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Pharmacognostic Study of the Involucre of *Centaurea cyanus* L.: Morphological, Anatomical, and Phytochemical Characteristics

Yeluzaveta Shevchenko, Iryna Cholak

Bogomolets National Medical University, Kyiv, Ukraine

Corresponding Author:

Yeluzaveta Shevchenko

+380666859050

liza.v.shevchenko09@gmail.com

Abstract: *one of the relevant directions for the rational use of raw material resources and reducing the cost of pharmaceuticals is the comprehensive processing of plant materials in the pharmaceutical industry. Often, during harvesting and preparation of medicinal plants, a significant amount of waste remains unused in practice, leading to the loss of valuable biologically active compounds. One such plant is the cornflower (*Centaurea cyanus* L.), belonging to the Asteraceae family. This plant has long been known for its medicinal properties and is widely used in both traditional and official medicine. In medicinal practice, funnel-shaped and tubular flowers of cornflower are mainly utilized due to their pronounced anti-inflammatory, diuretic, antibacterial, immunomodulatory, antioxidant, and capillary-strengthening effects, making them effective for cardiovascular disease prevention. These pharmacological properties result from the presence of a wide range of biologically active compounds, including flavonoids, hydroxycinnamic acids, tannins, and polysaccharides. However, during the harvesting of cornflower blossoms, the involucre is typically discarded, as its pharmacological potential has not been sufficiently investigated. Preliminary studies, however, indicate the promising use of this part due to its high content of biologically active substances. Thus, this study aimed to comprehensively investigate the involucre of cornflower blossoms to determine its chemical composition and evaluate its potential for use in pharmaceutical practice. To achieve this goal, microscopic and phytochemical methods of analysis according to the requirements of the State Pharmacopoeia of Ukraine (SPhU) were employed. Microscopic analysis, conducted using a scanning electron microscope (SEM, JSM-6060LA, Japan), revealed characteristic ultrastructural features, including spiral-twisted multicellular trichomes and elongated stomata located along the central vein of the involucre leaf. Additionally, distinctly visible accumulations of calcium oxalate crystals were identified on the leaf surface. Phytochemical analysis showed a high content of polyphenolic compounds (79.46 ± 0.65 mg/g), significantly exceeding their levels in other plant parts. Additionally, water-soluble polysaccharides and pectins were identified, which are known for their anti-inflammatory, detoxifying, antibacterial, and immunostimulating properties. The obtained results suggest the cornflower involucre as a promising raw material for developing new medicinal products with pronounced therapeutic properties. Further research should focus on clarifying the full spectrum of biologically active substances, thoroughly investigating their pharmacological activities, and optimizing extraction technologies, thereby significantly contributing to the development of the domestic pharmaceutical industry.*

Keywords: [Centaurea](#); [Microscopy](#); [Polysaccharides](#); [Phenols](#); [Pectins](#); [Flowers](#).

Introduction

Comprehensive processing of plant raw materials in the pharmaceutical industry is one of the relevant approaches for the rational use of resources, the development of medicinal products with various biological activities, and the reduction of production costs. Often, during the harvesting and preparation of medicinal plants, a significant amount of waste remains unused.

One such plant is the cornflower (*Centaurea cyanus L.*) from the Asteraceae family. For medicinal purposes, the ligulate and tubular flowers are used, which exhibit anti-inflammatory, diuretic, antibacterial, and immunomodulatory effects. These activities are attributed to polyphenolic compounds, such as flavonoids, phenolic acids, tannins, and polysaccharides [1,2,3,4,5,6,7].

In the harvesting of cornflower flowers, the peripheral, blue, ligulate, and partially tubular, violet flowers are plucked. The receptacle and involucre are discarded, as these parts of the plant are insufficiently studied and not utilized [8,9,10].

Since one of the main directions of modern pharmaceutical industry is the zero-waste processing of medicinal plant raw materials, studying the chemical composition of the cornflower involucre is a relevant and timely issue.

