

Research Article

## The Study of Biological Active Substances of Thistle Curled (*Carduus crispus L.*)

Omirbaeva A.E., Datkhaev U.M., Asfendiarovs' National Medicinal University, Almaty,  
Republic of Kazakhstan, Grudko V.A., Bevz N.Yu., Gladukh Eu. V., Iudina Iu. V.

*National University of Pharmacy, Kharkov, Ukraine*

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### ABSTRACT

Currently in Kazakhstan Republic actively introduced fitopreparations based on domestic vegetable raw materials. One of the promising plant is curled thistle (*Carduus crispus L.*). The aim of this work was to study biologically active substances thistle curly herb by physico-chemical methods (UV spectrophotometry and thin layer chromatography) and by chemical reactions. The studies found that the studied raw material contains about 0.25% of total flavonoids in terms hyperaside and is promising in terms of the search of herbal drugs with hepatoprotective action.

**Keywords:** Curled thistle (*Carduus crispus L.*), raw material, biological active substances, flavonoids, physical and chemical methods of analysis.

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### INTRODUCTION

One of the directions of Kazakhstan pharmaceutical science development is the creation of new herbal drugs. The object of our research is curled thistle (*Carduus crispus L.*), widespread on almost the entire territory of the Kazakh Republic. Preparations on the basis of this plant have long been used in folk medicine and have anti-inflammatory, antioxidant and hepatoprotective effect<sup>1</sup>. Such wide spectrum of activity is determined by a complex of biologically active substances contained in the plant. However, until now there were no purposeful studies of the chemical composition of the plant.

The aim of this work is to study biologically active substances of curly thistle herb, harvested in the period of mass flowering in the territory of South Kazakhstan region.

#### *Objects and methods of research*

The object is a dry powdered aerial part of thistle curled. Raw materials harvested in 2014 (South Kazakhstan region) during the plants mass flowering.

For work was used volumetric ware of class A and reagents meet the requirements of the State Pharmacopoeia of the Republic of Kazakhstan, analytical balances «AXIS», spectrophotometer Evolution 60S, thin-layer plates with a layer of silica gel GF<sub>254</sub>.

Thin-layer chromatography (2.2.27) [2,3].

*Test solution.* 0.100 g of the extract was dissolved in 25 ml of 96% alcohol.

*Reference solution.* 5 mg quercetin R and 5 mg of rutin R dissolved in 5 ml of 96% alcohol.

On the start line of TLC plate with a layer of silica gel P applied as strips 20 microliters of test solution and reference solution. The plate is placed in a chamber with a solvent system acetic acid glacial R - water R - acetate

R (20:20:60). When the solvent has reached 10 cm from the starting line, the plate is removed from the chamber, air dried, sprayed with 5% ethanolic solution of aluminum chloride, dried for 10 minutes before the appearance of spots and viewing under UV light.

On the chromatograms of the test solution are observed area at the zone level of the reference solution, corresponding to routine and quercetin. On the chromatogram of the test solution can be detected additional zones.

#### *Quantitative determination*

The basic solution. 1.00 g of the powdered raw material (355), (2.9.12), 1 ml of a solution of 5 g / L hexamethylenetetramine R, 20 ml of acetone R and 2 ml of hydrochloric acid R1 was placed in a round bottom flask of 100 ml, refluxed in for 30 min and filtered through a cotton swab into the flask. A cotton swab was placed in a round bottom flask and the residue was extracted with acetone P in two portions of 10 ml, each time conducting boiling with reflux for 10 min and than cooled. Each extract was filtered through a cotton swab into the flask. Cooled and united acetone extracts were filtered through filter paper into a volumetric flask. Rinsing the flask and washing filter with acetone P, the solution volume was adjusted up to 50.0 ml. 20.0 ml of this solution placed into a separatory funnel, add 20 ml of water and shake the mixture with 15 ml of ethyl acetate R, adding 2.0 g of finely ground powder of sodium chloride, and then - with three portions of ethyl acetate P of 10 ml. The ethyl acetate extract were combined in a separatory funnel, washed with 2 portions of water R of 50 ml, filtered through filter paper, containing a layer of 10 g of anhydrous sodium sulphate R in the flask and the

