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PHYTOCHEMICAL STUDY OF *CENTAUREA CYANUS* L.

TATIANA CHIRU

The State Medical and Pharmaceutical University "Nicolae Testemitanu"

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Abstarct

In this work it was proposed to undertake the study of phenolic compounds present not only in the species of Centaurea cyanus L. with blue flowers (which are used in medicine), but also with the flowers of other colors (red, pink), which are not valued until now. By qualitative determination, using chemical reaction and chromatography, some groups of phenolic compounds were established: phenilpropanic substances, flavonoids, anthocyanins and tannins. The total of phenilpropanic compounds, flavonoids and anthocyanins was determined spectrophotometrically, the total of tannins – using titration. These analysis have clarified the differences between the concentration of active principles in the blue flowers and those of another color. It was determined that anthocyanins, the main metabolites that confer a C. cyanus L. its pharmacological effects - diuretics, antiinflamateur, healing and so on, are more concentrated in red inflorescences (4-5 times more anthocyanins then in blue inflorescences). Other phenolic compounds also have a higher amount in the red flowers then in the blue.

INTRODUCTION

Centaurea cyanus L. (cornflower) is one of the species of *Asteraceae* that has not been the subject of many investigations. It is an annual plant, growing to a height of 1-1.5 m, with strong stems and grayish slightly furry leaves, with small clusters of bright blue flowers [5]. Cornflower is native to Europe, where it is a weed in fields. It is considered to be a good companion, in small quantities, for cereal crops, though another report says that its greedy roots deprive the cultivated plants of nutrients and its tough stem dulls the reaper's sickle. However, it is also used as an ornamental plant because of its intense blue flowers, which are often used in dried-flower arrangements because they retain their color well [2]. Cornflower has a long history of herbal use, though it is seldom employed nowadays. Externally it is used as an anti-inflammatory and astringent herb for eye ailments and skin cleansing. An eye wash made with cornflower blossoms is used for conjunctivitis as well as to relieve strained, tired or puffy eyes. Blue blossoms infused in water have both curative and calming action for nervous disorders. Eye wash is reputed to strengthen weak eyes. Traditionally it is said to work best on blue eyes. The dried flowers are antipruritic, antitussive, astringent, weakly diuretic, emmenagogue,

ophthalmic, very mildly purgative, and tonic. An infusion can be used in the treatment of dropsy, constipation, or as a mouthwash for ulcers and bleeding gums. This infusion is also taken as a bitter tonic and stimulant, improving the digestion and possibly supporting the liver as well as improving resistance to infections. Water distilled from the marginal flowers was formerly in repute as a remedy for weak eyes and a soothing lotion for conjunctivitis. The seeds are used as a mild laxative for children. Cornflower leaves are used to create a cleansing facial steam for dry sensitive skin. A decoction of the leaves is antirheumatic. These effects are determined by the presence of the phenolic compounds (flavonoids, tannins, caffeic and chlorogenic acids), which are very poorly studied in *C. cyanus* L. [9].

Phenolic compounds are ubiquitous constituents of higher plants found in a wide range of commonly consumed plant foods such as fruits, vegetables, cereals and legumes. They are diverse in structure but are characterized by hydroxylated aromatic rings. These compounds are secondary metabolites of plants generally involved in defense against ultraviolet radiation or aggression by pathogens. They have been the subject of a great number of chemical, biological, agricultural and medical studies. Plant phenolics have received considerable attention because of their potential antioxidant activity as well as hepatoprotective, hypoglycemic and antiviral activities. Human consumption of antioxidants has many alleged health benefits, including protection against cardiovascular diseases, and, most recently, cancer [1, 4]. Therefore the studies of the plants, which are sources of phenolic compounds represent a great actuality.

MATERIAL AND METHODS

Plant materials (aerial parts, inflorescences, marginal and disc flowers) were collected during the flowering period from the South of Basarabia in June, 2008. After powdering, these materials have been exposed to physico-chemical analysis.

The phenilpropanic compounds were identified by thin-layer chromatography [7]. 1 g of plant material was extracted by refluxing with 10 ml methanol and concentrated to 4 ml. Operating conditions were as follows:

- mobile phase: ethyl acetate–acetic acid–formic acid–water (100:11:11:26);
- stationary phase: silica gel plate;
- etalon solutions: caffeic and chlorogenic acids;
- identification in visible and UV specter.

The total of the phenilpropanic compounds in blue and purple inflorescences, in aerial parts with blue an purple inflorescences of *C. cyanus* L., was determined using spectrophotometric analysis, at 500 nm wavelength (caffeic acid as reference substance) [7]: 4.0000 g powdered plant material with 80 ml ethylic alcohol 50% is put in a bottle, weighted and extracted in reflux during 30 min. After cooling, the bottle is restored to the initial weight with ethylic alcohol 50% and filtrated

(solution A). 5 ml of solution A is diluted in a 50 ml marked bottle with ethanol 50%. To 1 ml of this solution are added: 1 ml chlorhydric acid 0.5 n, 1 ml Arnow reactive, 1 ml NaOH 1 n and it's filled with water till the 10 ml mark in a marked bottle (resulting a red solution). After 10 min the absorbance is determined in comparison with the following solution: 0.5 ml solution A, 1 ml HCl 0.5 n, 1 ml NaOH 1 n and water till 10 ml mark.