Analysis of the literature shows that the extract from the cornflower involucre contains hydroxycinnamic acids – caffeic, succinic, and syringic acids. Subsequent studies of the biological activity of this extract revealed its antioxidant and antibacterial effects [11].

Therefore, it was appropriate to perform a quantitative study of the polyphenolic compounds in the raw material, as well as to fractionate the polysaccharides - whose medicinal properties are associated with cornflower - and to study the macro- and microscopic characteristics of the involucre for the development of quality control methods [12].

Aim

To investigate the quantitative content of polyphenolic compounds in the cornflower involucre, to perform fractionation of polysaccharides with subsequent quantitative

determination of the content of water-soluble polysaccharides and pectin, and to establish the morphological and anatomical characteristics of the raw material.

Materials and methods

The raw material of the cornflower was collected in Kyiv region, Brovary district, near Knyazhichy village. The involucre, flowers, and herb of cornflower were taken for the study.

The quantitative content of polyphenolic compounds in the involucre was determined in methanolic extracts of the raw material using a spectrophotometric method on a 6850 UV/VIS JENWAY spectrophotometer at a wavelength of 750 nm. Folin–Ciocalteu reagent (Sigma-Aldrich®, Germany), a sodium carbonate solution, and distilled water were used as reagents.

Before the spectrophotometric measurements, a calibration curve was constructed for the dependence of the concentration of gallic acid on the optical density of the solution. The total phenolic content was determined in mg/g, expressed as gallic acid equivalents based on the air-dry raw material [13].

For comparative analysis, the content of polyphenolic compounds was also determined in methanolic extracts from the flowers and herb of cornflower.

The quantitative content of water-soluble polysaccharides (WSP) in the involucre was determined by a gravimetric method according to the SPhU monograph “Althaeae radix” [14]. The residue remaining after obtaining the WSP was used to obtain pectic substances (PS). The extraction was carried out twice using a mixture of 0.5% oxalic acid solution and 0.5% ammonium oxalate solution in a 1:1 ratio at a temperature of 80–85°C for 2 hours. Subsequently, the extracts were combined, concentrated, and the pectic substances were precipitated by adding three volumes of 96% ethanol. The precipitate was washed with 96% ethanol, dried, and weighed [15].

For the study of the external characteristics of the cornflower involucre, intact, undamaged raw material was selected.

Microscopic studies of the raw material were carried out using a scanning electron microscope (SEM, JSM-6060LA, Japan). The

ultrastructure of the surface of the cornflower involucre was examined at 10× magnification. The raw material was fixed on brass stubs and coated with a thin layer of gold and platinum in a vacuum chamber.

Results and discussion

The results of the quantitative determination of polyphenols in the methanolic extracts from the involucre, flowers, and herb of cornflower are presented in Fig. 1.

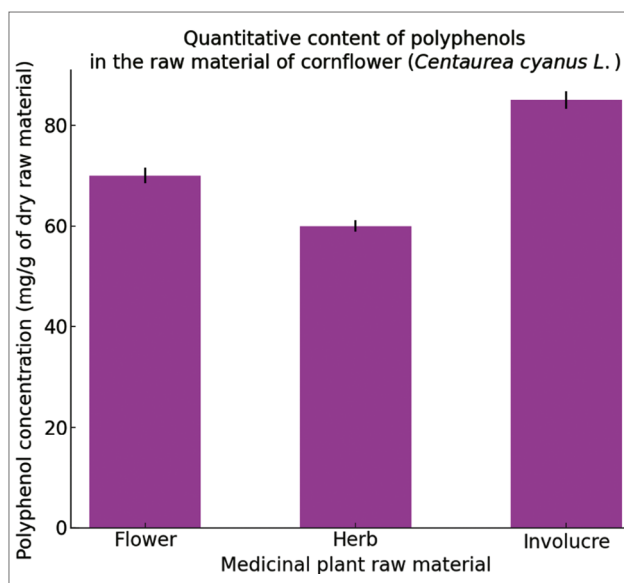


Fig. 1. Quantitative content of polyphenols in the raw material of cornflower (*Centaurea cyanus L.*).

