ORIGINAL ARTICLE

A Comparative Study of Morphological and Anatomical Characteristics of Herbal Powder and Cut-pressed Granules Derived from Tripartite Bur-marigold

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Abstract

Aim: To confirm the medicines derived from tripartite bur-marigold herb compliance with quality parameters, including characteristic plant-specific microscopic features. At present, there's a strong need in studies related to the development of resource-saving technologies aimed at widening the range of herbal medicines manufactured in various forms. **Materials and Methods:** Comparative analysis of anatomical and diagnostic features of two dosage forms (powder and cut-pressed granules) derived from tripartite bur-marigold herb was conducted. **Results:** In the course of analysis diagnostic features specific to tripartite bur-marigold herb was identified in cut-pressed granules: The presence of epidermal cells of leaves with sinuous walls and numerous anomocytic stomata, eruciform hairs, thick-walled hairs, mesophyll with secretory passages filled with brown content, aerenchyma, epidermal cells of membranous bract leaf with slightly sinuous and moniliform thickened cell walls, tightly packed rectangular cells of epidermis stem, large cells of the parenchyma of the stem, prickly pollen of rounded polyhedral shape, square, pentagonal and hexagonal cells with uniformly thickened walls of achenes' cover fabric and unicellular, and thick hairs of achenes. **Conclusions:** The results of the analysis allow us to conclude that the preparation of cut-pressed granules preserves basic anatomical and diagnostic features of tripartite bur-marigold herb. In other words, the production process does not affect the manifestation of features, which can be distinguished during the microscopic analysis of the dosage form, and cut-pressed granules of tripartite bur-marigold herb can be assessed, in accordance with the State Pharmacopoeia, by their microscopic features.

Key words: Comparative study, cut-pressed granules, microscopic features, tripartite bur-marigold

INTRODUCTION

To manufacture herbal drug preparations in various forms (packs, filter bags, etc.), most of domestic pharmaceutical companies use entire raw materials as well as raw materials of various fineness degrees including coarse powder.

The process of raw materials grinding often results in the loss of biologically active substances contained in the brittle parts of plants, because they are firstly pulverized during the grinding process and are turned into dust-like particles, which afterward is sifted and removed. Moreover, the crushed medicinal plant raw material represents a difficult product for industrial packaging as it shows a wide range of physical characteristics that depend on its botanical and morphological attributes.

Manufacturing of new dosage forms by means of resource saving technologies allows both to improve the technological properties of the raw material such as flow ability,

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homogeneity, and uniformity of mass during packaging, and to widen the range of medicines of plant origin.

In 2015, general monograph 1.4.1.0022.15 "cut-pressed granules" was included in the Russian Federation State Pharmacopoeia (XIII edition) for the first time.[1] The cut-pressed granules are cylindrical, spherical, or irregularly shaped pieces, derived from medicinal plants, that are intended for preparation of aqueous extracts. To manufacture the cut-pressed granules and treat cut-chopped medicinal herbs or powder of medicinal plants a special multistep technological process is used.[1]

Standardization and quality control of cut-pressed granules is conducted in accordance with the Russian Federation State Pharmacopoeia (XIII edition) requirements.[1] Some of the quality characteristics correlate with the technological parameters, such as granule size, loss on drying, disintegration, mass of package content, and others. Whereas granules are made directly of herbal raw material, quality control is provided in terms of parameters, which are specific to herbal raw material: Identification (determination of macro- and microscopic features, which are specific to starting material, presence of main groups of active substances); total ash, acid-insoluble ash, etc., Moreover, the assessment is also conducted in terms of microbiological purity, level of pest contamination, content of radionuclides and heavy metals, and pesticide residues.

The genus bur-marigold (*Bidens* L.) of the sunflower family (Asteraceae) includes 230 types, which are well-known in Europe, Northern Asia, Africa, and America.[2]

According to official medical practice, only tripartite bur-marigold (*Bidens tripartitae* herba)[3] is used in chronic dysentery and chronic enteritis treatment[4] as a mild diaphoretic and a diuretic in the treatment of bladder and kidney disorders that result in hematuria,[5] and as an astringent agent in ulcerative colitis and diarrhea treatment.[6] A large number of closely related species represent an admixture to this type of medicinal plants.

To assess the authenticity of medicinal plants, the macroscopic and microscopic analyses are used[7] that justifies the importance of morphological, anatomical, and diagnostic features study.

Authenticity assessment is one of the basic steps of herbal drug standardization. An optimal test for authenticity should demonstrate the difference between the test object, related species and/or foreign organic matter. The test should also be herbal drug-specific and combine several techniques depending on characteristics of the material under test. In cases when preparations are derived from pulverized medicinal plants, the main method of authenticity assessment is a microscopic analysis. This method can reliably determine the authenticity of the herbal drug and identify the presence of impurities.[8] The Russian Federation State Pharmacopoeia and leading foreign pharmacopoeias widely recommend the method of microscopic analysis.[7,9-12] The techniques of preparation of medicinal plant material (and derived herbal drugs) samples for microscopic examination vary and depend on a morphological group of the object under test, as well as its fraction and the degree of grinding – uncut, milled or powdered.

