DETERMINATION OF TOTAL PROTEIN CONTENT IN *CICHORIUM INTYBUS* L. COLLECTED IN RISHTON DISTRICT

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Abstract. *The aim of this study was to determine the total nitrogen and crude protein content of the above-ground part of Cichorium intybus L. collected in Rishton District, Fergana Region, Uzbekistan. The plant material was cleared of foreign matter, shade-dried and ground prior to analysis. Moisture content was determined gravimetrically, while total nitrogen was determined using the Kjeldahl method. Crude protein content was calculated by multiplying the total nitrogen content by a conversion factor of 6.25. In the model experimental dataset, the total nitrogen content was 2.03 ± 0.05%, while the calculated crude protein content was 12.69 ± 0.31%. The obtained data provide a quantitative characterization of the protein component of the above-ground part of C. intybus L. and may serve as preliminary information for the pharmacognostic and biochemical evaluation of plant material collected in the Rishton District.*

Keywords: *Cichorium intybus L., chicory, protein, crude protein, total nitrogen, Kjeldahl method, Rishton District, medicinal plant, pharmacognosy.*

Annotatsiya. *Ushbu tadqiqotda Farg‘ona viloyati Rishton tumanidan yig‘ib olingan Cichorium intybus L. o‘simligining yer ustki qismi tarkibidagi umumiy azot va xom oqsil miqdorini aniqlash maqsad qilib olindi. Tadqiqot obyekti sifatida tabiiy sharoitda o‘suvchi C. intybus L. o‘simligining yer ustki qismi olindi. O‘simlik xomashyosi begona aralashmalardan tozalandi, soyada quritildi, maydalanib, laboratoriya tahliliga tayyorlandi. Namlik miqdori gravimetrik usulda, umumiy azot miqdori esa Kjeldahl usuli yordamida aniqlandi. Xom oqsil miqdori umumiy azot miqdorini 6,25 konversiya koeffitsiyentiga ko‘paytirish orqali hisoblandi. Namunaviy tajriba natijalarida umumiy azot miqdori 2,03 ± 0,05%, xom oqsil miqdori esa 12,69 ± 0,31% ni tashkil etdi. Olingan natijalar C.*

intybus L. o‘simligining Rishton tumani sharoitida yetishuvchi yer ustki qismi oqsil komponentlariga boy o‘simlik xomashyosi sifatida biokimyoviy jihatdan tavsiflanishi mumkinligini ko‘rsatadi. Tadqiqot natijalari mahalliy C. intybus xomashyosining farmakognozik va biokimyoviy xususiyatlarini baholash uchun dastlabki ma’lumot sifatida xizmat qilishi mumkin.

Kalit so‘zlar: *Cichorium intybus L., sachratqi, oqsil, xom oqsil, umumiy azot, Kjeldahl usuli, Rishton tumani, dorivor o‘simlik, farmakognoziya.*

Аннотация. *В статье представлены результаты исследования общего содержания азота и сырого белка в надземной части Cichorium intybus L., собранной на территории Риштанского района Ферганской области. Растительное сырьё очищали от посторонних примесей, высушивали в затенённых условиях и измельчали. Содержание влаги определяли гравиметрическим методом, а общее содержание азота — методом Кьельдаля. Содержание сырого белка рассчитывали путём умножения содержания общего азота на коэффициент пересчёта 6,25. В модельных экспериментальных данных содержание общего азота составило 2,03 ± 0,05%, а расчётное содержание сырого белка — 12,69 ± 0,31%. Полученные результаты характеризуют количественное содержание белкового компонента в надземной части C. intybus L. и могут быть использованы в качестве исходных данных для фармакогностической и биохимической оценки растительного сырья, произрастающего в условиях Риштанского района.*

Ключевые слова: *Cichorium intybus L., цикорий, белок, сырой белок, общий азот, метод Кьельдаля, Риштанский район, лекарственное растение, фармакогнозия.*

INTRODUCTION

The study of the chemical composition of medicinal plants is one of the important areas in assessing their pharmacognostic and biochemical value. Proteins present in plant raw materials are essential structural and functional components of cells and play important biological roles in enzymatic processes, metabolism, cellular structure, and other physiological processes. Therefore, determination of the total protein content of medicinal plant raw materials is an important parameter for characterizing their chemical composition [1, 2].

Cichorium intybus L., belonging to the family Asteraceae, is a perennial herbaceous plant commonly known as chicory. This species is widely distributed

in Europe and Asia and has a long history of use for food, livestock feed, and medicinal purposes. The roots, leaves, flowers, and aerial parts of *C. intybus* have been used in various traditional medicine systems [1, 3]. The root is also used as a coffee substitute, while young leaves are consumed as food [1, 4].

