Isolation of major flavonoids from Kazakhstan endemic plant
Crataegus almaatensis Pojark by high speed counter-current chromatography and their quantification by HPLC.

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Introduction: Hawthorn (Crataegus L.) is a well-known widely used medicinal plant with more than 280 species that are spread in all over the world [1]. There are seven species of Crataegus growing in the Kazakhstan flora, from which only Crataegus almaatensis Pojark (C. almaatensis) is an endemic one. It is a dense, spiny tree up to 3-4 m tall producing dense white colored flowers. It is spread in the foothills of Ile-Alataumountains in Almaty [2,3]. Crataegus is used as an alternative medicine for ailments of the cardiovascular, digestive and endocrine systems[4]. These plants are rich in biologic active compounds, such as flavonoids, hydrocinnamic acids, sugars, organic or phenolic acids, terpenes and essential oils [5]. There are a few scientific studies on the biochemistry of cultivated C. almaatensis fruits, focused on the determination of carotene, bioflavonoids, sugar and organic acids contents [6,7]. There is no full-scale study of the C. almaatensis to date. The authors of this work, from Kazakhstan and from two other European Universities are carrying out joint scientific full-scale studies with this endemic plant.

The aim of this study is to present preliminary results on isolation and quantification of the main constituents of C. almaatensis flowers, leaves and fruits by means of high speed counter current chromatography (HSCCC) and HPLC respectively.

Methods and Results: The HSCCC was performed using an instrument IntroPrep™ (Quattro). The solvent system HEMWat was chosen according to the partitioning of the constituents of the plant material on a TLC analysis. Identification of the isolated compounds was carried out by means of different NMR spectra. Reverse phase HPLC system (Waters) was used for the quantification of the isolated compounds using the following mobile phase: (A) 40mM potassium dihydrogen phosphate (pH 2.3 with 85% Orthophosphoric acid); (B) Methanol, 0-52.5 min 95%A-5%B; 52.5-57 min 58%A-42%B; 57-60 min 95%A-5%B, with a flow of 1ml/min.

Two main flavonoids hyperoside and quercetin-3-O-rhamnoside were isolated and identified. The quantity of hyperoside was determined to be 19.79±0.36 mg/g, 14.70±0.37 mg/g and 0.27±0.01 mg/g for flowers, leaves and fruits respectively.

The concentration of quercetin-3-O-rhamnoside in the extract was found to decrease from leaves (51.00±0.92 mg/g) to flowers (1.23±0.05 mg/g) to fruits (almost undetectable).
Conclusions:
At this stage of studies we can conclude that *C. almaatensis* is a prospective medicinal plant, which can be used for the development of domestic phytomedicines, and we can point out that the leaves of this plant accumulate more flavonoids than other parts of the plant, which could suggest their use as raw materials in phytopharma industry.

**Key words:** Hawthorn, *Crataegus almaatensis*, Kazakhstan, HSCCC, HPLC