The aim of the research was to conduct a comparative study of anatomical and diagnostic characteristics of tripartite bur-marigold herb powder and cut-pressed granules from tripartite bur-marigold.

MATERIALS AND METHODS

The powder derived from tripartite bur-marigold, which complied with the requirements of the pharmacopoeial monograph 2.5.0048.15 "*B. tripartitae* herba,"[3] and the cut-pressed granules derived from this powder were used as the objects of the study.

For cut-pressed granules manufacturing the cut raw material of tripartite bur-marigold (particles passing through a 2 mm sieve) was used. The raw material was exposed to saturated steam (vapor pressure 3.5-5.5 kg/cm^2) for 3-4 min under constant stirring (for uniform moisture distribution). The moistened raw material was transferred to a compression machine. After pushing moistened mass through a 5-7 mm sieve, it came out in the form of extruded cylinders 10-30 mm in length, which were placed in the dryer. Forced-air cooled, the material was transferred on a roller machine, where it was crushed to granules passing through a 2 mm sieve.

Samples for microscopic examination were prepared using derived cut-pressed granules. About 0.1 g of the material under test was weighted into 50 ml beaker, 5-10 ml of 5% sodium hydroxide solution was added and the content was boiled for 2-5 min, followed by fractional washing of the powder by means of 100-150 ml portions of purified water, which was decanted after complete sedimentation of the particles. After last careful decantation, the powder was transferred into a drop of inclusive fluid (glycerol-water solution [1:1]) by a spatula or a scalpel, covered with a cover glass, slightly pressed by the reverse side of a dissecting needle and examined under a microscope.

To obtain photographs Olympus CX41 microscope (Olympus, Japan), equipped with 10× eyepiece and 4×, 20×, and 40× lenses, and a digital head (Olympus DIGITAL CAMERA C-3000 ZOOM [Japan]), were used. The photographs were processed using Adobe Photoshop 7.0 (Adobe, USA).

Main part

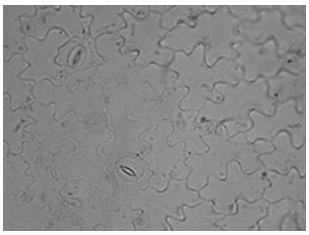
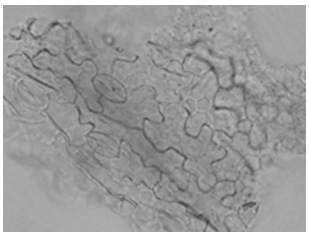
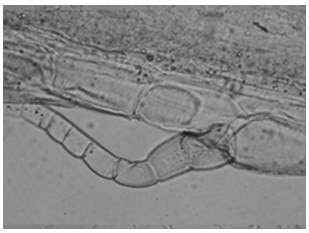
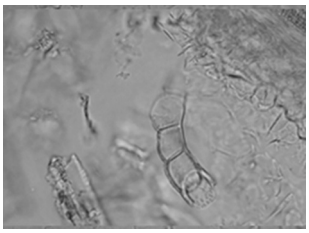
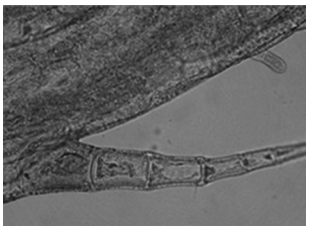
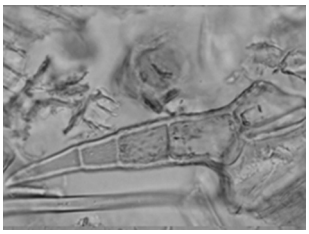
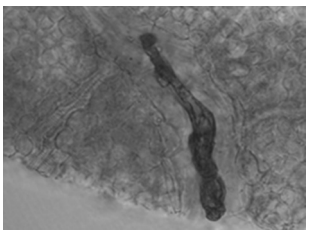
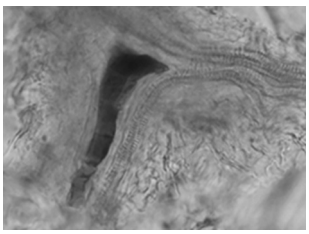
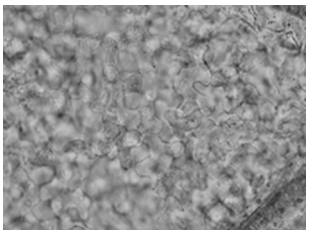
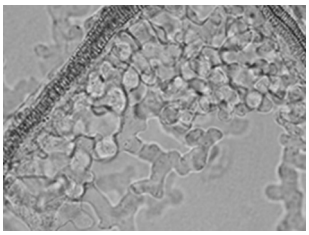
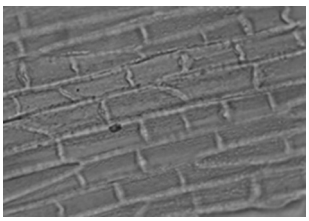
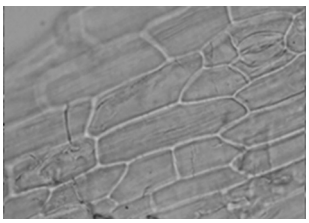
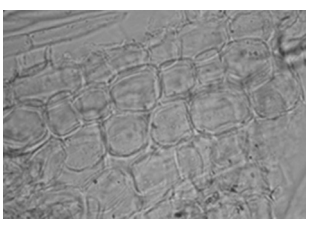
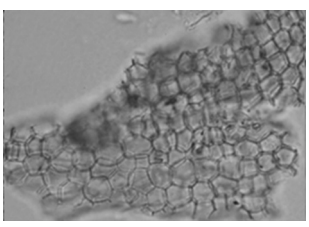
During microscopic analysis of the herb morphological group the following anatomical and diagnostic features were paid attention to:

1. Anatomical and diagnostic features of leaves: Character of the cuticle of the upper and lower epidermis, the form of cells of the upper and lower epidermis, tortuosity of the cells' walls of the upper and lower epidermis, level of tortuosity, thickening of the cells' walls of the upper and lower epidermis; presence of stomata, their shape (round, oval), size, frequency of occurrence of the upper and lower epidermis, stomata type; presence and structure of the hairs on the upper and lower epidermis, their size, trait of the points of attachment(wall outlets), thickening of the walls, the nature of the cuticle; the presence of glands in the upper and lower epidermis, their structure, size; presence of secretory channels, lacticifers, conceptacles (in parenchyma beneath the epidermis); availability and structure of crystalline inclusions and their location, size; availability of spare nutrients inclusions: Mucus, inulin, etc., mesophyll structure, leave structure; structure of leave's conductive system (form of the main vein, number, shape, and arrangement of vascular bundles in the vein, structure of vascular bundles - xylem and phloem location, and the presence of mechanical tissue); presence of mechanical tissue (collenchyma, sclerenchyma fibers, stone cells, bast fibers, etc.); structure of the stem: On a cross section of petiole its shape in the middle, basal, and apical parts (round, triangular, grooved, crescent-shaped, slightly webbed, and wide-winged), number and location of the vascular bundles, presence of mechanical tissue (collenchyma, sclerenchyma) are indicated.
2. Anatomical and diagnostic features of flowers (petals, sepals, spatha leaves, and epidermis of peduncles): Nature of the cuticle of the upper and lower epidermis, shape of cells of the upper and lower epidermis, tortuosity of the cells of the upper and lower epidermis, thickening of the cells' walls of the upper and lower epidermis; presence of stomata, their shape, their size on the upper and lower epidermis, stomata type; number of peristome cells; availability and characteristics of hair on the upper and lower epidermis, their size, trait of the points of attachment; presence and structure of the glands on the upper and lower epidermis, their sizes; the presence of secretory channels, lacticifers, conceptacles (in the parenchyma beneath the epidermis); presence and structure of crystals and their size; presence of inclusions mucilage, inulin, carotenoids, and others; pollen form, nature of its surface, and size of pollen.
3. Anatomical and diagnostic features of fruits: Characteristics of epidermis: Nature of the cuticle (wax deposition), form of the epidermal cells (hypanthium, fruit, and seed); tortuosity of the cells' walls of the epidermis; nature of the thickening of the walls of the epidermal cells; characteristics of the stomata: Presence of stomata in the epidermis, their shape and size; type of stomatal apparatus, number of peristome cells; stomata absorption in the epidermis; presence of lenticels in the epidermis; presence and nature of trichomes (hairs), their size, traits of the points of their attachment; secretory channels, lacticifers, conceptacles; character of mesocarp parenchyma (cell shape and size, uniformity, density of location); aerenchyma presence; nature of conduction system (location and structure of the vascular bundles); nutrients storages and their size; presence of mechanical tissue (stone cells, sclerenchyma fibers).
4. Anatomical and diagnostic features of stem: Nature of the cuticle; form of the epidermal cells; tortuosity of the cells' walls of the epidermis and the level of tortuosity; thickening of the cells' walls of the epidermis (presence of beaded thickening); presence of stomata and their shape, size; type of stomata apparatus; availability, characteristics and size of the hairs, traits of the points of attachment (wall outlets), walls' thickening (thick, thin walls), nature of the cuticle (smooth, warty, streaks); availability and structure of glands, their sizes; presence of secretory channel, lacticifers, conceptacles; presence of crystals, their structure and location, size; presence of inclusions: Mucus, inulin, carotenoids and others, presence of the aerenchyma.

