

Changes in Quantitative and Qualitative Composition of Biologically Active Substances in *Bidens tripartita* L. during Granulation Process

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Abstract:

Development of novel dosage forms is the common way of expanding the product range of herbal medicines. Cut-pressed granules, a recently developed in the Russian Federation novel dosage form of herbal drugs, provide better performance (improved dose consistency) of the product during manufacturing than the commonly produced herbal powders. However, only herbal raw material, the quality of which is not altered during granulation, can be used for cut-pressed granules manufacturing. Previously, we have shown that the manufacturing process does not affect morphological and anatomical characteristics of trifid bur-marigold (*Bidens tripartita* L.) – a plant, which is extensively used in folk medicine. Current study is aimed at the assessment of changes in qualitative and quantitative composition of biologically active substances in *B. tripartita* that accompany granulation process. The results of the study demonstrate that chromatographic profiles of both herbal raw material and cut-pressed granules from *B. tripartita* are practically identical. Granulation process does not affect qualitative composition of phenolic compounds in the herbal raw material, and the total flavonoid content also remains unchanged.

Keywords: herbal raw material, dosage form, cut-pressed granules, flavonoids, polysaccharides, trifid bur-marigold, *bidens tripartita*.

INTRODUCTION

Development of novel dosage forms is one of the ways to achieve better provision of patients with herbal drug products. Recently, a unique dosage form of herbal medicinal products – cut-pressed granules – has been developed in the Russian Federation. Cut-pressed granules are essentially compressed pieces (cylindrical, round, or irregular) of granulated herbal raw material, used to obtain aqueous extracts [1]. The necessity for their development is based on the fact that the most common form of herbal raw material processing is simple cutting or powdering. Both cut herbal raw material and herbal powder demonstrate inadequate technological behavior, e.g. poor flowability, which may negatively affect their dosing, especially on modern high-performance processing lines. Thus, some kind of pre-treatment is required if the drug is released in filter tea bags. In case of cut-pressed granules, pre-treatment consists of moistening the powdered herbal raw material with water steam under specified pressure, followed by direct granulation and obtaining of granules of required size [2-4]. It should be mentioned that the manufacturing of cut-pressed granules does not require any of the excipients; only water steam is used.

However, only plant species that do not lose their activity during granulation [1], and that can be standardized in terms of their qualitative and quantitative composition [5, 6], can be used in cut-pressed granules manufacturing.

Previous studies have shown that cut-pressed granules from trifid bur-marigold (*Bidens tripartita* L.) still possess main morphological and anatomical characteristics of the unprocessed herbal raw material, i.e. the processing does not alter these characteristics. Therefore, quality of the granules can be assessed in terms of morphology and anatomy [7].

The aim of our work was to investigate changes in quantitative and qualitative composition of biologically active substances in *B. tripartita* during manufacturing of cut-pressed granules.

MATERIALS AND METHODS

Several commercial batches of powdered trifid bur-marigold (quality complies with the requirements of corresponding pharmacopoeial monograph) [1] and pilot batches of cut-pressed granules obtained from trifid bur-marigold were

used in the study. Analytical samples of the powder and granules were ground to particles passing through a 0.5 mm sieve.

Comparative qualitative analysis of phenolic compounds in trifid bur-marigold powder and cut-pressed granules was performed by thin-layer chromatography (TLC), using "TLC Silica gel 60 F254" aluminum TLC plates (Merck, Germany). Ascending chromatography was performed in TLC chamber (Camag, Switzerland), using ethyl acetate – anhydrous formic acid – water (40:4:6) mixture as a mobile phase. A "Reprostat 3" TLC imaging system (Camag, Switzerland) was used to obtain photographs of the chromatogram. The photographs were processed using Adobe Photoshop 7.0 (Adobe, USA). Spectrophotometric measurements were carried out using Cary 50 UV-Vis spectrophotometer (Varian, USA). Weighing of samples was performed using GH-252 (AND, Japan) analytical balance.

All reagents and solvents used in the study were of analytical grade and were used as received without further purification. Rutin (CAS 153-18-4) and quercetin (CAS 6151-25-3) reference standards (RS) were provided by PhytoLab (Germany).

