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TOTAL PHENOLIC AND FLAVONOID CONTENT IN THE DRY MATTER OF *DROSERACEAE* SPECIES DURING LONG-TERM STORAGE

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1. Introduction

Over the last few decades, large amounts of scientific data have been accumulated regarding the beneficial properties of medicinal plants. Flavonoids and other phenolic compounds of plant origin possess significant pharmaceutical value. This class of plant secondary metabolites is widely known for its antioxidant, antibacterial, anti-inflammatory, antitumor, immunostimulatory, and cardioprotective properties [Tungmunnithum, 2018]. In recent years, efforts to develop phytopharmaceuticals based on medicinal plant extracts have intensified. However, despite their impressive potential, such drugs preparations face difficulties entering the mainstream pharmaceutical industry. One of the main problems is the inconsistency in the quality of finished products and the raw materials used for their production, which directly affects the efficacy and safety of the medicinal product. Therefore, studying the stability of both raw materials and the final product is

extremely important for the development of stable preparations with standardized dosage [Khalid, 2011].

Carnivorous plants of the family *Droseraceae* are relatively easy to cultivate *in vitro* and are capable of accumulating significant amounts of secondary metabolites with high biological activity in their tissues [Wójciak, 2023].The sensitivity of carnivorous plants to cultivation conditions and the use of elicitors make it possible to significantly increase the content of secondary metabolites in the biomass [Makowski, 2020, 2024]. This makes carnivorous plants promising candidates as bioreactors for the industrial production of plant phenolics for pharmaceutical needs. Currently, most studies are focused on optimizing cultivation conditions and biochemical profiling of carnivorous plants. However, the question of the potential shelf life of medicinal raw materials obtained from these plants remains unexplored. Therefore, the aim of this study is to determine the effect of long-term storage on the total content of phenols and flavonoids in the dry matter of representatives of the family *Droseraceae*.

2. Materials and Methods

2.1. Plant Material

Carnivorous plants *Dionaea muscipula* 'Cupped Trap' and *Drossera spatulate* were used in this study. The plant material and equipment were kindly provided by the staff of the Tissue Culture Laboratory of the Plant Breeding and Genetics Institute – National Center of Seed and Cultivar Investigation, Odesa.

The plants were cultivated for thirty days in vitro in glass containers on Quoirin and Lepoivre nutrient medium (pH 5.8) [Quoirin, 1977] without the addition of growth regulators.

2.2. Drying and Preparation of Plant Tissue Extracts

The plants were weighed and then dried to constant weight in a drying oven at 105 °C. Extraction was carried out by maceration for 72 hours at 5 °C using 80% methanol. The first portion of the material was extracted and analyzed immediately after drying. The second portion of the dried plant material was stored in a metal container in a dark, dry place at 15–20 °C for one year.

2.3. Spectrophotometric Analysis of Samples

Total phenolic content (TPC) was assessed using Folin–Ciocalteu's reagent, with modifications according to Makowski et al. Plant material (10 mg DW) was homogenized in 1 mL of 80% methanol at 4 °C. Samples were centrifuged for 15 min (25,155×g, 4 °C). The diluted extract was mixed with 0.2 mL of Folin's reagent (Sigma-Aldrich Chemie, GmBH, Steinheim, Germany) and 1.6 mL of 5% Na_2CO_3. After 20 min of incubation at 40 °C, the absorbance of samples was measured at 740 nm. Gallic acid was used as the reference standard [Makowski, 2020].

Total flavonoid content (TFC) was estimated using the method of Fukumoto and Mazza, with modifications [Tokarz, 2018]. Plant tissue was extracted in the same manner as for TPC determination. The supernatant was mixed with 0.25 mL of 0.1% HCl in 96% ethanol and 4.55 mL of 2% HCl in water. Samples were

incubated at room temperature in darkness for 20 min. Absorbance was measured at a wavelength of 360 nm. Quercetin was used as the reference standard [Makowski, 2020].

Spectrophotometric analysis of the samples was performed using a KFK-2 photoelectric colorimeter (Ukraine). All measurements were carried out in five biological replicates.

Results from the experiments were subjected to Student's t-test at a significance level of $p < 0.05$.

3. Results and Discussion

After one year of storage, the total phenolic content (TPC) in the dry matter of *Dionaea muscipula* decreased by 22.8%. In contrast, no statistically significant changes in TPC were observed for *Drossera spatulata*.

The total flavonoid content (TFC) in the dry matter of *Dionaea muscipula* decreased by 54.5% over the year, while in *Drossera spatulate* it decreased by 45.7%.

	TPC	TPC after 1 year	TPC reduction	TFC	TFC after 1 year	TFC reduction
D.muscipula	51,3±5,1	39,6±1,5	22,8 %	78,1±9,1	34,8±4,0	54,5%
Dr.spatulata	20,0±3,7	24,0±5,3	-	30,7±3,3	16,7±3,3	45,7%

Table 1. Total phenolic and flavonoid content in the dry matter of *Droseraceae* plants after one year of storage.