It should be taken into account that cut-pressed granules are derived from herbal raw material grinded to a coarse powder state. Hence, during microscopic analysis, the anatomical and diagnostic features are mostly present in the form of fragments, so separate hairs, gland, crystals, etc., can be found. If fragments of the powder are more than 0.5 mm in size, then almost all characteristic features of the raw material can also be seen on the fragments under test.

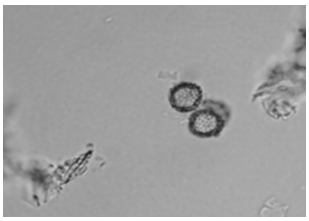
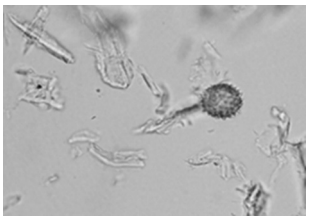
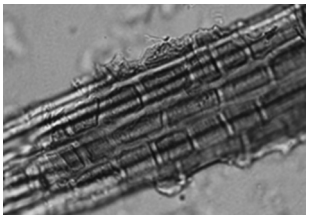
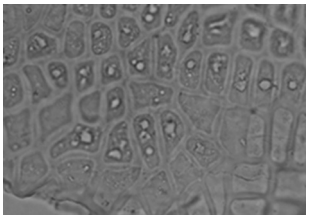
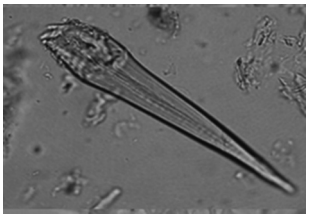
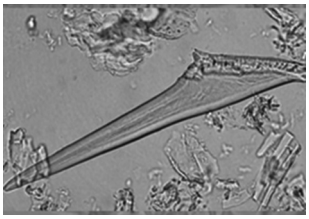
During microscopic examination of tripartite bur-marigold powder preparations, pieces (in longitudinal section) of stems, petioles, achenes; fragments of leaves, bracts, leaves and flowers of the involucre, fragments of epidermal cells with sinuous walls and numerous anomocytic stomata were visible. There were remnants of eruciform and thick-walls hairs with a large cell elongated at the base, sometimes with brown contents inside; fragments of mesophyll secretory passages filled with brown contents; fragments of aerenchyma. There have also been seen fragments of membranous bracts with slightly sinuous and beaded-thickened cell's walls, the remains of the stem and the leave stalk with rectangular tightly closed cells of epidermis and large cells of parenchyma. Furthermore, pieces of epidermis of the corolla's leaves of tubular flowers with spiral vessels and patches of thorny pollen of rounded polyhedral shape were found. Pieces of achenes and their spines with a cover cloth consisting of square, pentagonal and hexagonal cells with uniformly thickened walls, and with the remains of single-celled, thick hairs sometimes can be seen.

Table 1: Anatomical and diagnostic features of tripartite bur-marigold herb (*Bidentis tripartitae* herba) (×200 zoom)

Anatomic and diagnostical features	Powder	Cut-pressed granules
Fragments of the leaf epidermis cells with sinuous walls and numerous anomocytic stomata		
Eruci form hairs		
Think-wall hairs		
Mesophyll fragments with secretory passages filled with brown content		
Aerenchyma		
Rectangular tightly closed cells of stem of epidermis		
Large parenchymal stem cells		

(Contd...)

Table 1: *(Continued)*

Anatomic and diagnostical features	Powder	Cut-pressed granules
Spiny pollen of rounded polyhedral shape		
Square, pentagonal and hexagonal cells with unevenly thickened walls of achenes cover fabric		
Single-celled, thick hairs of achenes		

The characteristics of anatomical and diagnostic features of the powder and the cut-pressed granules of tripartite bur-marigold are presented in Table 1.

CONCLUSION

Microscopic analysis has confirmed the presence of anatomical and diagnostic features, which are specific to tripartite bur-marigold herb, in its powdered form and cut-pressed granules derived from the herbal powder.

This finding indicates that the basic anatomical and diagnostic features of the tripartite bur-marigold herb are preserved during the production of cut-pressed granules: The technological process does not affect the manifestation of features, which can be distinguished during the microscopic analysis of the dosage form. Therefore, cut-pressed granules of tripartite bur-marigold herb can be assessed, in accordance with the State Pharmacopoeia, by their microscopic features.

The results can be used in normative documentation of the examined object and for further research on improving the quality control of medicinal plant preparations.

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