For the quantitative determination of tannins (in aerial parts and blue inflorescences), was employed the titrimetric method [3], based on its oxidation with potassium permanganate: 2 g of plant materials with 50 ml boiling water are warmed up. After 30 min the solution is filtrated in a bottle with 250 ml volume. The extraction is repeated until tannins reaction (with ferric alum) is negative. The bottle containing the cold solution is filled with water to the marked level (250 ml) and is transferred in a 1 l bottle, 750 ml water and 25 ml acid indigosulphonic solution are added. Then it is titrated with potassium permanganate 0.1 n until the solution becomes yellow. Similarly the control probe (25 ml indigosulphonic acid in 750 ml water) is titrated. The total quantity of tannins is calculated using the formula:

$$x = \frac{(V_1 - V_2)K \cdot D \cdot V \cdot 100 \cdot 100}{mV_3(100 - \omega)}$$

V_1 – used volume of potassium permanganate 0.1 n, ml; V_2 – used volume of potassium permanganate 0.1 n for the titration of the control probe, ml; K – the correction to the titer (via oxalic acid); D – the coefficient of tannin; V – the total volume of the extract, ml; m – the exact mass of plant material, g; V_3 – volume of extract used for titration, ml.

RESULTS AND DISCUSSION

By qualitative determination, using chemical reaction and chromatography, some groups of phenolic compounds were established: phenilpropanic substances [7], flavonoids [6, 10], anthocyanins [6, 8] and tannins [3] (reaction with ferric alum proves that tannins are condensated). The caffeic and chlorogenic acids were determined qualitatively in blue and purple inflorescences, in aerial parts with blue an purple inflorescences of *C. cyanus* L., using thin–layer chromatography.

The results of quantitative analysis, obtained in our actual and previous studies [6], are listed in table 1.

In the scientific medicine only the blue flowers of *C. cyanus* L., but not others exemplars (with purple, pink or white inflorescences), are employed. Strikingly, these exemplars generally are ignored and not studied, their unique utilization is as ornamental plants. According to our investigations in the purple flowers the content of active agents is higher than their level in the blue flowers (table 1). In this case

the obtained pharmaceutical products from purple flowers of *C. cyanus* L. can be more economically (efficiently). Sure is the fact that additional studies are needed.

Table 1

Results of quantitative analysis

Plant material		**Anthocyanin (%)**	**Phenilpropanic compounds (%)**	**Tannins (%)**	**Flavonoids (%)**
Inflorescences	purple	1.3574	0.360		0.206
	blue	0.2418	0.150	0.291	0.193
	pink	0.0700			0.218
Disc flowers	purple	2.2534			0.212
	blue	0.4916			0.175
	pink	0.1909			0.193
Marginal flowers	purple	3.6313			0.218
	blue	0.6716			0.112
	pink	0.2330			
Aerial parts	purple		0.240		0.262
	blue		0.313	4.656	0.218
radix					0.093
fruits					0.112

CONCLUSIONS

Our investigations prove that:

1. A great importance in the content of active principles makes the colour of the flowers:
   - the highest concentration of flavonoids are determined in purple flowers (0.21 – 0.22%), next – pink flowers (0.19%) and the lowest – in blue flowers (actually used in medicine); in pink – 0.22%, purple – 0.2% and blue inflorescences – 0.17%;
   - anthocyanins highest concentration is in purple (1.36 – 3.63%), in blue – (0.24 – 0.67%) and in pink flowers and inflorescences – (0.07 – 0.23%);
   - in purple inflorescences the concentration of phenilpropanic compounds is higher (0.36%) than in blue inflorescences (0.15%), but in aerial parts with purple inflorescences is lowest (0.24%) than in aerial parts with blue inflorescences (0.31%).

2. Also has an importance the part of the plant:
   - the total of flavonoids in aerial parts is the highest (0.22 – 0.26%), followed by inflorescences (0.19 – 0.21%), marginal and disc flowers (0.11 – 0.22% and 0.17 – 0.21% respectively), fruits (0.11%) and radix (0.09%);
   - the total of tannins in aerial parts from the exemplars with blue inflorescences is higher than only in inflorescences (4.656% and 0.291% respectively).

These results stimulate us to continue our investigations, to make better the elaborating and standardization of new pharmaceutical products.

REFERENCES

1. Cheynier V., 2005. *Polyphenols in foods are more complex than often thought*. Am. J. Clin. Nutr. 81(suppl.) (pp. 223S-229S).
2. Chevalier A., 2001. *The Encyclopedia of Medicinal Plants*. London, Dorling Kindersley.
3. Ladîghina E.I., L.N. Safronic, V.E. Otriaşenkova, 1993. *Analiza chimică a plantelor medicinale.* Chişinău (pp. 112-116).
4. Manach C., A. Scalbert, C. Morand, 2004. *Polyphenols: food sources and bioavailability.* Am. J. Clin. Nutr. 79 (pp. 727-747).
5. Negru A., 2007. *Determinator de plante din flora Republicii Moldova*. Chişinău (pp. 259-262).
6. Nistreanu A., T. Chiru, 2007. *Centaurea cyanus L. - sursă de diverşi compuşi fenolici.* Anale ştiinţifice ale USMF Nicolae Testemiţanu. Chişinău (pp. 271-274).
7. Oniga I., Hanganu D., 2004. *Analiza produselor naturale medicinale.* Cluj-Napoca (pp. 85-102).
8. *Государственная Фармакопея СССР*. Москва, 1990, с.238-239.
9. *Растительные ресурсы СССР. Семейство Asteraceae.* Санкт-Петербург, 1993, с.83-93.
10. 1993, *Farmacopeea Română.* Ed. X. Bucureşti (pp. 334-335).