It was found that the highest content of polyphenols was observed in the extracts from the involucre (79.46 ± 0.65 mg/g), while the lowest was in the extracts from the herb (52.61 ± 0.35 mg/g). Since polyphenolic compounds include flavonoids, tannins, phenolic acids, etc., it is reasonable to conduct a more detailed study of these substances to further investigate the effects of extracts from the cornflower involucre on human biological processes.

After fractionation of the polysaccharides from the involucre, fractions of WSP and PS were obtained. The yield of the polysaccharide fraction from the raw material is shown in Fig. 2.

As seen from Table 2, the highest amount of the WSP fraction was present in the flowers and involucre— $12.48 \pm 0.69\%$ and $7.04 \pm 0.017\%$, respectively, while the lowest was in the herb - $3.91 \pm 0.14\%$. In terms of PS content, the flower and herb predominated with $10.3 \pm 0.49\%$ and $8.57 \pm 0.21\%$, respectively, whereas the involucre contained only a negligible amount ($4.82 \pm 0.015\%$).

Macroscopic examination of the cornflower involucre revealed that it has a bell-shaped, multi-row, imbricate structure. The bracts forming the involucre are ovate, acuminate, slightly pubescent, dark green with a dark purple margin at the apex (Fig. 3).

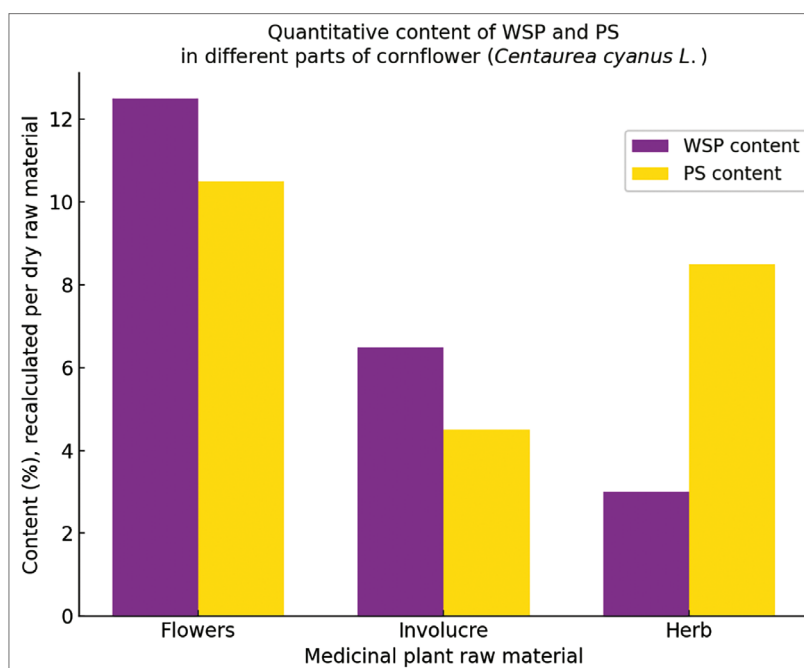


Fig. 2. Quantitative content of WSP and PS in different parts of cornflower (*Centaurea cyanus L.*)



Fig. 3. Involucre of cornflower

Scanning electron microscopy was used to study the ultrastructure of the surface of the involucre leaves. The abaxial surface is densely covered with spirally twisted, multicellular, non-glandular, long covering trichomes (Figs. 4a, 4c) mainly located at the base of the leaf in its central part. These trichomes consist of 3–4 short cylindrical cells and a terminal, spirally twisted long cell. In addition, the surface is also covered with short, unicellular, hook-like trichomes with an acuminate apex (Fig. 4b). Numerous well-

defined, small, elongated stomata are arranged on the abaxial surface, oriented along the midrib (Fig. 4d).

In the central part of the involucre leaf, four-lobed clusters of calcium oxalate crystals are visible (Fig. 5a). The cuticle is well developed and folded. The wax is present in the form of plates and scales in which the stomata are embedded (Fig. 5b).