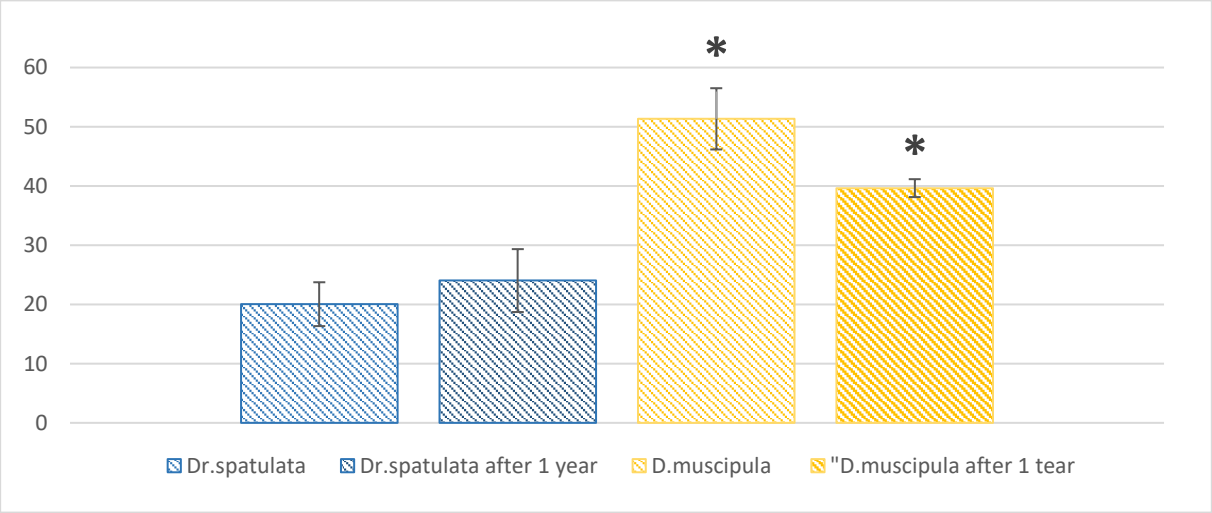

Figure 1. Total phenolic content in the dry matter of *Droseraceae* plants after one year of storage.

Note: Values marked with * are statistically significant ($p < 0.05$)

The average loss of phenolic compounds during the storage of dry medicinal plant material typically ranges from 30% to 50% [Dziadek, 2022; Mrázková, 2023; Tsantili, 2011]. In our experiment, *Dionaea muscipula* showed a smaller decrease in

TPC, only 22.8%, which can be considered relatively favorable. However, the total flavonoid content in *Dionaea muscipula* decreased by 54.5%—more than half compared to fresh plant material. *Drossera spatulate* also lost nearly half of its total flavonoid content, with a reduction of 45.7%. This indicates an uneven rate of degradation among different phenolic compounds under stable storage conditions.

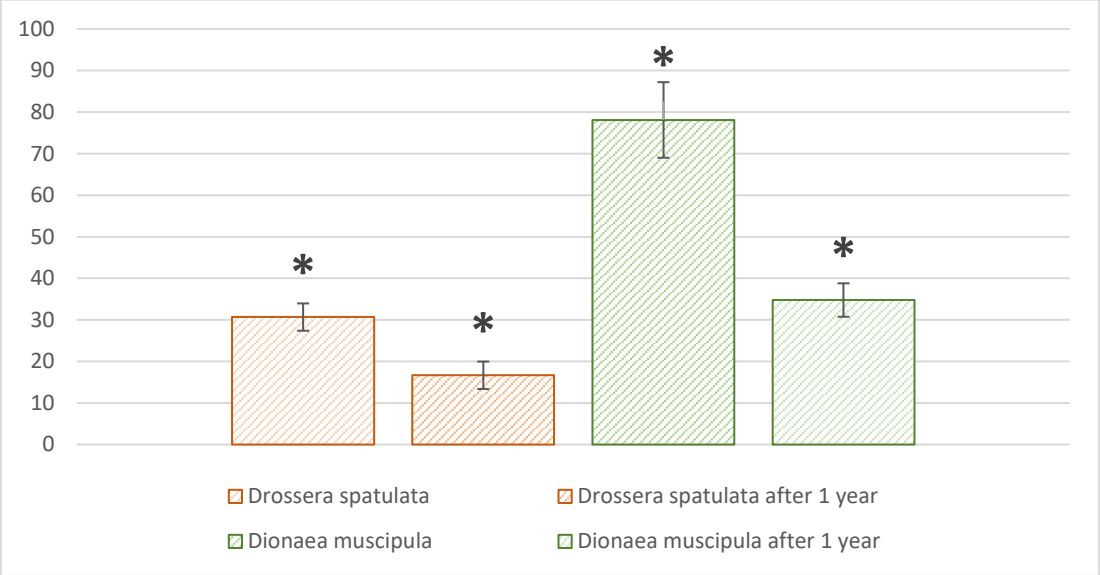

Figure 2. Total flavonoid content in the dry matter of *Droseraceae* plants after one year of storage.

Uneven decreases in the content of bioactive compounds in plant medicinal raw materials pose a risk to the stability and safety of preparations derived from them. Therefore, further research is necessary to optimize the long-term storage conditions of dry material from *Droseraceae* species.

Conclusions

A decrease in the total phenolic content of the dry matter of *Dionaea muscipula* by 22.8% was observed after one year of storage. Additionally, the total flavonoid content decreased in *Dionaea muscipula* and *Drossera spatulata* by 54.5% and 45.7%, respectively.

These results indicate the need for further research and the development of methods to stabilize the quality of dry material from *Droseraceae* species during long-term storage, aiming at the production of reliable phytopharmaceuticals.

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