Manufacturing of cut-pressed granules consisted of the following steps: powdered herbal raw material, passing through a 2 mm sieve, was moistened for 3-4 minutes using saturated steam (vapor pressure – 3.5-5.5 kgf/cm²) under constant stirring for even moist distribution. After that the material was transferred to a compression machine in which the moistened mass was pushed through a 5-7 mm sieve. The material was extruded from the machine in form of 10-30 mm cylinders, which were transferred to the dryer. After drying, the material was force-cooled with air and was transferred to a roll grinder in which it was crushed to granules passing through a 2 mm sieve.

About 1.0 g of the powdered herbal raw material or cut-pressed granules was placed in a 100 ml ground glass flask, 10 ml of 96% ethyl alcohol were added, and the flask was heated under backflow condenser on a boiling water bath for 10 minutes. After cooling to room temperature, the content of the flask was filtered through a paper filter obtaining *Test Solution*.

In order to obtain *Rutin Reference Solution*, about 0.005 g of rutin were dissolved in 10 ml of 96% ethyl alcohol. *Quercetine Reference Solution* was prepared in the same manner.

Test Solution (30 µl), *Rutin Reference Solution* (5 µl), and *Quercetin Reference Solution* (5 µl) were applied to the 10x10 chromatographic plate as 10x3 mm bands (both *Reference Solutions* were applied as a single band). The plate was left to dry at room temperature for 5 minutes, then placed into TLC chamber, which was lined out with filter paper and saturated with mobile phase for 30 minutes. After solvent front has traveled about 80-90% of the TLC plate, the plate was removed and air-dried until evaporation of solvent residues, then the plates were placed in the temperature chamber at 100-105 °C for 2-3 minutes. Following heating, the plates were consequently sprayed with 1% alcoholic solution of diphenylboric acid aminoethyl ester (CAS 524-95-8) and 5% alcoholic solution of polyethylene glycol 400 (CAS 25322-68-3)

Quantitative composition of biologically active substances in *B. tripartita* powder and cut-pressed granules was assessed in terms of Total Flavonoid Content (TFC) and Total Polysaccharide Content (TPC), according to the "Bidens tripartita herb" pharmacopoeial monograph [1].

TFC (expressed as rutin) was assayed using the following procedure: about 1.0 g (exact weight) of the powdered material were placed in a 250 ml ground glass flask, 50 ml of 70% ethyl alcohol were added, and the flask was weighed to the nearest 0.01 g. The flask was connected to a backflow condenser and heated on a boiling water bath for 1 hour, then cooled to room temperature and weighed once again, bringing the content to its original mass with 96% ethyl alcohol, if necessary. The content of the flask was filtered through a paper filter, discarding first 25 ml of the filtrate (*Solution A*).

One (1.0) ml of the *Solution A* was transferred to a 25 ml volumetric flask and 5 ml of 2% alcoholic solution of aluminum chloride were added. The solution was brought to volume with 96% ethyl alcohol and mixed (*Solution B*). The solution was left for 40 minutes, then its absorbance was measured at 415 nm in 10 mm cuvette. The solution containing 1.0 ml of *Test Solution A* and 0.1 ml of concentrated acetic acid, mixed in a 25 ml volumetric flask and brought to volume with 96% ethyl alcohol, was used as the *Reference solution*.

TFC, expressed as per cent (X) of rutin equivalent per g of dry material, was calculated using the following formula:

$$X = \frac{A \cdot 25 \cdot 50 \cdot 100}{A_{1\text{cm}}^{1\%} \cdot a \cdot 1 \cdot (100 - W)}$$

where:

A – absorbance of *Solution B*;

$A_{1\text{cm}}^{1\%}$ – specific absorbance of rutin-AlCl₃ complex at 415 nm (=260);

a – sample mass, g;

W – moisture content, %.

TPC was assayed as follows: about 10 g (exact weight) of powdered material were placed into a 250 ml ground glass flask, 100 ml of water were added, the flask was placed on a hot plate and boiled under reflux condenser for 30 minutes, stirring continuously. The extraction was repeated four more times, each time using another 100 ml of water. Aqueous extracts were centrifuged at 5000 RPM for 10 minutes, then decanted into 500 ml volumetric flask through five layers of gauze, pre-wetted and

placed into a funnel. The filter was rinsed with water and the solution was brought to volume with water (*Solution A*).

