 is the optical density of the test solution;
D_0 is the optical density of the rutin solution of the standard sample;
a_1 is the weight of the preparation, in g;
a_0 is the weight of a sample of the standard sample routine, in grams

Note: Preparation of the working standard sample solution (RDF) routine.

About 0.05 g (exact weight) of rutin (FS 42 Uz-0137-2007), previously dried at a temperature of (130-135) ° C for 3 hours, is placed in a 100 ml volumetric flask and dissolved in 85 ml 96% ethyl alcohol when heated in a water bath. After cooling to room temperature, the volume of the solution is adjusted to the mark with the same solvent and stirred. 1.0 ml of the solution obtained is placed in a volumetric flask with a capacity of 25 ml and then also comes when preparing the test solution starting from the words "... add 3 ml of 2% aluminum chloride solution ..." to "... without adding aluminum chloride solution". The shelf life of the reagent is 3 months.
The results of the quantitative determination of flavonoids are presented in table 2.

Table 2: Metrological characteristics of the results of the quantitative determination of flavonoids in jujube fruits using the SF method.

f	*x*	$\bar{x}$	S^2	*S*	*t(pt)*	$\Delta\bar{x}$	$E_1^{\%}$	$E^{\%}$
5	0,061 0,062 0,063 0,063 0,062	0,063	0,0000007	0,000836	2,78	0,0003741	3,7	1,6

As the results of the study showed, the content of flavonoids in jujube fruits varies from 0.061-0.063%. Based on the data obtained, the rate of tannins is set to not less than 0.05%. The other numerical indicators listed above were determined according to the methods described in the GF XI.

Based on the results of chemical and merchandising analyzes of raw jujube, the norms of numerical indicators governing its quality are established.

Table 3: Numerical data of Jujube Fruits.

Name of the data	Norm for raw materials
Tannins, %, not less	4
The amounts of flavonoids in terms of rutin,%, not less	0,05
Humidity,%, not more	10
Total ash,%, not more	10
Ash insoluble in 10% solution of hydrochloric acid,%, not more	3
Fruits, poorly cleared from the remnants of leaves, not more	1
Organic impurity,%, not more	1
Mineral impurity,%, not more	1

The stability of the raw material has been studied under conditions of its natural storage for 3 years. During storage, the content of flavonoids and tannins in raw materials for 2.5 years does not change significantly. Therefore, it is recommended to use jujube fruit within 2 years from the moment of preparation.

Heavy metal content. Tin order to 1 g of the fruits of jujube, it should be added 1 ml concentrated sulfuric acid, carefully burned, calcined. The resulting residue is treated with heating with 5 ml of a saturated ammonium acetate solution, filtered through a no filter, washed with 5 ml of water and the filtrate is adjusted to 200 ml. 10 ml of the resulting solution must withstand the test for heavy metals (no more than 0.01% in the preparation). The results of studies on the content of heavy metals showed the ecological purity and the possibility of safe use of jujube fruits.

Taking into account that medicinal products, including vegetable ones, not sterilized during the production process. It can be contaminated with microorganisms; we investigated the microbiological purity of the offered raw materials in accordance with the requirements of Global Fund XI and amendment to the article of Global Fund XI "Methods of Microbiological Drug Control" dated September 29, 2005, category 4A. The results of analysis were shown in table 4.

Table 4: Indicators of microbiological purity of jujube fruit.

Indicators	Requirements of regulatory documents (GF XI, issue 2, p. 193.)	Results of analysis	Compliance of ND
Total number of aerobic bacteria (in 1 g sample)	Not more than 10^7 (total)	400 CFU	Corresponding
Total number of yeast and mold fungi (in 1 g of the sample)	Not more than 10^5 (total)	100 CFU	Corresponding
Enterobacteriaceae, Pseudomonas aeruginosa и Staphylococcus aureus	Must be absent	Absent	Corresponding

Based on the data obtained, it can be concluded that the fruits of jujube meet the requirements for microbiological purity.

Thus, as a result of the research, scientifically based characteristics of authenticity and good quality of the proposed raw materials of jujube have been developed, which will be used in the design of the VFS project and other regulatory documentation.

CONCLUSIONS

For the first time, studies were conducted to substantiate the system of standardization of the fruits of jujube: morphological and anatomical features that characterize the authenticity of the raw materials were determined. Methods have been developed for the qualitative and quantitative determination of the main active ingredients and scientifically based criteria for the quality of jujube raw materials.

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