The adaxial side of the leaf is bare, covered with wax. Stomata and trichomes are absent (Figs. 6a, 6b).

The established main morpho-anatomical diagnostic features of the cornflower involucre (*Centaurea cyanus L.*) can be used for the development of quality control methods for this medicinal plant raw material.

Conclusions

The obtained results offer a solution to the problem of developing resource-saving technologies through the use of waste from the pharmaceutical industry. For the comprehensive utilization of cornflower, we demonstrated

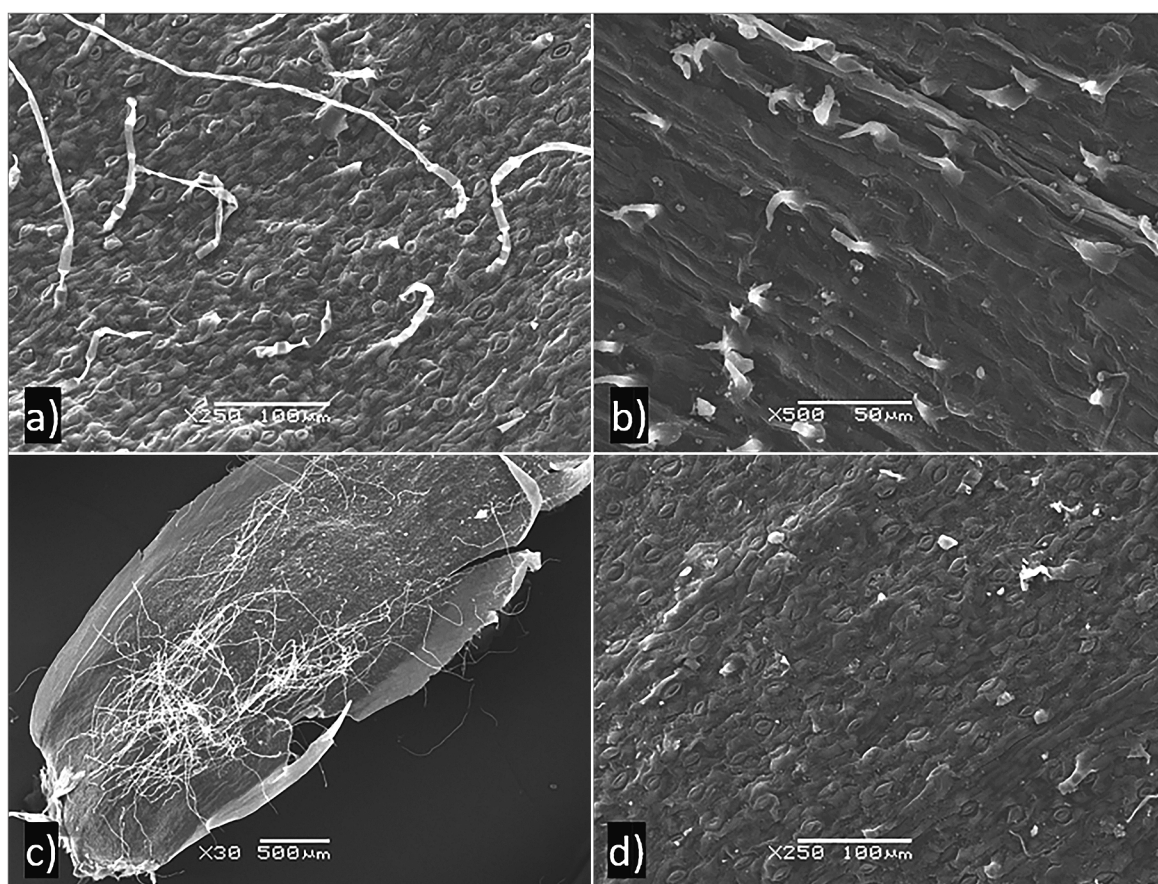


Fig. 4. Scanning electron microscopy of the abaxial surface of the cornflower involucre.