Twenty five (25.0) ml of the *Solution A* were placed into a 100 ml centrifuge test tube and 75 ml of 96% ethyl alcohol were added. The content was mixed and heated on a water bath at 60 °C for 5 minutes. The mixture was allowed to stand for 30 minutes, then centrifuged at 5000 RPM for 30 minutes.

Supernatant was filtered under vacuum (13-16 kPa residual pressure) through a 40 mm POR 16 glass filter, previously dried to constant weight at 100-105 °C. After that, the precipitate was quantitatively transferred to the same filter and washed with 15 of 96% ethyl alcohol-water mixture (3:1). The filter was first air-dried, then dried at 100-105 °C to constant mass.

TPC, expressed as per cent (X) per g of dry material, was calculated using the following formula:

$$X = \frac{(m_2 - m_1) \cdot 500 \cdot 100 \cdot 100}{a \cdot 25 \cdot (100 - W)}$$

where:

m_1 – filter weight, g;

m_2 – filter weight with precipitate, g;

a – sample mass, g;

W – moisture content, %.

RESULTS AND DISCUSSION

The chromatogram of the RS mixture shows two zones: yellow, yellow-orange or orange zone (rutin RS) and, close to the finish line, another yellow, yellow-orange or orange zone (quercetin RS).

The chromatogram of test solutions demonstrates two red or violet-red absorption zones, located between zones due to rutin RS and quercetin RS; yellow absorption zone between these two red or violet-red zones; yellow or pink-yellow zone at the level of quercetin RS zone. Additional red or violet-red zones might be observed (Figure 1).

In UV light (365 nm), the chromatogram of RS mixture also shows two yellow, yellow-orange or orange zones due to rutin RS and quercetin RS, the latter being located close to the finish line.

The chromatogram of test solutions shows the following zones due to phenolic compounds: two absorption zones with red-brown fluorescence or non-fluorescent (dark) zones between zones due to rutin RS and quercetin RS; two orange-yellow or pink-yellow fluorescent absorption zones below and at the level of quercetin RS zone; and two absorption zones with blue fluorescence, located between orange-yellow or pink-yellow zones. Additional zones might be observed (Figure 2).

It can be seen, that the chromatographic profiles of trifid bur-marigold powder and pilot batches of cut-pressed granules are similar. Thus, it can be concluded that granulation process does not affect phenolic compound composition of trifid bur-marigold.

The analysis of pilot batches of cut-pressed granules from *B. tripartita* showed that TFC (expressed as rutin) and TPC remained within limits, described in the pharmacopoeial monograph for the herbal raw material (not less than 0.5% and not less than 3.5%, respectively) [1]. It can be concluded that the process of granulation does not negatively alter biologically active compounds content (Table 1).

Table 1. Total flavonoid content and total polysaccharide content of *B. tripartita* powder and cut-pressed granules (mean of 3 measurements).

<i>B. tripartita</i> herb			<i>B. tripartita</i> cut-pressed granules		
Batch No.	TFC expressed as rutin, %	TPC, %	Sample	TFC expressed as rutin, %	TPC, %
20217	0.84	3.98	cut-pressed granules from batch 20217	0.83	3.96
60418	0.71	4.11	cut-pressed granules from batch 60418	0.72	4.08