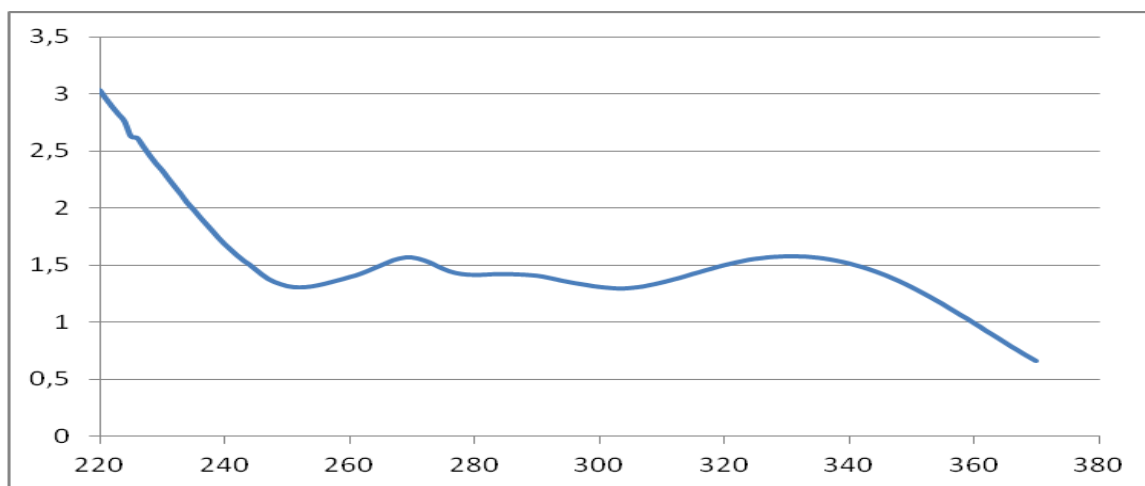


Figure 1: Adsorption spectrum of the alcohol extract of the thistle curly herb

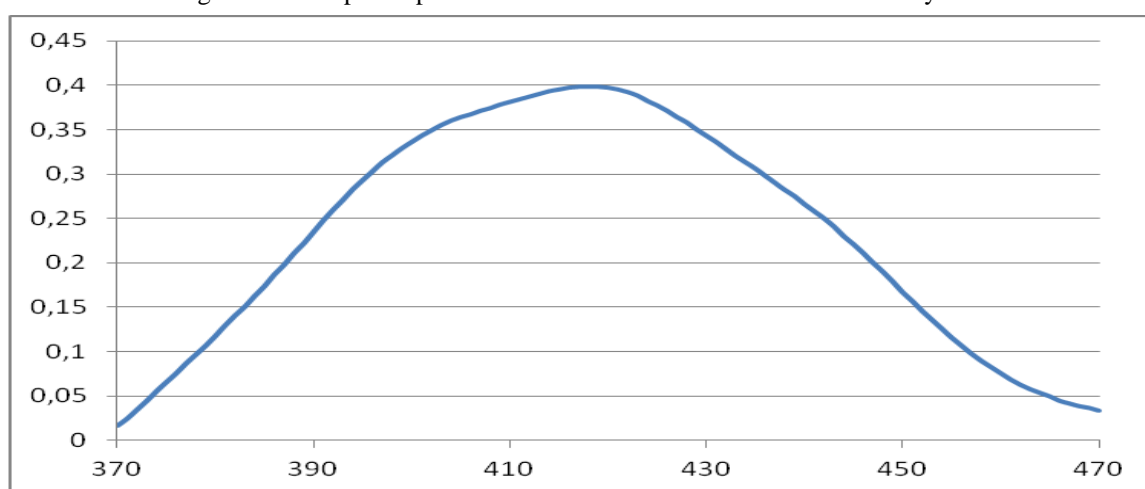


Figure 2: The adsorption spectrum of the reaction product of the thistle herb alcohol extract and aluminum chloride

solution volume was adjusted with ethyl acetate R up to 50.0 mL.

The test solution. To 10.0 ml of the basic solution was added 1 ml of the aluminum chloride P and the solution volume adjusted by 5% (v / v) solution of glacial acetic acid P in the ethyl alcohol 96% to about 25.0 ml.

Compensating solution. 10.0 ml of the basic solution was adjusted by 5% (v / v) solution of glacial acetic acid in 96% ethyl alcohol R to a volume of 25.0 ml.

Measure the optical density (2.2.25) of the test solution at a wavelength 425 nm 30 minutes after its preparation using compensating solution.

The content of total flavonoids in terms of hyperoside (C<sub>21</sub>H<sub>20</sub>O<sub>12</sub>; M. 464.40) in dry raw material is 0.25%.

## RESULTS AND DISCUSSION

To study the *Carduus crispus* extract compose was analyzed adsorption spectrum of 0.02% alcoholic solution, described in the 220 nm to 370 nm. The spectrum is characterized by peaks at wavelengths of 270 nm and 285 nm, typical for absorption of aromatic compounds, which suggests the presence of polyphenolic compounds in the extract. Absorption maximum at 330

nm can indicate the presence of hydroxycinnamic acids in the sample (Fig. 1). Based on the location of absorption bands observed in the absorption spectrum in the region from 270 nm to 285 nm and 330 nm can assume the presence of natural flavonoid compounds<sup>4</sup>.

To further establish the composition of the biologically active substances in the extract was analyzed using thin layer chromatography. Determination was carried out on plates "Sorbfil" in solvent system glacial acetic acid - water - ethyl acetate (20:20:60) as detector was used 5% alcoholic solution of aluminum chloride. Comparison was carried out with a mixture of standard solutions of rutin and quercetin. Chromatograms viewed in the UV range. It was found that in the sample are observed zones at the rutin and quercetin zone level, which may indicate the presence of similar structure flavonoids.

The presence of flavonoids was established also by qualitative reactions (reaction of pyrylium salts formation with an alcoholic solution of alkali and alcohol solution of aluminum chloride).

We have found that the absorption spectrum of reaction product of analyzed thistle herb alcohol extract with aluminum chloride reagent in the medium of acetic acid

(Fig. 2) is characterized by a maximum absorption at 418 nm, which allows standardization of thistle extract by the presence of flavonoids.

Therefore, for a quantitative assessment of flavonoids in the thistle herb extract was used method based on the initial hydrolysis of flavonoid glycosides and determining optical density of the solution obtained after aglycones reaction with aluminum chloride in acetic acid medium at a wavelength of 420 nm. The content of flavonoid aglycones was calculated using the specific absorption index in terms of hyperoside and it was in dry herb 0.25%.

The results will be used to develop methods of quality control of analyzed raw material. It is planned to conduct pharmacological studies of raw materials, as well as extract thistle curled. Since flavonoids contained in the raw material, the active drug will presumably have a hepatoprotective and anti-inflammatory properties.

## **CONCLUSION**

The studies found that the analyzed raw materials – herb of thistle curled (*Carduus crispus* L.) contains in its composition substances of polyphenolic nature.

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