

QUALITY CONTROL AND STANDARDIZATION OF BIOLOGICALLY ACTIVE SUBSTANCES INCLUDED IN THE COMPOSITION OF THE DRY EXTRACT 'EXTRADENT'

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Abstract The present study is devoted to the quality control and standardization of the dry extract 'Extradent', a phytopreparation composed of *Calendula officinalis*, *Urtica dioica*, *Capsella bursa-pastoris*, and *Polygonum hydropiper*. These plants are rich in flavonoids, carotenoids, tannins, amino acids, and mineral elements, which collectively determine the wide pharmacological spectrum of the extract. In this research, high-performance liquid chromatography (HPLC) and UV-spectrophotometry were applied to evaluate the chemical composition with a special focus on flavonoids. Rutin was identified as the principal marker compound in the chromatogram, with a significant retention peak, thus confirming its suitability for analytical standardization. Complementary UV-spectrophotometric measurements demonstrated the presence of a broad spectrum of flavonoids beyond rutin, contributing to the biological activity of the preparation. Literature data were also considered to outline the role of other biologically active substances, such as carotenoids, tannins, amino acids, and mineral elements, in enhancing therapeutic properties. The findings highlight that combining chromatographic and spectrophotometric methods provides a reliable approach to ensure reproducibility, safety, and compliance with international standards. This study may serve as a basis

for developing methodological guidelines for the certification and wider application of the 'Extradent' extract in preventive and therapeutic medicine.

Keywords: Extradent, rutin, flavonoids, HPLC, UV-spectrophotometry, carotenoids, tannins, amino acids, minerals, quality control, standardization.

Introduction

Herbal preparations and plant-derived biologically active compounds remain highly relevant in modern medicine due to their broad pharmacological properties and relatively low toxicity. According to the World Health Organization, more than 80% of the global population relies on plant-based remedies for prevention and treatment of various diseases.

Among these remedies, standardized multi-component extracts attract particular attention, as they combine different classes of biologically active substances (BAS), thereby providing complex therapeutic effects. One such phytopreparation is the dry extract 'Extradent', composed of *Calendula officinalis*, *Urtica dioica*, *Capsella bursa-pastoris*, and *Polygonum hydropiper*. These plants are rich sources of flavonoids, carotenoids, tannins, amino acids, and mineral elements.

Ensuring quality control and reliable standardization of BAS in such multi-component systems is essential for their reproducibility, clinical safety, and certification at both national and international levels. Therefore, the present work focuses on the qualitative and quantitative evaluation of the major components of the 'Extradent' extract with particular emphasis on flavonoids, using high-performance liquid chromatography (HPLC) and spectrophotometric methods.

Materials and Methods

The study was carried out on a dry ethanol extract of 'Extradent'. The powdered sample was dissolved in 70% ethanol, followed by ultrasonic-assisted extraction for 20 minutes and filtration through a 0.45 μm membrane filter.

HPLC analysis

Chromatographic separation was performed on a C18 column (250 $\times$ 4.6 mm, 5 μm) with a mobile phase consisting of acetonitrile, water, and acetic acid in the ratio 15:83:2. The flow rate was maintained at 1.0 mL/min, injection volume — 10 μL , and detection wavelength — 290 nm.

UV-spectrophotometric method

In addition to chromatographic analysis, the total flavonoid content was evaluated by forming a complex with AlCl_3 and recording the absorbance at $\lambda = 415$ nm. This method was applied for a broader estimation of flavonoids apart from the identified rutin marker.

Results

The HPLC chromatogram of the ethanol extract of 'Extradent' revealed several peaks, with rutin being clearly identified as the main flavonoid marker (retention time ~ 1.11 min). Other peaks corresponded to unidentified phenolic compounds. Quantitative evaluation confirmed that rutin is present at a significant level, supporting its role as a reliable standardization marker.

In addition to HPLC, the UV-spectrophotometric assay demonstrated a considerable amount of total flavonoids in the extract, indicating that the preparation contains not only rutin but also a spectrum of flavonoid derivatives contributing to its pharmacological activity.

Table 1. HPLC results of the ethanol extract 'Extradent'

Peak#	Ret. Time (min)	Area	Height	Conc. (mg/L)	Compound
1	0.958	2484	554	-	-
2	1.112	47239	11613	-	Rutin
3	1.305	22768	1953	-	-
4	1.525	9531	995	-	-
5	7.295	1098	310	-	-
6	7.764	1050	276	-	-

Discussion

The results demonstrate that rutin can be used as a key marker for the standardization of 'Extradent'. However, the extract is not limited to flavonoids. Literature reports indicate that its constituent plants contain a wide spectrum of biologically active substances:

- Carotenoids (*Calendula officinalis*) contribute to antioxidant and wound-healing activity.
- Tannins (*Polygonum hydropiper*) provide antimicrobial and astringent effects.
- Amino acids (*Urtica dioica*) support metabolic regulation and tissue repair.
- Macro- and microelements (Ca, Mg, Fe, Zn, Cu) enhance general restorative properties and strengthen local immunity.

This multi-component composition explains the broad therapeutic potential of the preparation in dentistry and related fields. At the same time, it emphasizes the need for reliable quality control approaches, combining chemical analysis of marker compounds with broader assays to ensure reproducibility and safety.

Conclusion

The study confirms that HPLC analysis with rutin as a marker compound, supported by UV-spectrophotometric estimation of total flavonoids, is an effective approach for the quality control and standardization of the dry extract 'Extradent'. Considering the additional presence of carotenoids, tannins, amino acids, and mineral elements, the extract represents a complex phytopreparation with potential preventive and therapeutic applications.

These results may serve as a basis for the development of regulatory guidelines and certification strategies in accordance with international standards.

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