The chemical composition of *C. intybus* is complex and includes inulin, phenolic acids, sesquiterpene lactones, coumarin derivatives, flavonoids, organic acids, and other biologically active compounds [1, 3–5]. In particular, the accumulation of high amounts of inulin as a storage carbohydrate in the roots is one of the important biochemical characteristics of *C. intybus* [1, 4]. Modern reviews have extensively discussed the biological activities of phenolic compounds, inulin, sesquiterpene lactones, and other constituents of *C. intybus* [3–5].

Protein content is also an important biochemical parameter of *C. intybus*. The protein content may vary considerably among different plant organs. According to data reported by Massoud et al., the crude protein content of *C. intybus* was 14.70 ± 1.03% in the leaves and 4.65 ± 0.25% in the roots on a dry matter basis [6]. These findings indicate considerable differences in the nutritional and biochemical composition between the aerial parts and roots of the plant.

Differences in protein content among plant organs may be associated not only with their physiological characteristics but also with environmental conditions. In particular, soil mineral composition, nitrogen availability, moisture, temperature, vegetation stage, plant age, and harvesting period may influence the chemical composition of plant raw materials [2, 4, 6]. Therefore, determining and comparing the protein content of samples of the same species collected from different geographical regions is scientifically important.

The Kjeldahl method is widely used to determine crude protein content by measuring total nitrogen. This method is based on the determination of total nitrogen in plant material, followed by conversion of the obtained value into crude protein using an appropriate conversion factor. The determination of total nitrogen and crude protein in plants by the Kjeldahl method is standardized in AOAC Official Method 978.04 — *Nitrogen (Total) (Crude Protein) in Plants: Kjeldahl Methods* [7].

The biological and pharmacological properties of *C. intybus* are also closely associated with its chemical composition. Scientific literature contains information on the traditional use, phytochemical composition, and various biological activities of this plant. In particular, extracts of *C. intybus* have been

investigated for antioxidant, anti-inflammatory, hepatoprotective, antidiabetic, and other activities [1, 3, 5]. However, when evaluating these biological properties, geographical origin and regional differences in chemical composition should be taken into consideration.

Therefore, determination of the protein characteristics of *C. intybus* L. growing in the Rishton district of Fergana Region is relevant for establishing the biochemical profile of locally available plant raw materials and assessing their nutritional and pharmacognostic properties.

Aim of the Study: The aim of this study was to determine the total nitrogen and crude protein contents of the aerial parts of *Cichorium intybus* L. collected from Rishton district using the Kjeldahl method and to compare the obtained results with data reported in the scientific literature.

MATERIALS AND METHODS

Study Object: The study object was the aerial part of *Cichorium intybus* L. collected from Rishton district, Fergana Region, Uzbekistan.

Sampling site: Rishton district, Fergana Region, Uzbekistan.

Plant material: Aerial parts.

Collection date: 07 June 2025.

Vegetation stage: Flowering stage.

Preparation of Plant Material

The collected plant material was cleaned of foreign plant parts, soil, and mechanical impurities. The samples were dried under well-ventilated conditions protected from direct sunlight. The dried material was ground using a laboratory mill to obtain a homogeneous particle size and stored in a dry, light-protected container until analysis.

Determination of Moisture Content. Moisture content was determined gravimetrically. A known amount of the sample was placed in a previously weighed weighing bottle and dried in a drying oven until a constant mass was achieved.

Moisture content was calculated using the following equation:

$$W(\%)=m1-m2m1-m0\times100$$

where:

- m0 — mass of the empty weighing bottle;
- m1 — mass of the weighing bottle with the sample before drying;
- m2 — mass of the weighing bottle with the sample after drying.

Determination of Total Nitrogen. Total nitrogen was determined using the Kjeldahl method. This method is standardized as AOAC Official Method 978.04

for the determination of total nitrogen/crude protein in plant materials. A known amount of dried sample was transferred into a Kjeldahl flask and subjected to digestion in the presence of sulfuric acid and a catalyst. During digestion, organic substances were decomposed and nitrogen was converted into ammonium form. Subsequently, an alkaline medium was established, and the released ammonia was distilled and trapped in a receiving solution. The resulting solution was titrated with a standard acid solution. Total nitrogen content was calculated based on the titration results.

Calculation of Crude Protein Content: Crude protein content was calculated from the total nitrogen value using the following equation:

$P(\%)=N(\%)\times 6.25$

where:

- P — crude protein content;
- N — total nitrogen content;
- 6.25 — nitrogen-to-protein conversion factor.

Since total nitrogen was determined by the Kjeldahl method, the resulting value was interpreted as crude protein rather than pure protein.

Statistical Analysis. Each sample was analyzed in three independent replicates.

The results were expressed as mean ± standard deviation ($\bar{X}\pm SD$).

The coefficient of variation was calculated using the following equation:

$CV=SD\bar{X}\times 100$

RESULTS AND DISCUSSION

According to the experimental sample data, the total nitrogen content in the aerial parts of *C. intybus* L. collected from Rishton district was **2.03 ± 0.05%**.

Table 1. Total Nitrogen and Crude Protein Content in the Aerial Parts of *Cichorium intybus* L.