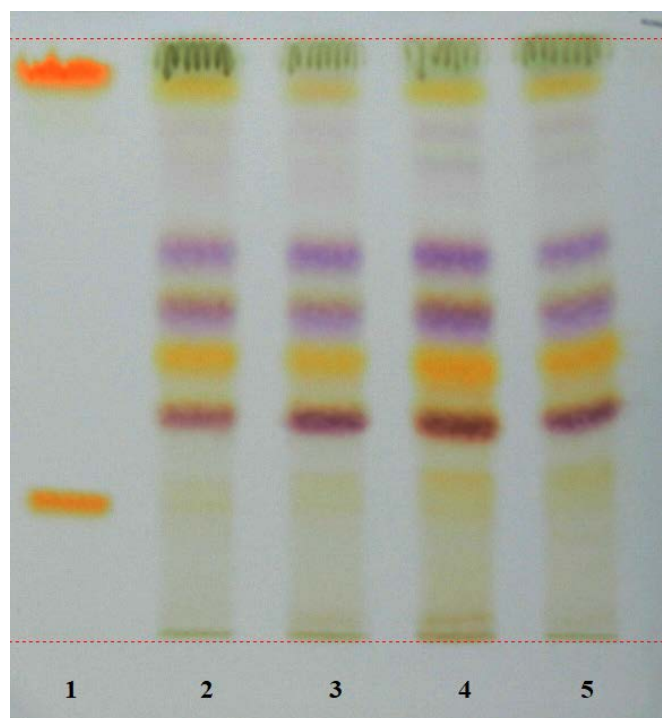


Figure 1. Photo of phenolic compounds chromatogram obtained using *B. tripartita* cut-preserved granules and powder after spraying with 1% alcoholic solution of diphenylboric acid aminoethyl ester and 5% alcoholic solution of polyethylene glycol 400 (daylight):

- 1 – mixture of reference standards (0.05% alcoholic solution of rutin RS 0.05% alcoholic solution of quercetin RS, 5 μ l of each);
- 2 – extract from trifid bur-marigold cut-preserved granules, Batch No. 20217, 30 μ l;
- 3 – extract from trifid bur-marigold herbal raw material, Batch No. 20217, 30 μ l;
- 4 – extract from trifid bur-marigold cut-preserved granules, Batch No. 60418, 30 μ l;
- 5 – extract from trifid bur-marigold herbal raw material, Batch No. 60418, 30 μ l.

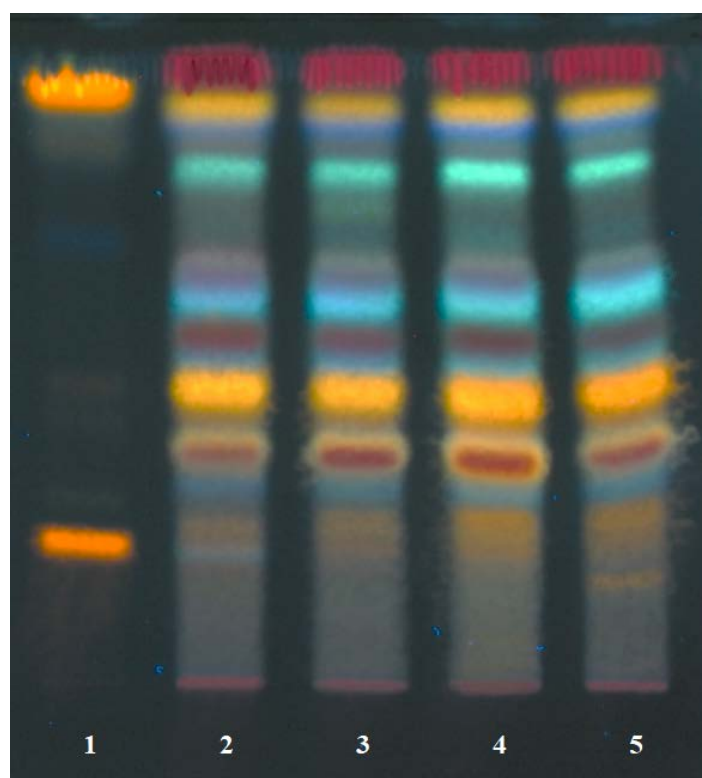


Figure 2. Photo of phenolic compounds chromatogram obtained using *B. tripartita* cut-preserved granules and powder after spraying with 1% alcoholic solution of diphenylboric acid aminoethyl ester and 5% alcoholic solution of polyethylene glycol 400 (365 nm UV light):

- 1 – mixture of reference standards (0.05% alcoholic solution of rutin RS 0.05% alcoholic solution of quercetin RS, 5 μ l of each);
- 2 – extract from trifid bur-marigold cut-preserved granules, Batch No. 20217, 30 μ l;
- 3 – extract from trifid bur-marigold herbal raw material, Batch No. 20217, 30 μ l;
- 4 – extract from trifid bur-marigold cut-preserved granules, Batch No. 60418, 30 μ l;
- 5 – extract from trifid bur-marigold herbal raw material, Batch No. 60418, 30 μ l.

CONCLUSION

It was established that the chromatographic profiles of trifold bur-marigold powder and cut-pressed granules are similar, i.e., granulation process does not affect phenolic composition of the herbal products. It was also shown, that the granulation process does not alter biologically active substances content.

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