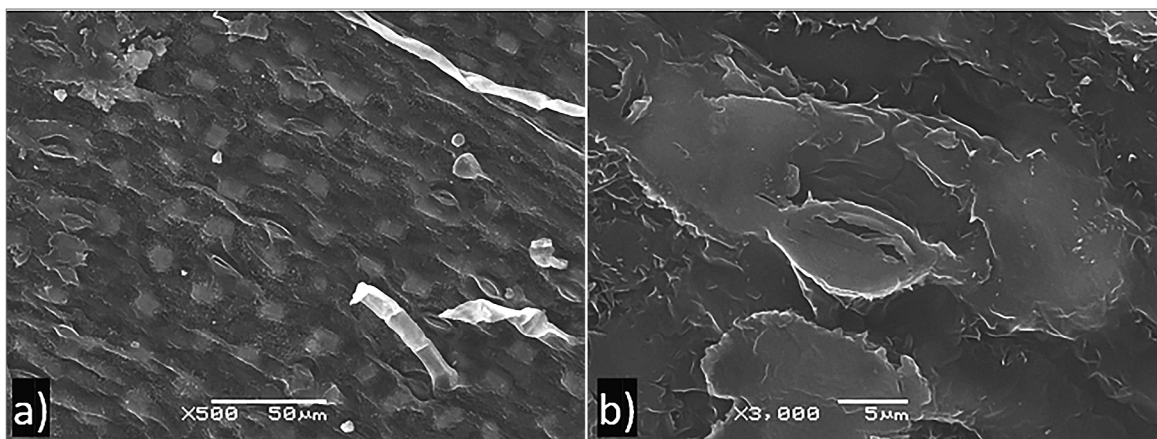


Fig. 5. Scanning electron microscopy of the abaxial surface of the cornflower involucre.

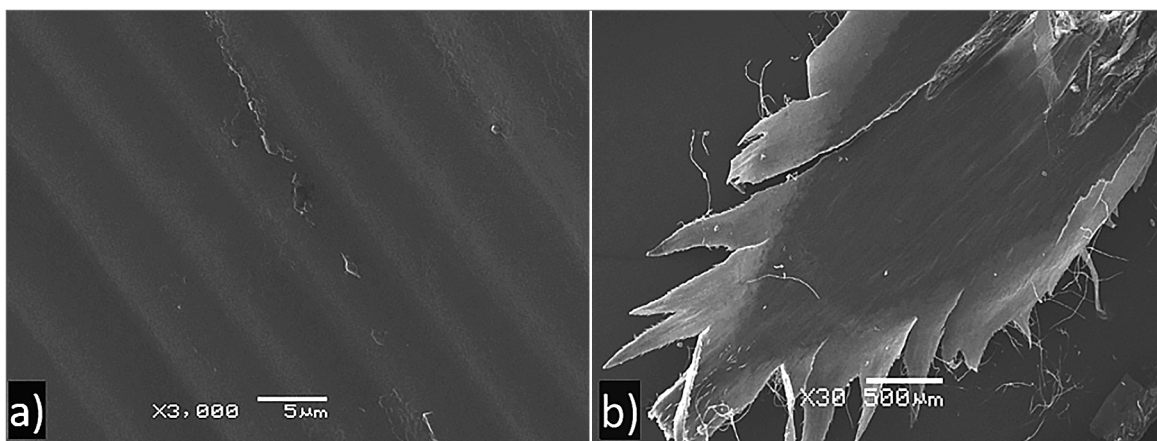


Fig. 6. Scanning electron microscopy of the adaxial surface of the cornflower involucre.

that the involucre possesses a rich chemical composition: polyphenolic compounds, WSP and PS. In terms of polyphenol content, the involucre surpasses the ligulate and tubular flowers as well as the herb, all of which have practical significance. As is well known, these substances exhibit anti-inflammatory, antioxidant, analgesic, and antibacterial activities.

Therefore, it is advisable to continue more detailed studies of the cornflower involucre in order to expand Ukraine's raw material base.