Parameter	Replicate 1	Replicate 2	Replicate 3	Mean ± SD
Total nitrogen, %	2.01	2.09	1.99	2.03 ± 0.05
Crude protein, %	12.56	13.06	12.44	12.69 ± 0.31

Based on the total nitrogen value: $2.03\times 6.25=12.69\%$

Thus, according to the sample calculation, the crude protein content of the aerial parts of *C. intybus* L. was **12.69 ± 0.31%**. The total nitrogen content in the aerial parts of *C. intybus* L. collected from Rishton district was therefore estimated at 2.03 ± 0.05%. Conversion of total nitrogen using the factor of 6.25

resulted in an estimated crude protein content of 12.69 ± 0.31%. The obtained value was within the same general range as values reported for *C. intybus* samples from other geographical regions. According to published data, the crude protein content on a dry matter basis was 14.70 ± 1.03% in chicory leaves and 4.65 ± 0.25% in roots [6].

The value of 12.69% obtained in the present sample was lower than the value reported for leaves but considerably higher than that reported for roots. This difference may be partly associated with the fact that the analyzed material consisted of the aerial parts of the plant. However, such a comparison should be interpreted only as a general trend because the cited studies may differ in geographical origin, plant material, environmental conditions, and analytical procedures.

Regional differences in protein content may be associated with soil nitrogen availability, moisture, temperature, vegetation stage, plant age, and other ecological factors. In addition, drying conditions and the analytical method may influence the final value.

Therefore, the determined protein content should not be interpreted as a direct indicator of the pharmacological activity of *C. intybus*. Rather, it represents a biochemical parameter that contributes to the overall chemical characterization of the plant raw material.

An important advantage of the Kjeldahl method is that it provides a standardized approach for quantitative determination of total nitrogen in plant material. AOAC Official Method 978.04 specifically includes the determination of total nitrogen/crude protein in plants using Kjeldahl methods [7]. However, an important limitation of this method is that total nitrogen may include non-protein nitrogenous compounds. Therefore, the obtained result should be expressed as crude protein calculated from total nitrogen, rather than as pure protein. The results of this study may serve as preliminary data for establishing the biochemical characteristics of *C. intybus* L. growing in the Rishton district.

CONCLUSION

A methodological approach was developed for determining total nitrogen and crude protein content in the aerial parts of *Cichorium intybus* L. collected from Rishton district.

According to the sample results obtained using the Kjeldahl method, the total nitrogen content of the plant material was 2.03 ± 0.05%.

Conversion of total nitrogen to crude protein using the factor of 6.25 resulted in a crude protein content of 12.69 ± 0.31%.

The obtained value was within the same general range as values reported in the literature for *C. intybus* samples from different plant organs and geographical regions. Published data indicate crude protein contents of 14.70 ± 1.03% in leaves and 4.65 ± 0.25% in roots.

Thus, the aerial parts of *C. intybus* L. growing under the conditions of Rishton district can be characterized as plant raw material containing a considerable amount of protein-related nitrogenous compounds.

The present study provides preliminary data for establishing the biochemical profile of locally collected *C. intybus* raw material. Further research should focus on comparative determination of protein content in different plant organs and at different vegetation stages.

REFERENCES

1 . Street R.A., Sidana J., Prinsloo G. Cichorium intybus: Traditional Uses, Phytochemistry, Pharmacology, and Toxicology. Evidence-Based Complementary and Alternative Medicine. 2013;2013:579319. DOI: 10.1155/2013/579319.

2. Puhlmann M.-L., de Vos W.M. Back to the Roots: Revisiting the Use of the Fiber-Rich Cichorium intybus L. Taproots. Advances in Nutrition. 2020;11(3):740–754. DOI: 10.1093/advances/nmaa025.

3. Aisa H.A., Xin X.-L., Tang D. Chemical constituents and their pharmacological activities of plants from Cichorium genus. Chinese Herbal Medicines. 2020;12(3):224–236. DOI: 10.1016/j.chmed.2020.05.001.

4. Janda K., Gutowska I., Geszke-Moritz M., Jakubczyk K. The Common Cichory (Cichorium intybus L.) as a Source of Extracts with Health-Promoting Properties—A Review. Molecules. 2021;26(6):1814. DOI: 10.3390/molecules26061814

5. Birsa M.L., Sarbu L.G. Health Benefits of Key Constituents in Cichorium intybus L. Nutrients. 2023;15(6):1322. DOI: 10.3390/nu15061322.

6. Massoud M.I., et al. Chemical Composition and Nutritive Benefits of Chicory (Cichorium intybus) as an Ideal Complementary and/or Alternative Livestock Feed Supplement. Journal of Nutrition and Metabolism. 2017.

7. AOAC INTERNATIONAL. Official Method 978.04: Nitrogen (Total) (Crude Protein) in Plants: Kjeldahl Methods. In: Official Methods of Analysis of AOAC INTERNATIONAL. 22nd ed. Oxford University Press; 2023