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Conflict of interest

There is no conflict of interest in this article.

No rewards received.

Consent to publication

All authors of the article are acquainted with the final version of the manuscript and have no objections to its publication. The article does not use personal data and information about patients.

ORCID ID and autor contributions

[0009-0006-5258-3721](https://orcid.org/0009-0006-5258-3721) (B,C,D) Yeluzaveta Shevchenko

[0000-0003-1833-321X](https://orcid.org/0000-0003-1833-321X) (A,E,F) Iryna Cholak

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of article.

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Фармакогностична характеристика обгортки волошки синьої (*Centaurea cyanus* L.): морфологічні, анатомічні та фітохімічні особливості

Єлизавета Шевченко, Ірина Чолак

Національний медичний університет імені О.О. Богомольця, Київ, Україна

Corresponding Author:

Yeluzaveta Shevchenko

+380666859050

liza.v.shevchenko09@gmail.com

Анотація. Одним з актуальних напрямків раціонального використання сировинних ресурсів та зниження собівартості лікарських засобів є комплексна переробка рослинної сировини в фармацевтичній промисловості. Часто при заготівлі та підготовці до використання лікарських рослин залишається значна кількість відходів, які надалі не знаходять застосування у практиці,

що веде до втрати цінних біологічно активних сполук. До таких рослин належить волошка синя (*Centaurea cyanus* L.), що належить до родини Айстрові (*Asteraceae*). Ця рослина давно відома своїми лікувальними властивостями та активно застосовується в офіційній та народній медицині. В лікарських цілях переважно використовуються лійкоподібні та трубчасті квітки волошки, які мають виражену протизапальну, сечогінну, антибактеріальну, імуномодуючу, антиоксидантну та капілярозміцнювальну дію, що дозволяє ефективно застосовувати їх у профілактиці серцево-судинних захворювань. Ці фармакологічні властивості обумовлені наявністю широкого спектра біологічно активних сполук: флавоноїдів, гідроксикоричних кислот, дубильних речовин та полісахаридів. Проте в процесі заготівлі квіток рослини обгортку зазвичай відкидають, оскільки її фармакологічна цінність не була достатньо досліджена. Водночас попередні дослідження вказують на перспективність використання цієї частини рослини завдяки високому вмісту біологічно активних речовин. Саме тому в рамках цієї роботи було проведено комплексне фармакогностичне дослідження обгортки квіток волошки синьої з метою визначення її хімічного складу та оцінки потенційних можливостей використання в фармацевтичній практиці. Для досягнення цієї мети застосовували мікроскопічні та фітохімічні методи досліджень відповідно до вимог Державної фармакопеї України (ДФУ). Мікроскопічний аналіз, проведений за допомогою сканувального електронного мікроскопа (*SEM, JSM-6060LA, Японія*), дозволив виявити характерні ультраструктурні ознаки, зокрема наявність спіральних закручених багатоклітинних трихом та видовжених продихів, розташованих уздовж середньої жилки листка. Крім цього, було виявлено чітко виражені скупчення кристалів оксалату кальцію на поверхні листків обгортки. Фітохімічний аналіз показав високий вміст поліфенольних сполук ($79,46 \pm 0,65$ мг/г), що значно перевищує показники в інших частинах рослини. Також у складі обгортки були ідентифіковані водорозчинні полісахариди та пектини, відомі своїми протизапальними, детоксуючими, антибактеріальними та імуностимулюючими властивостями. Отримані результати дозволяють розглядати обгортку волошки синьої як перспективну сировину для створення нових лікарських препаратів з вираженими терапевтичними властивостями. Подальші дослідження мають бути зосереджені на уточненні повного спектра біологічно активних речовин, поглибленому вивченні їх фармакологічної активності та оптимізації технологій екстракції, що стане важливим внеском у розвиток вітчизняної фармацевтичної промисловості.

Ключові слова: *Centaurea*, мікроскопія, полісахариди, фенольні сполуки, пектини, квітки.



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